University of Alberta

Factors Affecting Nifedipine Bioavailability

by

John Stewart Grundy

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

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Dear Sir or Madam:

This letter has been written as confirmation and permission for John S. Grundy to use the information in the following nine journal articles, in which we shared authorship, for the preparation and publication of his PhD thesis at the University of Alberta:

1. J.S. Grundy, R.T. Foster. The nifedipine gastrointestinal therapeutic system (GITS): Evaluation of pharmaceutical, pharmacokinetic and pharmacological properties. *Clin. Pharmacokin.*, 30(1), 28-51, 1996.

2. J.S. Grundy, R. Kherani, R.T. Foster. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing UV detection. *J. Chromatogr. B. Biomed. Appl.*, 654, 146-151, 1994.

3. J.S. Grundy, R. Kherani, R.T. Foster. Photostability determination of commercially available nifedipine oral dosage formulations. *J. Pharm. Biomed. Anal.*, 12, 1529-1535, 1994.

4. J.S. Grundy, R.Z. Lewanczuk, D.W. Grace, R.T. Foster. Observation of time-dependent and variable subject kinetics in a nifedipine gastrointestinal therapeutic system bioequivalency study. *J. Contr. Rel.*, Accepted for publication, 1996.

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9. J.S. Grundy, R.T. Foster. Grapefruit juice does not influence the extent of in vitro nifedipine protein binding in human plasma. *Biopharm. Drug Dispos.*, Submitted for publication, 1996.

If you have any additional questions, please do not hesitate to contact me (Office: 403 487 1600) at your earliest convenience.

Sincerely,

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July, 4, 1996

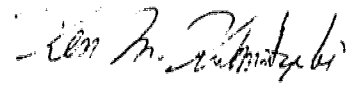
To Whom It May Concern:

This letter gives John Grundy permission to include, *Grapefruit juice and orange juice effects on the bioavailability of nifedipine in the rat*, in his Ph.D. thesis.

If you have any questions please feel free to contact me at 482-6001, if I am not in please leave a message.

Thank you.

Yours truly,



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Dear Sir or Madam:

This letter has been written as confirmation and permission for John S. Grundy to use the information in the following two journal articles, in which we shared authorship, for the preparation and publication of his PhD thesis at the University of Alberta:

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If you have any additional questions, please do not hesitate to contact me (Lab: 403-492-6829; Home: 403-430-0559) at your earliest convenience.

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This letter has been written as confirmation and permission for John S. Grundy to use the information in the following two journal articles, in which we shared authorship, for the preparation and publication of his PhD thesis at the University of Alberta:

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If you have any additional questions, please do not hesitate to contact me (Office: 492-3478) at your earliest convenience.

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Dear Sir or Madam:

This letter has been written as confirmation and permission for John S. Grundy to use the information in the following journal article, in which we shared authorship, for the preparation and publication of his PhD thesis at the University of Alberta:

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If you have any additional questions, please do not hesitate to contact me (403-492-6277) at your earliest convenience.

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FACULTY OF MEDICINE • UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA • T6G 2N8 • PHONE: 403-492-6277

DEDICATION

For my wife (Starr) and both our parents (Stewart and Margaret Grundy; John and Deanna Dutton), whose love and support made the work and effort that culminated in the writing of this thesis possible.

ABSTRACT

Nifedipine, prototype of the 1,4–dihydropyridine class of calcium channel blockers, is typically used for treatment of hypertension or ischaemic heart disease. Peroral nifedipine bioavailability is similar in humans and rats, ranging from about 40% to 68%. The elucidation of specific factors that influence nifedipine bioavailability (including both the rate and extent of drug absorption) is important for the design of new formulations of nifedipine or similar compounds (e.g., felodipine, isradipine, nicardipine etc.) to maximize drug delivery and clinical effects, while minimizing inter– and intrasubject variability and adverse effects.

To quantify nifedipine concentrations in aqueous and biological samples, a sensitive and specific HPLC assay method was developed and proved to be reliable and suitable for use in subsequent studies. A series of in vitro and in vivo studies were conducted to investigate several factors that could potentially affect nifedipine bioavailability, including: photostability, chronopharmacokinetics, dissolution characteristics, gut wall presystemic metabolism, grapefruit juice effects and plasma protein binding.

Photodegradation of nifedipine in intact commercially available formulations was found to be clinically insignificant even after long–term artificial sunlight exposure. Hence, if differences in bioavailability are observed between different nifedipine formulations then photodegradation of the drug is probably not a contributing factor.

Chronopharmacokinetic properties of nifedipine appeared to be observed in a bioequivalency study with the nifedipine gastrointestinal therapeutic system (GITS). Alternatively, the results may have shown that the position of this dosage form in the GI tract affected nifedipine bioavailability. In addition, large inter– and intrasubject variability of typical pharmacokinetic measures was found.

A two-phase dissolution test system was developed for the nifedipine GITS and found to improve in vitro–in vivo correlations. Dissolution of the released nifedipine particles was found to be an important factor for determining the rate and duration of drug absorption.

Pharmacokinetic studies in the rat showed that nifedipine bioavailability following po dosing was influenced by both hepatic and gut wall metabolism. The rat was also found to be a suitable model for exploring the interaction of grapefruit juice with nifedipine as observed in human clinical trials.

Grapefruit juice did not affect the extent of nifedipine human plasma protein binding, although effects on the affinity of binding could not be ruled out.

The results found illustrate the variety of factors which can influence nifedipine bioavailability, and may be important considerations for optimizing nifedipine delivery and the therapeutic effects of this compound in human beings.

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a	Absorbed dose
A	Amount of drug
α	Level of significance (probability of making a type I error)
ANOVA	Analysis of Variance
AUC	Area under the plasma concentration-time curve from zero to infinity
AUC(0-t)	Area under the plasma concentration time curve from 0 to time t
b	body
BP	Blood Pressure
BP_r	Whole blood–to–plasma concentration ratio
CAM	Carboxylic Acid Metabolite
CC	Coat–Core
χ^2	Chi–Square statistic
C_B	Bound drug concentration
C_F	Free (unbound) drug concentration
C_{max}	Maximum (peak) plasma drug concentration after single–dose administration
C^{ss}	Steady–state drug concentration in plasma during constant rate infusion or zero–order absorption
C_T	Total drug plasma concentration (bound and unbound)
C_{UF}	Ultrafiltrate drug concentration
$C(t)$	Input response function
$C_\delta(t)$	Impulse response function
CL	Total body (systemic) clearance
CL_{h_b}	Hepatic clearance of drug from blood
CL_{h_p}	Hepatic clearance of drug from plasma
CL_i	Intrinsic clearance
CL/F	Oral clearance of drug from plasma
CR	Controlled–Release
CV	Coefficient of Variation
CYP	Cytochrome P450
°C	Degrees Celsius
D	Dose
DNIF	Dehydronifedipine
E	Organ extraction ratio
ER	Extended–Release
ev	Extravascular
eV	Electron–volt(s)
F	Absolute bioavailability
f_{ra}	Fraction remaining to be absorbed
$f(t)$	input rate function
f_u	Free (unbound) fraction of drug in plasma

FDA	Food and Drug Administration (United States)
g	Gram(s) or Gut wall
GC	Gas Chromatography (Chromatographic)
GI	Gastrointestinal
GITS	Gastrointestinal Therapeutic System
GJC	Grapefruit Juice Concentrate
GJRS	Grapefruit Juice Regular Strength
h	hepatic
HPB	Health Protection Branch (Canada)
HPLC	High–Performance Liquid Chromatography (Chromatographic)
HR	Heart Rate
HSA	Human Serum Albumin
IBD	Inflammatory Bowel Disease
ic	Intracolonic
i.d.	Inside Diameter
INDAS	Insoluble Drug Absorption System
ip	Intraperitoneal
IR	Immediate–Release
IS	Internal Standard
IU	International Unit(s)
iv	Intravenous
K_a	Association constant
K_d	Dissociation constant
k_{el}	Terminal first–order elimination rate constant
kg	Kilogram(s)
K_m	Michaelis constant
l	gut lumen
L	Litre
λ_z	Terminal phase
LLD	Lower Limit of Detection
LLQ	Lower Limit of Quantitation
M	Molar
MAT	Mean Absorption Time
MATH	Modern Approach to the Treatment of Hypertension
µg	Microgram(s)
µL	Microliter(s)
µM	Micromolar(s)
mg	Milligram(s)
mL	Milliliter(s)
mmol	Millimole(s)
MRT	Mean Residence Time
MTBE	Methyl–t–butyl ether
n	Number of observations
N	Normal
NDNIF	Nitroso–analog of Dehydronifedipine
ng	Nanogram(s)
nm	Nanometer(s)

nmol	Nanomole(s)
o.d.	Outside Diameter
OJC	Orange Juice Concentrate
OJRS	Orange Juice Regular Strength
OROS	Osmotic Release Oral System
OTC	Over–the–Counter
p	Plasma
P	Probability of rejecting the null hypothesis when it is true
PA	Prolonged–Action
PD	Pharmacodynamic
%	Percent
%B	Percent binding of drug to plasma proteins
%E	Percent evaporative loss
pH	Negative logarithm of concentration of H^+ ions
PK	Pharmacokinetic
pK_a	Negative logarithm of the equilibrium constant (acid or base)
po	Peroral
P_T	Total plasma protein concentration
Q	Organ blood flow
r	Correlation coefficient
r^2	Coefficient of determination
R	Chromatographic resolution
R_0	Zero–order release rate
RDC	Rotating Dialysis Cell
s	Standard deviation
$s_{\bar{x}}$	Standard error of the mean
SIF'	Simulated Intestinal Fluid, USP, without pancreatin
SR	Sustained-Release
t_{max}	Time to reach peak or maximum concentration after drug administration
$t_{1/2}$	Elimination half-life associated with the terminal slope (λ_z) of a semilogarithmic concentration-time curve
TEA	Triethylamine
T/P	Trough/Peak
US	United States
USP	United States Pharmacopeia
uv	Ultraviolet
V'_z	Pharmacokinetic volume of distribution during terminal (λ_z) phase
V'_{ss}	Volume of distribution at steady state
W_{AC}	Weight of loaded ultrafiltration unit after centrifugation
W_{BC}	Weight of loaded ultrafiltration unit before centrifugation
W_{BL}	Weight of ultrafiltration unit before loading with sample

Chapter 1

INTRODUCTION

General Definition of Bioavailability

Bioavailability refers to both the rate and extent of drug absorption, although this term is typically used to characterize the extent (amount) of therapeutically active drug reaching the systemic circulation.[1,2] Complete bioavailability is assumed to be obtained following intravenous (iv) administration since a drug is considered to be placed directly into the systemic circulation. Absolute bioavailability, F, or the fraction of an extravascular dose (ev) reaching the general circulation can be determined from plasma data using one of the following relationships:[1]

$$F = \left(\frac{AUC_{ev}}{AUC_{iv}}\right)\left(\frac{D_{iv}}{D_{ev}}\right) \tag{1.1}$$

and

$$F = F_a \cdot F_g \cdot F_h \tag{1.2}$$

where AUC_{ev} and AUC_{iv} are the areas under the plasma concentration–time curves following ev and iv dosing, respectively; D_{ev}, D_{iv} are the corresponding ev and iv doses; F_a, F_g, and F_h are the fraction of the ev dose absorbed, fraction escaping gut wall metabolism (enteral absorption) and fraction escaping hepatic (liver) metabolism, respectively. These calculations assume that systemic clearance (CL_s) remains constant and that the lung does not contribute to drug clearance.

Relative bioavailability is determined from comparison of different formulations of a drug, different routes of administration or different conditions (e.g., diet, disease state), but typically refers to the systemic availability of a test formulation (e.g., a 'second–entry product') as compared to a recognized reference standard formulation (e.g., a 'brand–name product') of a drug:[1,2]

$$\text{Relative Bioavailability} = \frac{F_A}{F_B} = \left(\frac{AUC_A}{AUC_B}\right)\left(\frac{D_B}{D_A}\right) \tag{1.3}$$

where A, B refer to the test and reference standard drug products, F_A, F_B are the absolute bioavailabilities of drug products A and B, AUC_A, AUC_B are the AUC's of products A and B, and D_A, D_B are the doses of products A and B, respectively. As with the calculation of absolute bioavailability, systemic clearance is assumed to remain constant.

Deconvolution methods are commonly used to estimate drug absorption parameter values (including the absorption rate constant, k_a),[3,4] especially when it is suspected that absorption is not a first–order process. Deconvolution, which makes no assumptions about the nature of the absorption process, allows information about absorption to be calculated by subtracting data obtained after iv administration from data collected after ev administration. Deconvolution methods include both model dependent [Wagner–Nelson (one–compartment model) or Loo–Riegelman (multicompartment model) methods] and model independent techniques.[3,4]

The Wagner–Nelson method assumes a one–compartment pharmacokinetic model for disposition.[3] From the mass balance equation,

$$A_a = A_b + A_e \tag{1.4}$$

where A_a is the amount of drug absorbed, A_b is the amount of drug in the body and A_e is the amount of drug excreted, the following expression can be derived,

$$\frac{A_a}{V} = C + k_{el} \cdot \int_0^t C \cdot dt \tag{1.5}$$

where V is the apparent volume of distribution, C is the drug plasma concentration, t is time, and k_{el} is the terminal first–order elimination rate constant. A plot of fraction (or percent) remaining to be absorbed (f_{ra}) versus time can be obtained by dividing A_a/V by the total amount absorbed/V and subtracting this from unity,

$$f_{ra} = 1 - \frac{C + k_{el} \cdot \int_0^t C \cdot dt}{k_{el} \cdot \int_0^\infty C \cdot dt} \tag{1.6}$$

An estimate of k_a for a zero– or first–order process is determined from the linear or log–linear slope of this plot, respectively.

Model–independent deconvolution techniques are based on the expression,

$$C(t) = C_\delta(t) \cdot f(t) \tag{1.7}$$

where, $C(t)$ is the drug concentration following oral administration of a solid dosage form (input response), $C_b(t)$ is the drug concentration after iv administration (impulse response) and $f(t)$ is the input rate.[3,5] Therefore, estimates of $f(t)$ can be obtained using a suitable computer program if the input response and impulse response data are known.

As indicated by equation 1.2, the value of F following po delivery is dependent upon the absorbable fraction of the given dose and the fraction that passes through both the gut wall and liver, which, in addition to the rate of drug absorption, can be influenced by a number of different factors. These factors include: the physicochemical properties of the drug (e.g., solubility, pK_a, n–octanol:water partition coefficient, crystal habit), the nature of the drug formulation [e.g., immediate–release (IR), sustained–release (SR) or controlled–release (CR) formulations], stability of the drug in the gastrointestinal (GI) tract (e.g., pH or microflora effects), the extent of first–pass metabolism (intrinsic clearance) within the gut wall and liver, the time of administration (chronopharmacokinetics), hepatic and GI blood flow, the extent and affinity of plasma protein binding, and the influence of various disease states, foods and drugs. In addition, the sensitivity, specificity and accuracy of the analytical method used to determine blood, plasma or serum drug concentrations, as well as the integrity, purity and accuracy of the labeled drug content (dose) as indicated by the manufacturer can be very important factors for accurate determination of drug bioavailability.

Nifedipine

Nifedipine is a calcium channel blocking drug characterized by a short duration of action, due to its relatively short terminal plasma elimination half–life (1 to 2 hours),[6-8] and poor–to–moderate absolute bioavailability (0.4 to 0.68) following po administration in human beings.[7-10] However, the large degree of inter– and intrasubject variability in bioavailability and differences in the types of oral nifedipine formulations make it difficult to reliably predict plasma concentrations for this compound. This is an important issue since several reports[11-16] appear to show a linear plasma concentration–effect relationship for this drug, although this point still remains controversial.[15,16] In human beings and rats, nifedipine is thought to be cleared mainly by the liver via transformation (oxidation) to an inactive metabolite by cytochrome P450 3A isozymes.[17,18] Hence, the liver is also thought to be mainly responsible for the presystemic metabolism of this drug following po administration. Nonetheless, gut wall metabolism may also be involved particularly after absorption from the small intestine,[19-

[21] but this has not yet been unequivocably established or quantified. Nifedipine also appears to possess chronopharmacokinetic properties, i.e., pharmacokinetic differences occur between morning and evening administration of an IR formulation in human beings, with both the rate and extent of nifedipine systemic absorption affected.[22,24] The type of nifedipine formulation used (i.e., IR, SR, CR) also impacts upon the bioavailability reported for this drug since the compound can be incorporated into these products as a solution (e.g., liquid–filled capsules), an amorphous powder [e.g., the insoluble drug absorption system (INDAS) SR tablets] or a specific crystalline (polymorphic) form [e.g., prolonged–action (PA) tablets]. Therefore, different nifedipine products possess distinct dissolution properties, release nifedipine at different rates, and release the majority of their labeled dose into different segments of the GI tract. The pharmaceutical, pharmacokinetic, and pharmacological properties of some oral nifedipine formulations, and specifically the nifedipine gastrointestinal therapeutic system (GITS), are discussed further in chapters 2, 4, 5, 6 and 7.

History

Nifedipine is the prototype of the 1,4–dihydropyridine class of calcium channel antagonists, which also includes such compounds as amlodipine, darodipine, felodipine, isradipine, lacidipine, nicardipine, nilvadipine, nisoldipine and nitrendipine.[25] The chemistry of the dihydropyridine compounds can be traced back to 1882 when Hantzch first reported them as stable intermediates in the pyridine synthesis bearing his name.[26] In the 1960's, the cardiovascular activities of 4-aryl, 1,4–dihydropyridine 3,5–dicarboxylic acid esters were discovered independently by the laboratories of Bayer and Smith, Kline and French. In 1966, F. Bosset first synthesized a compound designated Bay a 1040,[27] which was later introduced (1975) as the compound nifedipine.[26] Since then, nifedipine has become one of the major cardiovascular drugs with indications in the US and Canada for treatment of hypertension and ischaemic heart disease, depending upon the type of formulation selected.

Physicochemical Properties

The chemical structure of nifedipine ($C_{17}H_{18}N_2O_6$) is depicted in Figure 1-1. Nifedipine is a yellow, odorless and tasteless crystalline powder that is relatively thermostable and non–hygroscopic, and has a molecular weight of 346.3 daltons.[26] The compound is freely soluble in acetone (250 g L^{-1}), methylene chloride (160 g L^{-1}) and chloroform (140 g L^{-1}), soluble in

ethyl acetate (50 g L^{-1}), and slightly soluble in methanol (26 g L^{-1}) and ethanol (17 g L^{-1}).[26] The drug is practically insoluble in water as its solubility is only about 10 μg mL^{-1} (at 37°C), and its solubility in phosphate buffer solutions is ≤ 6 μg mL^{-1} (pH 1 to pH 13).[26] The melting point of nifedipine is 171 to 175°C.[28] The dissociation constant [pK_a–value (basic), measured with tetrabutyl ammonium hydroxide in dimethyl formamide using a potentiometric microtitration technique] is about –0.9; thus, nifedipine is not protonated in aqueous solution and must exert its action(s) via the unchanged form.[29] The n–octanol:water distribution coefficient for nifedipine is about 10,000.[26] The ultraviolet (UV) spectrum of nifedipine shows absorption maxima at 235 nm and about 340 nm in methanolic solution, and at 238

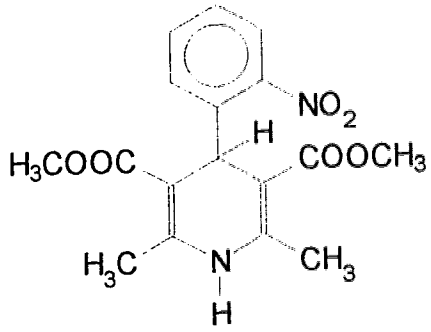

Figure 1-1. Chemical structure of nifedipine.

nm and about 340 nm in alkaline and acid solutions, respectively.[26,30] This compound is extremely sensitive to light in solution and even in solid form must still be protected from light.[31] Exposure of nifedipine to light, high temperature or the presence of oxidizing agents yields two major degradation products, a nitroso compound (visible and UV light) and a nitro compound (UV light).[31] Hence, all experimental work with nifedipine should be performed under yellow (sodium) or red light which have no influence on its decomposition.[26]

Rationale for Hypotheses

The pharmacokinetics of nifedipine and its different oral formulations have still not been adequately assessed in either man or rat due, in part, to problems developing a suitable plasma assay for this compound (including minimizing photodegradation or the production of degradation products due to thermoinstability) and the large variability typically observed following po dosing.[32] Similarly, as nifedipine is light sensitive (even in solid form) the

stability, integrity and accuracy of the labeled drug content of different nifedipine dosage forms needs to be assessed as a potential source of variability in bioavailability determinations [i.e., are significant amounts of photodegradation product(s) formed when commercial nifedipine dosage forms are exposed to sunlight for extended periods of time?].

Greater interest in the therapeutic uses of nifedipine has been generated due to the recent publication of controversial reports and discussion papers,[33-40] which describe and debate the increased risk of mortality apparently seen with the use of moderate–to–high doses of nifedipine IR capsules ($\geq$ 60 mg day^{-1}). Because nifedipine is one of the most commonly used cardiovascular agents, and generates total combined yearly revenues of several billion dollars world–wide for its manufacturers, there is a great deal of interest in developing a better understanding of the factors which influence the bioavailability of this compound, especially since an understanding of these factors may be important with regard to their potential influence on clinical outcome(s). As well, new studies may lead to a better understanding of the potential cause(s) of the drugs apparent increased mortality risk, and clarify whether differences exist between the numerous types of nifedipine formulations (particularly between IR and long–acting preparations with respect to both mortality risk and incidence of adverse effects).

A nifedipine oral dosage form of particular interest is a controlled–release formulation called the nifedipine GITS. This dosage form is typically taken once daily and provides relatively zero–order release of suspended drug and, hence, zero–order absorption following drug dissolution in the GI tract. This formulation acts like a controlled infusion pump operating in the GI tract and potentially allows an assessment of: nifedipine bioavailability from different segments of the GI tract (i.e., dosing of this formulation could provide evidence of gut wall nifedipine metabolism and demonstrate potential metabolic differences between the walls of the small and large intestine), nifedipine chronopharmacokinetic properties, and nifedipine dissolution characteristics. To date, relatively little information has been published on the dissolution and pharmacokinetic properties of this formulation, including a notable lack of progress in the development of a suitable dissolution test system which provides a 1:1 in vitro–in vivo correlation. Detailed descriptions of the known pharmaceutical, pharmacokinetic and pharmacological properties of this particular nifedipine dosage form, prior to the new investigations presented later in this report, are provided in the next chapter. In vitro and in vivo studies that are conducted with this product may aid future

development of nifedipine or other 1,4–dihydropyridine formulations that can provide optimal bioavailability, reduced inter– and intrasubject variability and improve the quality and duration of life.

Of additional interest is the impact that the presence of food and coadministered drugs have on the bioavailability of nifedipine. This is particularly true for the interaction observed in human clinical trials between grapefruit juice and nifedipine, which typically show changes in both the rate and extent of nifedipine absorption.[41-44] To date, the mechanism of action of grapefruit juice has not been unequivocally established and no animal model has yet been developed to further study this phenomenon. Although inhibition of nifedipine metabolism is the most likely effect, and the most studied, in vitro or in vivo studies determining the influence of grapefruit juice on nifedipine protein binding are lacking and this mechanism has not yet been ruled out. Furthermore, in vivo studies in the rat may allow a determination of both the potential role of the gut in nifedipine presystemic metabolism and may indicate that the rat is a suitable model for evaluating the effects of grapefruit juice on nifedipine bioavailability. Although the results obtained in rats cannot be necessarily extrapolated to man the data may be helpful in the design of specific human clinical studies.

Hypotheses

1. Different nifedipine formulations provide dissimilar degrees of protection against nifedipine photodegradation during long periods of exposure to artificial sunlight.
2. The clinical pharmacokinetic profiles of the nifedipine GITS will demonstrate the chronopharmacokinetic properties of the drug or, alternatively, provide evidence of gut wall presystemic nifedipine metabolism.
3. Problems obtaining satisfactory in vitro–in vivo correlations with the nifedipine GITS are due to the limitations of existing standard dissolution tests.
4. The rat model will provide evidence of gut wall nifedipine metabolism.
5. The rat model will show a similar interaction between grapefruit juice and nifedipine as observed in human beings.
6. Human plasma protein binding of nifedipine (either the extent or affinity of binding) is affected by some component(s) of grapefruit juice.

Objectives

1. To develop a rapid, sensitive and specific HPLC assay capable of accurately and precisely measuring nifedipine concentrations in biological or aqueous samples.
2. To evaluate the photodegradation of nifedipine in commercially available nifedipine dosage forms after long periods of exposure to direct artificial sunlight.
3. To delineate the pharmacokinetics of nifedipine in healthy volunteers following po administration of the nifedipine GITS, and to assess the results for evidence of large inter– and intrasubject variability, chronopharmacokinetics or gut wall metabolism.
4. To develop a simple and inexpensive dissolution test system for evaluating both drug release and dissolution characteristics of the nifedipine GITS in order to provide 1:1 in vitro–in vivo correlations.
5. To compare the pharmacokinetics of nifedipine in rat after dosing by various routes of administration to estimate absolute bioavailability and demonstrate gut wall metabolism.
6. To evaluate the rat as a suitable animal model for studying the interaction of grapefruit juice with nifedipine, as seen in human beings.
7. To explore potential transient effects of grapefruit juice on the extent and affinity of in vitro nifedipine human plasma protein binding.

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Chapter 2

THE NIFEDIPINE GASTROINTESTINAL THERAPEUTIC SYSTEM (GITS): EVALUATION OF PHARMACEUTICAL, PHARMACOKINETIC AND PHARMACOLOGICAL PROPERTIES[◆]

Introduction

A subject of debate among drug manufacturers, clinicians, pharmacists and the members of drug formulary review committees concerns the place in therapy of novel drug–delivery dosage forms, especially those displaying controlled–release (CR) properties. This debate arose, in part, due to the relatively expensive manufacturing processes inherent with CR products (hence, the typically higher acquisition costs), as well because of the relative paucity of blinded clinical comparisons between different drug formulations and 'newer generation' compounds within the same drug class. In addition, although some drugs may display enhanced properties after incorporation into a well designed CR matrix, others may not — because of their intrinsic pharmacokinetic/pharmacodynamic (PK/PD) characteristics or because pulse therapy is more desirable.

Nifedipine, the prototype 1,4–dihydropyridine calcium channel antagonist, is a relatively short–acting agent that blocks the influx of calcium through voltage dependent L–type calcium channels of cardiac and vascular smooth muscle cell membranes. The resulting effect is inhibition of cell contraction.[1] Nifedipine is usually administered orally and is typically indicated in the treatment of mild–to–moderate essential hypertension, angina resulting from coronary artery spasm and chronic stable angina. Other potential uses of nifedipine in certain subclasses of patients, include: treatment of Raynaud's phenomenon, congestive heart failure, hypertensive emergencies and prevention of atherosclerosis[2-4] — although it must be emphasized that these are not approved indications in the U.S. and Canada.

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Currently, three types of oral nifedipine formulations are available in North America: (1) immediate–release (IR), administered 3 to 4 times daily; (2) prolonged–action (PA), administered twice daily; and (3) CR, administered once daily. The indicated use is an important factor in determining the selection of a particular nifedipine formulation. Treatment of acute anginal attacks or hypertensive crises demands rapid drug absorption, thus for quick onset of the desired clinical effect an IR nifedipine formulation should be used. However, treatment of essential hypertension or prevention of angina attacks is aided by a uniform, long-lasting clinical effect. This may be achieved by once–daily administration of a long–acting nifedipine preparation, or another vasoactive compound 'naturally' possessing the desired PK/PD properties (e.g., amlodipine).[5]

This chapter reviews the rationale used to justify the development and marketing of the nifedipine gastrointestinal therapeutic system (GITS), a unique CR nifedipine formulation first introduced to North America in 1989. In addition, an evaluation of the benefits and risks that may be encountered after substitution of the GITS for other nifedipine formulations, and to a lesser extent second–generation 1,4–dihydropyridines, is presented. Consideration is given to GITS formulation factors and the pharmacokinetic and pharmacological properties of nifedipine, as well as to the physiology of the human gastrointestinal (GI) tract.

1. Rationale for Development and Marketing of the Nifedipine Gastrointestinal Therapeutic System (GITS)

The relatively short terminal elimination half–life ($t_{1/2}$) of nifedipine in plasma (1 to 2 hours) — determined from intravenous administration studies[1,6,9] — is responsible for the need to administer multiple daily doses of oral IR nifedipine formulations. However, such a regimen is inconvenient for most patients requiring long term therapy. In other words, the unfavorable ratio between the $t_{1/2}$ of nifedipine and a practical oral dose interval make a longer lasting formulation desirable.[10]

Additionally, frequent administration of IR nifedipine formulations (and to some extent PA tablets) has been associated with a relatively high incidence of adverse effects (e.g., tachycardia, flushing and headache), related to the marked rise and fluctuation in plasma nifedipine concentrations and rapid reduction of peripheral vascular resistance.[11]

The limitations of short–acting nifedipine dosage forms have stimulated the development of longer–acting formulations, and of newer 1,4–dihydropyridine molecules possessing

desirable PK/PD characteristics.[12] The former approach offers some advantages since nifedipine has proven efficacy and tolerability and well established PK/PD properties. However, several potential obstacles must be addressed to develop a CR drug formulation. A target concentration for the agent of interest must be established *a priori*, to specifically design a formulation that achieves clinically effective steady–state plasma concentrations. Furthermore, complicating factors resulting from the physical and chemical properties of the drug may need to be overcome (e.g., the poor aqueous solubility and, hence, dissolution properties of nifedipine).

Regardless of the difficulties, potential benefits to be gained with a CR drug formulation include: decreased frequency of administration and total daily dose, enhanced treatment compliance, reduced toxicity, stable drug concentrations and uniform clinical effects. On the other hand, potential disadvantages may include: delayed pharmacodynamic effects, reduced or unpredictable bioavailability, enhanced or modified first–pass metabolism, dose dumping, sustained toxicity, inflexible administration, rapid loss of efficacy in poorly compliant individuals, and higher manufacturing and acquisition costs.[13]

Fortunately, nifedipine possesses several 'ideal' characteristics which favor its incorporation into a CR matrix. These include: (1) a reasonable daily dose size (30 to 120 mg); (2) efficient absorption along the entire GI tract; (3) stability to pH changes; (4) stability to intestinal enzymes and bacterial flora; (5) nonsaturable presystemic metabolism over the typical therapeutic dose range; (6) a known minimum effective concentration — about 15 µg L^{-1};[1,6,14,15] and (7) an apparent correlation between the plasma nifedipine concentration and some clinical effects of the drug.[1,6,15,16]

Alternatively, the development of 'second–generation' compounds typically requires drug manufacturers to conduct expensive and time–consuming pre–clinical and clinical evaluations of hundreds, or perhaps thousands, of agents and derivatives to expose compounds with beneficial or deleterious PK/PD properties. Therapeutic agents emerging from this process may possess clinical and adverse effect profiles that are distinct from those of the prototype compound(s), and clinicians may be initially hesitant to prescribe such agents.[17] Therefore, despite the marketing of several, newer 1,4–dihydropyridine derivatives (e.g., felodipine, amlodipine, and isradipine), the nifedipine GITS remains an attractive therapeutic alternative.

It follows from the preceding comments, therefore, that the benefits and risks associated with the use of the nifedipine GITS can be best evaluated by considering its formulation

design, pharmacokinetic characteristics and pharmacological effects (including GI tract considerations), and influence on treatment compliance.

2. Formulation Design

The nifedipine GITS (Figure 2-1), uses a drug delivery system designed to provide zero–order drug delivery over a period suitable for once–daily administration. This device uses a proprietary mechanism involving a push–pull osmotic pump process; necessary since nifedipine release based upon diffusion is unreliable because of the relatively poor water solubility of the drug (≤ 10 mg L^{-1}).[16,18,19]

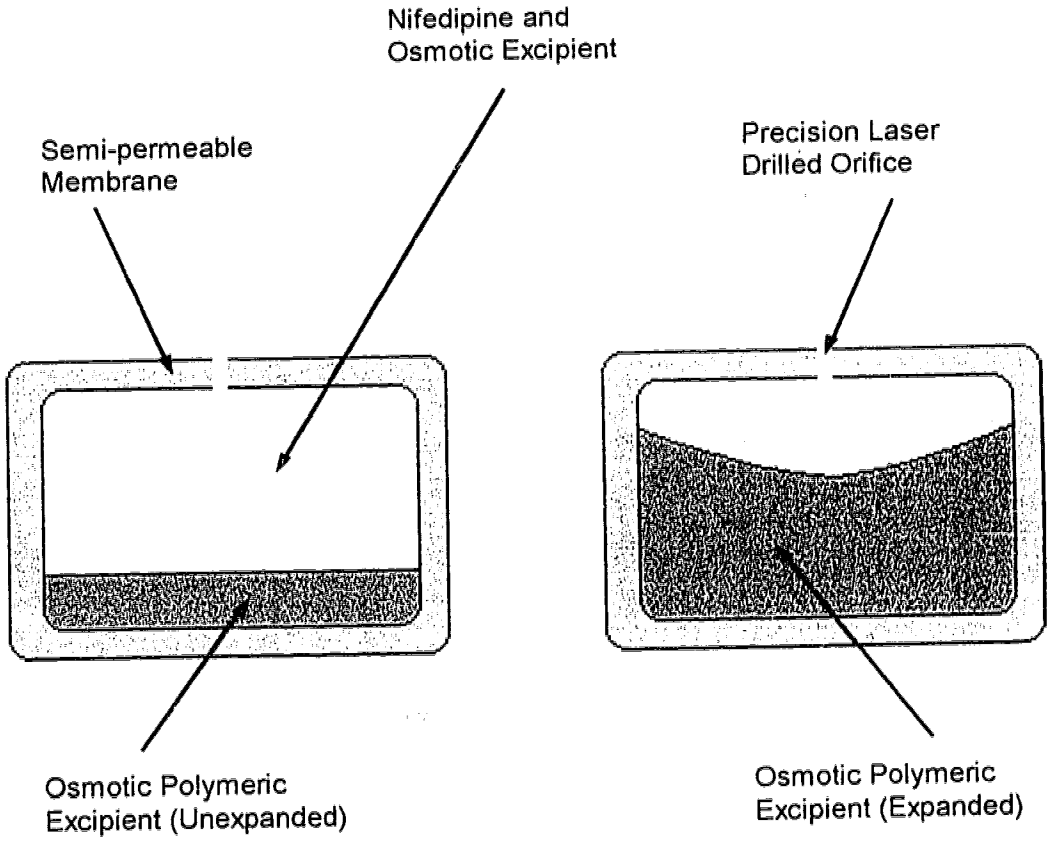

Figure 2-1. The nifedipine gastrointestinal therapeutic system illustrating the push-pull osmotic pump. Adapted from Swanson et al.[16]

The nifedipine GITS tablet is composed of a bilayer core ('push' and 'pull' layers) surrounded by a semipermeable membrane composed of cellulose acetates which allows only the entry of water.[20] A single precision laser drilled hole spanning the membrane is

positioned on the 'pull' layer side.[16] The lower 'push' layer contains osmotic polymeric excipients [a swellable hydrogel layer of poly(ethylene oxide) and hydroxypropylmethylcellulose] designed to drive a finely-powdered nifedipine suspension that continuously forms *in situ* in the upper 'pull' layer [containing nifedipine and a small amount of sodium chloride tableted in hydroxypropylmethylcellulose and poly(ethylene oxide)] out through the passageway in the tablet membrane.[20]

Factors influencing drug release from the system — and used by the manufacturer to 'program' the delivery rate — include the osmotic membrane permeability coefficient, membrane thickness, the area of the 'push' compartment, the total area of the dosage form, imbibition pressures of the 'push' and 'pull' compartments (resulting from water absorption), and the degree of hydration of the chosen osmotic polymer excipient.[16]

As a result of its inventive design the nifedipine GITS may be considered to be a 'controlled-delivery system'; since the drug release rate is determined solely by the dosage form, and appears from in vitro and in vivo tests to be independent of the biological milieu (e.g., pH changes, GI motility).[16,21,22] This system effectively overcomes the solubility and dissolution problems of nifedipine (with respect to release from a formulation matrix), with only about 10% of the dose remaining unreleased from the tablet under normal circumstances.[16] Additionally, as the shell of the tablet remains intact throughout its transit through the GI tract then dose dumping should not occur (It is worth noting that this latter feature may prompt concerned questions from patients who notice the tablet has been passed into the stools 'intact').

The nifedipine GITS requires an initial hydration phase (about 2 hours) before drug release can begin.[23] Thereafter, drug release from the nifedipine GITS is zero-order over about a 16 to 18 hour interval based on the results of in vivo (dog) and in vitro (modified USP) tests.[16] Similar results are also reported in another in vitro study using a flow-through dissolution method,[24] as drug release from a 30 mg nifedipine GITS tablet appears to be zero-order between 3 and 19 hours. Furthermore, a clinical study with 23 human healthy volunteers showed that the nifedipine GITS provides relatively constant plasma concentrations over at least an 18 hour interval.[23] This study suggests that drug release and absorption are zero-order within the human GI tract, and that once-daily administration appears to be a reasonable dosing schedule for the device.

For an oral drug formulation that delivers drug by a zero–order process, the in vivo steady–state plasma concentration (C^{ss}) can be determined using the following relationship:

$$C^{ss} = \frac{R_0 \bullet F}{Cl_p} \qquad (2.1)$$

where R_0 is the zero–order release– or delivery–rate of the drug (assuming rapid drug dissolution and absorption), Cl_p is total body plasma clearance and F represents oral bioavailability. Rates of drug release reported for currently marketed nifedipine GITS preparations are 1.7, 3.4 and 5.1 mg h^{-1} from 30 mg, 60 mg and 90 mg dosage strengths, respectively.[16] Substituting these values into equation 2.1 along with suitable population nifedipine pharmacokinetic parameter estimates of F and Cl_p [e.g., 0.5 (50%) and 600 mL min^{-1} (36 L h^{-1}), respectively] result in C^{ss} estimates (23.6, 47.2 and 70.8 μg L^{-1} for 1.7, 3.4 and 5.1 mg h^{-1} release rates, respectively) exceeding the minimum target concentration of 15 μg L^{-1}. Peak plasma nifedipine concentrations that have been measured in human trials[23,25-27] with these preparations (Table 2-1) are similar to, though interestingly somewhat lower than, the calculated values given above.

3. Pharmacokinetic and Dosage Considerations

3.1 Nifedipine Pharmacokinetics

Pharmacokinetics parameters reported for several different oral nifedipine formulations are summarized in Table 2-1 and Table 2-2 (single and multiple doses, respectively). Oral administration of IR nifedipine formulations (5 or 10 mg capsules) results in almost complete absorption from the gastrointestinal tract, although oral bioavailability averages only 40% to 68% due to presystemic metabolism of nifedipine to inactive metabolites.[6,7,17] This 'first–pass' effect is mainly attributed to the enzyme cytochrome P450 (CYP) 3A4 distributed within hepatocytes of the liver.[28,29] However, metabolic enzyme activity within intestinal enterocytes of the gut wall may also be a contributing factor.[30-32]

The mean steady state volume of distribution (V_{ss}) of nifedipine after oral administration is 1.32 L kg^{-1} and the drug is highly plasma protein bound (91% to 98%), particularly to albumin.[33] Nifedipine clearance is reported to be about 27 to 42 L h^{-1}, indicating that the rate of drug elimination depends on both drug metabolizing enzyme activity and hepatic blood flow.[6,15,32]

Linear pharmacokinetics are observed in the typical therapeutic dosage range employed for this drug. The reported $t_{1/2}$ of nifedipine is highly variable (1 to 17 hours), due mainly to differences in the route of administration and the type of formulation evaluated.[7,14,15,34-46] As stated in section 1, intravenous (iv) administration studies with nifedipine have determined its $t_{1/2}$ to be about 1 to 2 hours.[1,6,9] Therefore, the longer $t_{1/2}$ typically reported after oral administration (Table 2-1) is probably indicative of a slow drug absorption rate (i.e., 'flip–flop' kinetics[47]) — particularly from slow–release preparations — rather than a reflection of the $t_{1/2}$; however, considerable inter–subject variability may also be a factor.

3.2 Nifedipine GITS Pharmacokinetics

Several literature reports describe the single– or multiple–dose pharmacokinetics of the nifedipine GITS (see Table 2-1 and Table 2-2).[23,25-27,48] Detectable nifedipine plasma concentrations are not seen for at least 3 to 4 hours after administration of a single GITS dose.[23,25-27] Thereafter, nifedipine plasma concentrations rise until a 'plateau period' is reached — typically between 6 and 24 hours — after which plasma nifedipine concentrations begin to decline.[23,27]

The reported relative bioavailability of the nifedipine GITS formulation (compared with IR capsules) is 55% to 65% from a single–dose and 75% to 85% at steady–state (usually after 3 doses),[23] indicating about 33% accumulation.

Values for the mean maximum plasma nifedipine concentration (C_{max}) and area under the plasma concentration-time curve (AUC) after single and multiple doses of nifedipine GITS in different subject groups (e.g., young, elderly, normotensive, hypertensive, renally impaired, etc.) are included in Table 2-1 and Table 2-2. Apparent nifedipine C_{max} values (normalized to a 60 mg daily dose) after multiple doses of the nifedipine GITS are about 20% of those obtained with IR capsules, and average 56.5 μg L^{-1} (range 43.2 to 67.0 μg L^{-1}, see Table 2-2).[15,23,26,27,48,53]

Table 2-1. Pharmacokinetic properties of nifedipine in humans (oral dosage formulations, administered in single-doses)

References	*Dosage form*	*Dose (mg)*	*No. of study participants*	*Participant characteristics [age range, y]*	C_{max} *(μg L⁻¹)*	t_{max} *(h)*	*AUC (μg L⁻¹ • h) [time interval]*	$t_{1/2}$ *(h)*	*F (%)*	*Cl/F (L h⁻¹)*
Immediate-Release Formulation Studies										
Raemsch & Sommer[34]	Capsule	10	5 - 10	N,B	160 ± 49	0.5			62.4 ± 7.1	
Soons et al.[35]	Capsule	10	12	N,B [21-23]	122 ± 58	0.53 ± 0.15	179 ± 97	2.32 ± 0.70		55.9
Foster et al.[7]	Capsule	10	12	N [21-28]	73.5 ± 17.5	0.57 ± 0.06	125 ± 18	3.4 ± 10.4	45 ± 8	80.0
Reitberg et al.[36]	Capsule	10	15	N,M [18-36]	78.9 ± 42	0.97 ± 0.94	145 ± 48	1.71 ± 0.59		69.0
Pabst et al.[15]	Capsule	20 (2 x 10)	12	N,B [19-34]	201 ± 61	0.48 ± 0.13	376 ± 91	0.96 ± 0.42		53.2
Challenor et al.[37]	Capsule	20 (2 x 10)	6	N,M	96.7 ± 42	1.8 ± 1.1	343 ± 98	2.6 ± 0.4		58.3
Myers & Raemsch[38]	Capsule	20 (2 x 10)	6	H,M [63 ± 3]	231 ± 24	0.6 ± 0.1	540 ± 44	2.8 ± 0.3		37.0
Klembloesem et al.[5]	Capsule	20 (2 x 10)	6	N,M [22-28]	116 ± 15	1.4 ± 0.5			56 ± 25	
Ahsan et al.[39]	Capsule	20 (2 x 10)	27	N,B [19-46]	172 ± 107	0.8 ± 0.8	323 ± 116	2.8 ± 1.1		61.9
Qureshi et al.[40]	Capsule	20 (2 x 10)	10	N,M [26 ± 3]	186 ± 126	0.9 ± 0.4	360 ± 169	3.6 ± 1.3		59.6
Prolonged-Action/Retard Formulation Studies										
Robertson et al.[41]	Tablet	10	11	N,M [22-35]	22.3 ± 5.8	2.2 ± 1.2	136 ± 56	3.8 ± 1.4	46 ± 20	
Robertson et al.[41]	Tablet	10	6	N,M [73-83]	36.8 ± 11.8	2.9 ± 1.9	281 ± 64	6.7 ± 2.2		35.6
Taburet et al.[42]	Tablet	20	8	H,M [32-60]	62.6 ± 9	1.63 ± 0.26	537 ± 105	10.8 ± 2.4	65	
Scott et al.[43]	Tablet	20	12	N,B [18-36]	65.0 ± 9.6		332 ± 61	5.4 ± 0.7		60.2
Scott et al.[43]	Tablet	20	10	N,B [64-76]	53.2 ± 3.5		474 ± 73	7.8 ± 1.1		42.2
Pabst et al.[15]	Tablet	20	12	N,B [19-34]	40.3 ± 17.5	1.61 ± 1.27	305 ± 99	3.26 ± 2.19		65.6
Challenor et al.[37]	Tablet	20	6	N,M,F	37.9 ± 15.6	1.8 ± 1.2	255 ± 169	6.1 ± 3.6		78.4
Myers & Raemsch[38]	Tablet	20	6	H,M [63 ± 3]	50 ± 6	3.0 ± 0.7				
Klembloesem et al.[5]	Tablet	20	6	N,M [22-28]	25.9 ± 10.1	4.2 ± 0.7		9.9	52 ± 13	
Sugusch et al.[45]	Tablet	20	10	N,M [21-35]	27 ± 11	1.1 ± 0.6	162 ± 84	5.3 ± 1.6		123.5
Hoyo-Vadillo et al.[46]	Tablet	20	12	N,M-	48.7 ± 7.3	2.1 ± 0.7	526 ± 62	16.9 ± 5.1		38.0
Avgerinos & Gorrod[47]	Tablet	20	12	N,B [19-22]	27.6	4	658	16		30.4
Akopov et al.[44]	Tablet	20	44	H,B	46.9 ± 21.8	1.83 ± 0.58	360 ± 99 [0-12 h]	4.9 ± 2.9		
Ueno et al.[48]	Tablet	20	-	N,M[a] [27-36]	42.6 ± 31.8		315 ± 89.4 [0-12 h]			
Ueno et al.[48]	Tablet	20	-	N,M[a] [27-36]	86.6 ± 22.6		411 ± 67 [0-12 h]			
Grundy et al.[49]	Tablet	20	18	N,B [70.0 ± 4.6]	35.1 ± 12.7	2.3 ± 1.4	298 ± 125			
Grundy et al.[50]	Tablet	20	12	N,M [24 ± 3]	45.4 ± 27.1	1.88 ± 0.91	365 ± 196	6.17 ± 3.72		65.6

Controlled–Release Formulation Studies									
Chung et al.[23]	GITS	30	16	N	24[a]	12[a]	682[a]	6.22[a]	
Unpublished	GITS	30	12	N,B [34.2 ± 7.7]	19.8 ± 13.6	16.5 ± 8.2	419 ± 290 [0-36 h]		
Unpublished	GITS	30	12	N,B [34.2 ± 7.7]	22 ± 11	22.3 ± 10.6	468 ± 236 [0-36 h]		
Grundy et al.[25]	GITS	30	18	N,B [70.0 ± 4.6]	22.4 ± 8.9	19.7 ± 7.4	283 ± 87 [0-24 h]		
Chung et al.[23]	GITS	60	23	N	35[a]	6[a]	704[a] [0-24 h]		75-85[c]
Crome et al.[26]	GITS	60	23	N,M [18-31]	35.5	17.0	468 [0-24 h]		
Crome et al.[26]	GITS	60	24	N,B [65-82]	39.8	15.8	507 [0-24 h]		
Schneider et al.[27]	GITS	60	6	H,B [mean 55.8]	30.2	14.8	404 [0-24 h]		
Schneider et al.[27]	GITS	60	10	H,MRI,B [mean 57.6]	26.9	15.1	382 [0-24 h]		
Schneider et al.[27]	GITS	60	7	H,SRI,B [mean 56.0]	42.4	17.1	583 [0-24 h]		
Groenewoud et al.[46]	GITS	60	23	N [mean 72]	43.0		548 [0-24 h]		
Groenewoud et al.[46]	GITS	60	23	N [mean 21]	44.0		584 [0-24 h]		
Unpublished	GITS	60	12	N,B [34.2 ± 7.7]	36.9 ± 17.7	20.8 ± 9.0	915 ± 498 [0-36 h]		
Unpublished	GITS	60	12	N,B [34.2 ± 7.7]	40.9 ± 26.5	18.3 ± 8.9	877 ± 502 [0-36 h]		

[a] Of Mexican nationality.

[b] Participants in this study group were intentionally fasted to study the effects of food.

[c] Participants in this study group were intentionally fed to study the effects of food.

[d] Values estimated from figures presented in the study authors' publication.

[e] Relative to immediate–release capsules at steady–state.

Abbreviations: AUC = area under the plasma concentration–time curve; B = both sexes; C_{max} = maximum plasma drug concentration; Cl_{CR} = creatinine clearance; CL/F = apparent oral clearance; F = bioavailability; GITS = gastrointestinal therapeutic system; H = hypertensive (mild–to–moderate); M = men; MRI= moderate renal impairment (Cl_{CR} 1.5 to 3.6 L h^{-1}); N = normotensive; SRI= severe renal impairment (Cl_{CR} < 1.5 L h^{-1}); t_{max} = time to C_{max}; $t_{1/2}$ = elimination half–life associated with the terminal slope (λ_z) of a semilogarithmic concentration–time curve.

Table 2-2. Pharmacokinetic properties of nifedipine in human subjects (oral dosage formulations administered in multiple doses)

Reference	Dosage form	Dose (mg) [no. of days]	No. of study participants	Participant characteristics [age range, y]	C_{max} (μg L^{-1})	t_{max} (h)	AUC (μg L^{-1} • h)	$t_{1/2}$ (h)	F (%)	CL/F ($L\ h^{-1}$)
Immediate-Release Formulation Studies										
Khan et al.[51]	Tablet	10 tid [4 + 1 extra dose]	10	N,B	33 ± 14	2.03	105 ± 40	2.02 ± 0.89		111
Pabst et al.[15]	Capsule (hard)	20 bid [3]	12	N,B [19-34]	77.8 ± 26.7	1.97 ± 1.08	316.8 ± 70.6	1.66 ±1.05		63.1
Pabst et al.[15]	Capsule (soft)	20 (2 x 10) bid [3]	12	N,B [19-34]	235.4 ± 92.3	0.47 ± 0.45	365 ± 104	1.07 ± 0.52		54.7
Chung et al.[23]	Capsule	20 (2 x 10) tid [5]	23	N	200	~1.5				
Prolonged-Action/Retard Formulation Studies										
Scott et al.[43]	Tablet	20 qam [8]	12	N,B [18-36]	64.1 ± 5.0		334 ± 57	5.8 ± 1.1		59.9
Scott et al.[43]	Tablet	20 qam [8]	10	N,B [64-76]	51.4 ± 4.2		450 ± 46	8.8 ± 0.9		44.4
Debbas et al.[52]	Tablet	20 bid [3]	8	N,B	61.4 ± 21.9	1.5	300 ± 146	6.3 ± 2.0	46 ± 21	66.7
Pabst et al.[15]	Tablet	20 bid [3]	11	N,B [19-34]	45.1 ± 14	1.3 ± 0.98	253.3 ± 73.3	4.83 ± 2.44		79.0
Schall et al.[53]	Tablet	20 bid [6]	22	N,M [19-24]	66.9	3.21	608 (0-24 h)			65.8
Controlled-Release Formulation Studies										
Devane et al.[31]	INDAS	30 qd [3]	24	N,M	31.3 ± 23	3.9 ± 3.8	387 ± 103	11.8 ± 6.8		129.2

Schall et al.[53]	GITS	60 (2 x 30) qd [6]	24	N,M [18-38]	60.0 ± 1.36	8.52	875	68.6
Schall et al.[53]	GITS	60 qd [6]	24	N,M [18-38]	55.0 ± 1.41	9.02	844	71.1
Crome et al.[55]	GITS	60 qd [7]	23	N,M [18-31]	49.2	11.5	749	80.1
Groenewoud et al.[48]	GITS	60 qd [7]	23	N [mean 72]	67.0		1064	
Groenewoud et al.[48]	GITS	60 qd [7]	23	N [mean 21]	62.0		937	
Crome et al.[55]	GITS	60 qd [7]	24	N,B [65-82]	61.7	7.88	983	64.0
Schneider et al.[57]	GITS	60 qd [7]	6	H,B [mean 55.8]	53.7	4.7	795	75.5
Schneider et al.[57]	GITS	60 qd [7]	10	H,MRI [mean 57.6]	43.2	8.6	663	90.5
Schneider et al.[57]	GITS	60 qd [7]	7	H,SRI [mean 56.0]	60.2	13.0	837	71.7

Abbreviations: INDAS= insoluble drug absorption system; GITS= gastrointestinal therapeutic system; N= normotensive; H= hypertensive (mild–to–moderate); M= males; B= both sexes; qd= once daily; bid= twice daily; tid= three times daily; MRI= moderate renal impairment (CL_{CR} 25–60 mL min^{-1}); SRI= severe renal impairment (CL_{CR} < 25 mL min^{-1}).

Regarding the age of study participants, no or slight differences are reported for nifedipine GITS pharmacokinetic parameters after single or multiple doses are given to young and elderly volunteers, thus no dosage adjustment is apparently necessary.[26,48] Similarly, impaired renal function in patients does not appear to significantly affect the plasma concentrations of nifedipine even after long–term therapy with the nifedipine GITS and , hence, again dosage adjustment is not necessary.[27]

Food does not alter the absolute bioavailability of this device; however, an increase in the drug absorption rate with food is reported.[23] Some fluctuations in plasma nifedipine concentrations are apparent during multiple dosage with the GITS; due perhaps, in part, to carry–over from the previous days dose. However, these are minor fluctuations compared with other nifedipine formulation regimens and may not be clinically relevant.[23]

Boxenbaum states,[54] in a paper describing the pharmacokinetic determinants of sustained–release oral dosage form design, that zero–order in vivo release produces zero–order systemic absorption if: (1) the various gut segments are homogenous with respect to absorption; and (2) the drug release rate is rate–limiting for absorption. For the nifedipine GITS the latter assumption may hold due to its extended–release (ER) characteristics compared to the relatively fast absorption profile observed with IR nifedipine formulations [i.e., relatively short time to C_{max} (t_{max}); see Table 2-1]. However, the nifedipine GITS releases the drug in the form of a suspension which first has to undergo dissolution before absorption can begin and thus may be potentially rate–limiting. With respect to both assumptions, the single–dose mean plasma nifedipine concentration–time curves shown in papers by Chung et al.[23] (Figure 2-2) and Schneider et al.[27] appear to display the characteristic profile expected in vivo for the nifedipine GITS; given the zero–order nature of drug release from this device and the assumption of zero–order absorption along the entire gut.

Notwithstanding the results of these studies,[23,27] an interesting observation has been noted by our group from single–dose data collected in published[25]and unpublished clinical trials of the nifedipine GITS, and from review of two independent published reports.[26,55] In both our preliminary studies, 'apparent' C_{max} values occurred at 24 hours postdose (plasma samples collected between 08:00 and 09:00 the day after dosing) in about half of the study participants evaluated — with subjects receiving nifedipine GITS 30 or 60 mg tablets (Figure 2-3). The 'apparent' C_{max} was determined as the highest measured nifedipine concentration in the

plasma samples obtained at 6, 8, 12 and 24 hours (corresponding to the assumed nifedipine GITS plateau interval).

Although mean 24 hour plasma nifedipine concentrations were not significantly higher (ANOVA, $p > 0.05$) than those at other times within the assumed plateau interval (probably due to large interindividual variability), nonetheless, it was surprising to observe the trend described above. Especially considering that the nifedipine delivery rate is expected to decline about 20 hours after administration, as predicted from in vitro tests with the nifedipine GITS.[16,24]

Review of single–dose (nifedipine GITS 60 mg) mean plasma nifedipine concentration–time profiles reported by Crome et al.[26] indicate a similar phenomenon, as the mean 24 hour concentration values appear as the maximum values from data obtained in both young (n = 23) and elderly (n = 24) participants. Similar looking profiles are also seen in a paper by Melia et al.[55] who investigated 18 healthy volunteers given nifedipine GITS (60 mg) coadministered with either a placebo or orlistat (a lipase inhibitor).

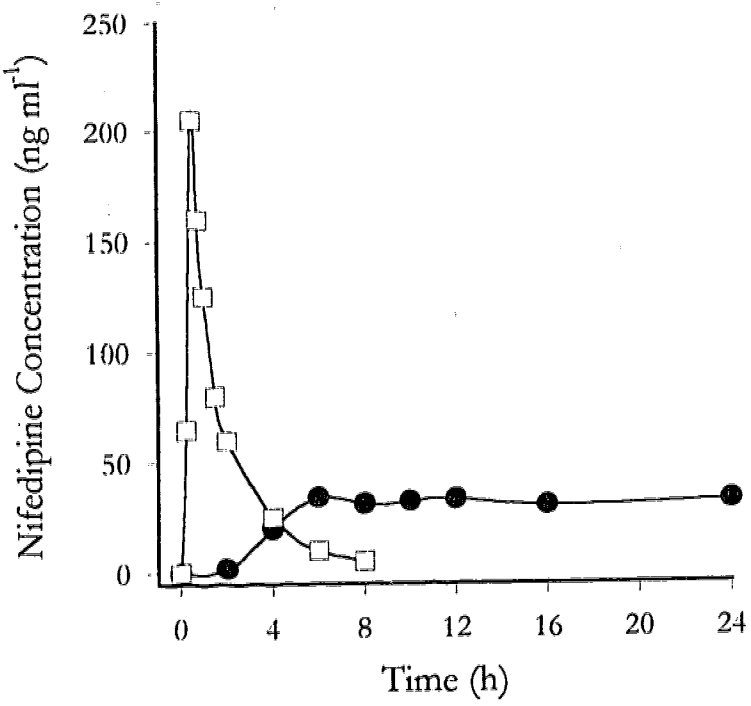

Figure 2-2. Mean plasma nifedipine concentration–time profiles in healthy volunteers (n = 23) after single–doses of a nifedipine gastrointestinal therapeutic system (GITS) tablet (60 mg, ●) or immediate–release (IR) nifedipine capsules (2 x 10 mg, □). Adapted from Chung et al.[23]

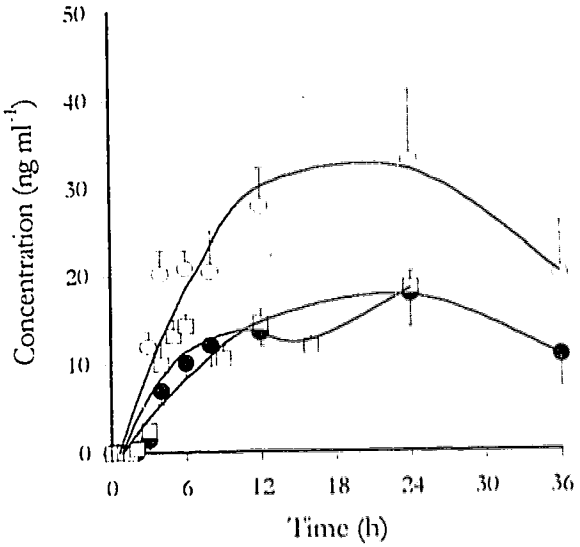

Figure 2-3. Mean plasma nifedipine concentrations ($\pm s_{\bar{x}}$) versus time profiles after single–doses of nifedipine gastrointestinal therapeutic system tablets administered to 12 young [30 mg (●) or 60 mg (○) dose] and 18 elderly [30 mg (□) dose] healthy volunteers (includes previously unpublished observations from our laboratory and data from Grundy et al.[25])

This apparent trend, while perhaps not clinically relevant, suggests at least 4 possibilities: (1) that steady–state nifedipine plasma concentrations were not achieved within the first 12 hours (in some study participants) — i.e., 3 to 5 $t_{1/2}$ > 12 hours; (2) predicted in vivo performance (from in vitro dissolution tests) may correlate poorly with actual in vivo performance — i.e., the rate of drug release in vivo may indeed gradually increase up to 24 hours post–administration; (3) the extent of nifedipine absorption may increase in the colon and rectum compared to the small intestine, perhaps due to reduced presystemic extrahepatic metabolism distally along the GI tract (gut wall metabolism?;[56] see section 3.7) or from drug bypassing hepatic first–pass extraction (i.e., rectal absorption into the middle and lower haemorrhoidal veins[57]); or (4) that nifedipine clearance or the extent of drug absorption may be time–dependent (chronopharmacokinetic properties? [58-61]).

Given the very short $t_{1/2}$ typically reported for nifedipine in iv studies, the first possibility can be assumed to be unlikely. Therefore, further investigations are warranted to confirm

these observations and to determine which, if any, of the 3 other mechanisms may be involved.

3.3 Converting Patients from Other Nifedipine Formulations

Converting patients from one drug dosage form to another requires consideration of the bioavailability and bioequivalence of the products concerned. Bioavailability is defined as the measurement of the drug absorption rate and the extent of drug reaching the general circulation intact; whereas, bioequivalence is a term which reflects specific requirements mandated by drug regulatory bodies [i.e., the Canadian Health Protection Branch (HPB) and U.S. Food and Drug Administration (FDA)] in order for some 'second–entry' drug products to be marketed.

Typically, two or more drug formulations are considered bioequivalent if they provide similar rates and extents of absorption. However, it is obvious that IR, PA and GITS nifedipine formulations differ with regard to the rate of drug absorption. Nonetheless, different nifedipine formulations might be considered bioequivalent under the following conditions: (1) the extents of absorption are similar; (2) the differences in the rate of absorption are intentional; (3) the rate differences are reflected on the labeling of the respective formulations; and (4) the rate differences are not detrimental to the efficacy and safety of the products.[62]

In 18 elderly volunteers,[25] administration of single–doses of the nifedipine GITS (30 mg) or nifedipine PA capsules (20 mg) produces similar AUC(0-24h) values for the 2 formulations but t_{max} is significantly different between them (see Table 2-1); indicating distinct rates but not extents of nifedipine absorption. Similarly, Schall et al.[53] report that nifedipine GITS and nifedipine PA formulations are bioequivalent with regard to the extent of nifedipine absorption after multiple dosing.

However, converting patients from short–acting nifedipine formulations to the nifedipine GITS requires that consideration also be given to the clinical effect(s) of reducing the drug absorption rate, especially since it has been shown that clinical response and adverse effect profiles of nifedipine are influenced by changes in its intravenous infusion rate.[6,8,63,64] Therefore, describing the bioequivalence of IR, PA and GITS nifedipine formulations solely from pharmacokinetic parameters ignores the issue of therapeutic equivalency (see section 4.4). However, this point was not raised to imply that converting patients from standard

nifedipine formulations to the nifedipine GITS is a serious clinical concern -- in fact, a recent study with 173 hypertensive patients switched from PA nifedipine (10 to 20 mg twice daily) to the nifedipine GITS (30 to 60 mg once daily), showed that the conversion may be achieved without loss of blood pressure control and that most patients require only a 30 mg daily dose.[65]

The point, however, is that it may be incorrect to assume a similar clinical response will be obtained after switching a patient from one type of nifedipine formulation to the next, solely by matching the total daily dose. Indeed, although manufacturers of the nifedipine GITS do not provide specific recommendations, a typical suggestion is to convert most patients using an initial dose of 30 mg regardless of their previous nifedipine dosing history, and to slowly titrate the dose as necessary up to a maximum of 120 mg once daily.[66]

3.4 Nifedipine GITS: Gastrointestinal Transit and Sites of Drug Absorption

The pharmacokinetic performance of the nifedipine GITS is reported to be unaffected by GI pH and motility; however, the potential for unusually rapid GI transit of the device and differences in extent of drug absorbed along the GI tract are important factors to consider. The GI transit time of intact formulations and the main site(s) of drug absorption in the GI tract is difficult to measure and, hence, published data are, as yet, unavailable for the nifedipine GITS. However, the performance, GI transit and residence time of similar osmotic devices (e.g., OROS formulations) and of solid indigestible capsules (e.g., the Heidelberg capsule) have been investigated.[67-71]

For example, Davis et al.[67] report that in 6 individuals the residence time of an osmotic pump device in the stomach is greatly increased by food intake, but that the release of a radiolabelled marker is unaffected by either food or the position of the device within the GI tract. In only 2 of the 6 study participants is the device detected in the stomach 3 hours after a light breakfast (1500 kJ); whereas, after a heavy breakfast (3600 kJ) the device is detected in the stomach more than 8 hours later in all 6 subjects.

Food can delay the gastric emptying of large units such as tablets and capsules for up to 6 to 12 hours.[11,69] In one study,[68] the gastric residence and small bowel transit times of a solid indigestible capsule given 15 minutes after a liquid, fatty meal (17 kJ mL^{-1} kg^{-1} bodyweight) ranges from 2.8 to 4.8 hours and 2.8 to 5.5 hours, respectively. Similarly, a radiolabelled osmotic tablet reaches the caecum on average 7 hours after dosing in fasted individuals.[69] In

a study with 6 fasted, healthy male volunteers,[70] investigators measured the position of a technetium-99m labeled, orally administered oxprenolol OROS drug delivery system in the GI tract. Results reported included: the mean gastric emptying time (0.8 ± 0.4 hours), mean time of arrival in the colon (3.8 ± 0.7 hours) and mean total transit time (24.7 hours, range 6 to 32 hours).[70] Therefore, the results of the preceding studies lead to the conclusion that majority of drug release, and subsequent drug absorption, from the nifedipine GITS would probably occur within the large intestine.

The term 'absorption window' is used to describe incomplete drug absorption (especially from CR formulations), with the reasoning that many drugs are absorbed only from specific segments of the GI tract.[72] It is well known that the small intestine possesses the largest surface area followed by the colon and stomach. Therefore, many drugs are assumed to be completely absorbed from the small intestine — i.e., entry and exit from the small intestine defines the boundaries of an 'absorption window' for some drugs. However, the pharmacokinetic profiles obtained after administration of the nifedipine GITS to humans attest to the fact that nifedipine is well absorbed along the entire GI tract.

A concern with CR preparations, particularly those designed for once-daily administration, is transit of the formulation through the GI tract in less than 24 hours due to excessive GI motility. The result of this could be less than optimal clinical outcomes in some patients. Mouth-to-anus transit times for intact capsules and radio-opaque markers in fasting individuals range between 17 to 72 hours.[73,74] Davis et al.,[70] report a mean total transit time of 24.7 hours for an oxprenolol OROS formulation in fasted individuals. Another study using oxprenolol and metoprolol OROS formulations reports a median GI transit time of 27.4 hours, with individual times ranging from 5.1 to 58.3 hours.[71] Thus, the inter- and intraindividual GI transit times of intact formulations appear to be variable and unpredictable.

Factors such as the formulation size or type, and the 'intrinsic' GI effects of the drug may also potentially influence GI transit. Although therapeutic failure with the nifedipine GITS may potentially occur in some patients, no studies to date have indicated that rapid GI transit of this formulation is a significant clinical problem.

3.5 Precautions in Patients with Gastrointestinal Disease States or Conditions

In a recent case report[75] a description is given of a female patient with a previous gastroplasty who later experienced gastric outlet obstruction resulting from long term administration of nifedipine GITS tablets. Another report[76] highlights 3 cases of false-positive results in a barium enema study; resulting in unnecessary colonoscopy due to misinterpretation of the nifedipine GITS tablets as colonic polyps.

These cases illustrate two of the many potential GI-specific problems that can arise when a solid dosage form (designed to remain intact) passes through the GI tract. Currently, only short bowel syndrome and pre-existing GI narrowing are listed as precautions in the Canadian nifedipine GITS product monograph.[77] This relatively short list is probably the result of incomplete study in the area, and also reflects the relatively short time that the formulation has been widely available. Nevertheless, several GI disease states could affect the performance of the nifedipine GITS.

Inflammatory bowel disease (IBD) and diarrhoea, may affect drug absorption within the large intestine.[78] Symptoms of IBD are variable but can include mild-to-severe diarrhoea (up to 20 bowel movements per day). Although specific data are unavailable, the transit time and retention of nifedipine GITS may be affected by patients experiencing IBD or diarrhoea, which would affect the extent of drug absorption and the duration of the clinical effect. Therefore, caution might be warranted when treating patients with GI motility disorders. Other conditions or disease states of potential concern include (but are not limited to): GI tract infections, diverticulitis, and irritable bowel syndrome.

3.6 Nifedipine GITS: Drug Interactions

Many patients must take multiple medications concurrently resulting in potential drug-drug interactions. Details of specific drug interactions with nifedipine have already been described elsewhere.[1,8] However, some drugs may affect the function of the autonomic nervous system and alter GI function, which may affect the performance of particular types of oral dosage forms including the nifedipine GITS.

For example: increased GI tone, motility and gastric acid secretions are observed after the use of cholinergic drugs. Other agents, including motility modifiers and laxatives, may also affect GI motility and transit. However, no studies have specifically evaluated the concomitant use of these agents with the nifedipine GITS. Although the rate of release of

nifedipine from the GITS appears unaffected by motility, the retention of the dosage form may be inadequate (< 24 hours), particularly when coadministered with drugs that decrease GI transit time.

3.7 Interaction of Grapefruit Juice and the Nifedipine GITS?

No specific studies have investigated the potential interaction between the nifedipine GITS and grapefruit juice, even though grapefruit juice can increase the extent of absorption of several 1,4–dihydropyridine drugs, including nifedipine.[79] In fact, the bioavailability of an IR nifedipine formulation after a single glass of grapefruit juice (compared with water) increased 34% to 47%.[80,81] Repeated administration of grapefruit juice also improves the bioavailability of a nifedipine PA formulation by 103%.[42]

The most likely mechanism of action for the interaction of grapefruit juice and nifedipine appears to involve inhibition of nifedipine first–pass metabolism by some component(s) of grapefruit juice (e.g., flavonoids).[79] However, it could be speculated that if nifedipine undergoes a significant degree of extrahepatic presystemic metabolism in humans, then the extent of the interaction between grapefruit juice and nifedipine may differ depending on the type of oral formulation given — since metabolic enzyme activity within the gut intestinal wall tends to decrease distally.[82] Immunoquantification of CYP enzymes in human extrahepatic microsomes shows the content of the isozyme CYP3A4 to be higher within enterocytes of the small intestine (particularly the duodenum) than those of the large intestine.[30] Therefore, drug released from CR formulations may be less affected by substances which influence gut wall metabolism.

Indeed, Edgar et al.[31] describes a formulation–dependent interaction between grapefruit juice and felodipine. After administration of water or grapefruit juice, the AUC (nmol L^{-1}·h) of a plain felodipine tablet (5 mg) increases from 23.7 ± 10.3 to 70.9 ± 24.6, respectively; whereas for an ER felodipine tablet (5 mg) the increase is only from 27.2 ± 12.0 to 49.2 ± 14.2. Thus, the AUC of felodipine from the plain tablet compared with the ER formulation increases significantly more when taken with grapefruit juice (approximately a 3:2 ratio).

However, ER felodipine is not a GITS formulation but is similar (with respect to the rate and duration of release) to the PA nifedipine formulation. Thus, most of the drug from an ER felodipine formulation is released relatively rapidly — the t_{max} is only 3.0 hours (range 2 to 6 hours).[83] Therefore, a large percentage of the felodipine dose would still be expected to be

absorbed from the small intestine and therefore subject to the inhibitory effects of grapefruit juice on gut wall enzymes. Hence, in theory, a felodipine or nifedipine GITS device could be relatively less affected by grapefruit juice than faster–releasing IR, PA or ER formulations.

4. Pharmacological Effects

4.1 Mechanism of Action of Nifedipine

The mechanism of action and pharmacodynamic properties of nifedipine (iv and conventional oral formulations) have been extensively reviewed.[1,2,84-86] Briefly, however, nifedipine blocks the influx of calcium mainly through voltage dependent L–type calcium channels of cardiac and vascular smooth muscle cell membranes, resulting in inhibition of cell contraction. Unlike verapamil and diltiazem, nifedipine does not suppress calcium influx into the atrioventricular and sinoatrial nodes at therapeutic doses.[87] The potency of nifedipine, as with most 1,4–dihydropyridines, is much greater in smooth muscle than in cardiac muscle; hence, the overall haemodynamic effects are dominated by peripheral vasodilation. Nifedipine is generally well tolerated, although the rapid achievement of maximal plasma nifedipine concentrations with conventional nifedipine formulations may contribute to a high incidence of vasodilator–related adverse effects (e.g., headache, flushing, dizziness and tachycardia).[6,88]

4.2 Results from Clinical Trials

Most clinical trials with the nifedipine GITS have assessed its efficacy in patients with mild–to–moderate essential hypertension. Table 2-3 summarizes the results from several clinical trials using different oral nifedipine formulations, and lists some of the clinical effects [e.g., changes in blood pressure (BP) or heart rate (HR)] measured after single or multiple doses were administered to patients and healthy volunteers.

From the data given in Table 2-3,[88-97] the overall mean change in sitting systolic and diastolic BP after long term therapy with the nifedipine GITS (30 to 180 mg daily) can be calculated as -18.0 and -13.0 mm Hg, respectively. In addition, HR does not appear to be significantly altered by the nifedipine GITS (see Table 2-3).[91,98]

In general, a once–daily nifedipine GITS regimen appears to produce equivalent (if not better) efficacy compared to other dosage forms of the drug. Some improvement in the incidence of certain vasodilator–related adverse effects has also been noted. Unfortunately,

the paucity of large, blinded clinical trials directly comparing the different formulations makes it difficult to make definitive recommendations.

The Modern Approach to the Treatment of Hypertension (MATH) trial, a large multisite study (1155 patients in total), was designed to evaluate the effectiveness of the nifedipine GITS given once daily as monotherapy in the treatment of essential hypertension.[94,99,100] Of those treated, 76% responded and 88% of the responders completed the 12–week study.[100] The final results showed the device to be an effective antihypertensive agent in a wide spectrum of patients (e.g., males/females; black/white; normal weight/obese; diabetic/nondiabetic).[100]

4.3 Trough/Peak Antihypertensive Effect Ratio

The goal of therapy for essential hypertension is to reduce the incidence and delay the onset of cardiovascular complications, and to prevent end–organ damage.[109] It is generally assumed that this is best achieved by consistent round–the–clock control of BP. Continuous 24–hour BP control helps to ensure that circadian variations in BP are maintained, albeit at lower baseline levels, and that large fluctuations in BP are minimized to prevent postural hypotension and ischaemic events.[110] Therefore, guidelines from the FDA and HPB have suggested a minimum trough/peak (T/P) effect ratio be established for antihypertensive drugs. A typical suggestion is that the trough effect be no less than one–half to two–thirds of the peak effect.[111,112]

It is clear, however, that many existing antihypertensive regimens do not meet these criteria. For example, a study of enalapril administration in patients with mild–to–moderate hypertension found that a 20 mg once–daily regimen resulted in a T/P ratio (BP reduction) of 33 ± 16%.[113] Similarly, a study comparing once–daily regimens of felodipine ER (10mg) and amlodipine (standard oral formulation, 5 mg) reported T/P ratios of 25% and 67%, respectively, for reduction of standing diastolic blood pressure; indicating that only the amlodipine regimen was appropriate.[114]

Table 2-3. Clinical Effects of nifedipine in human subjects (oral dosage formulations administered in single and multiple doses)

References	*Dosage Form*	*Daily dose (mg) [regimen, weeks]*	*Number of study participants*	*Participant characteristics [age range, y]*	*ΔSBP (mmHg)*	*ΔDBP (mmHg)*	*ΔHR (beats min⁻¹)*	*T/P Effect Ratio (%)*	*Incidence of Adverse Effects (%)*	*Incidence of oedema (%)*
Immediate–Release Formulation studies										
Kleinbloesem et al.[6]	Capsule	20 [single-dose]	6	N,M [22-28]			+16 ± 9 (sup)			
Myers & Raemsch[38]	Capsule	20 [single-dose]	6	H,M [63 ± 3]	-24 (sup)	-11 (sup)	+4 (sup)			
Soons et al.[35]	Capsule	10 [single-dose]	12	N,B [21-33]	-4.7 ± 8.8	-3.1 ± 4.4	+12 ± 8			
Frohlich et al.[93]	Capsule	30-120 [≥ 4]	10	H,B [39-71]	-25 (sit) -22 (std)	-16 (sit) -18 (std)	+9 (sit) +10 (std)	44		
Hilleman et al.[89]	Capsule	30-120 [≥ 12]	41	H,B [52 ± 11]	-9.0 (sit)	-5.0 (sit)	+4.0 (sit)		58	
Vertrovec et al.[101]	Capsule	30-120 [2]	91	A,B [46-81]					16	12[a]
Prolonged–Action/Retard Formulation Studies										
Kleinbloesem et al.[6]	Tablet	20 [single-dose]	6	N,M [22-28]			+7.5 ± 5.5 (sup)			
Myers & Raemsch[38]	Tablet	20 [single-dose]	6	H,M [63 ± 3]	-34 (sup)	-15 (sup)	+2 (sup)			
Hosie et al.[102]	Tablet	40 [2]	96	H,B [18-80]	-6 (sup) -8 (std)	-6 (sup) -6 (std)	0		41	14.6
Galletti et al.[103]	Tablet	40 [12]	16	H,B [52 ± 2]	-27 (sup)	-13 (sup)		72	44	25
Rogstad[104]	Tablet	40-80 [16]	53	H,B [24-68]	-13 (sit)	-8 (sit)	0 (sit)		43	13
Mörlin et al.[105]	Tablet	40-80 [12]	47	H,B	-25 (sit)	-15 (sit)	+0.7 (sit)		48.9	12.8

Controlled–Release Formulation Studies

Unpublished observations	GITS	30 [single-dose]	12	H,B [18-55]	-6 ± 12 (sup)	-5 ± 11 (sup)			
Unpublished observations	GITS	60 [single-dose]	12	H,B [18-55]	-1 ± 7 (sup)	-3 ± 6 (sup)			
Feig et al.[106]	CC	30 [10]	60	H,B [≥21]	-4.8 (sup) -6.1 (std)	-6.5 (sup) -6.1 (std)		49	50	16
Feig et al.[106]	CC	60 [10]	57	H,B [≥21]	-7.5 (sup) -9.6 (std)	-7.7 (sup) -7.6 (std)		67	60	23
Feig et al.[106]	CC	90 [10]	55	H,B [≥21]	-12 (sup) -13.4 (std)	-11.7 (sup) -10.0 (std)		61	62	29
Glasser et al.[107]	CC	60 [12]	117	H,B [24-87]	-15.1 (amb)	-9.0 (amb)	<3			22
Galletti et al.[103]	INDAS	50 [12]	16	H,B [54 ± 2]	-19 (sup)	-15 (sup)		78	31	19
Glasser et al.[107]	GITS	60 [12]	111	H,B [24-87]	-16.4 (amb)	-10.0 (amb)	<3			19
Hilleman et al.[89]	GITS	30-120 [≥12]	41	H,B [52 ± 11]	-21 (sit)	-20 (sit)	+2 (sit)		32	
Fagan et al.[90]	GITS	30-90 [6]	140	H,B [49 ± 9]	-16 (sit)	-13 (sit)			63	20
Frishman et al.[91]	GITS	30-60 [10]	24	H,B [56 ± 8]	-17 ± 13 (sit) -17 ± 15 (std)	-11 ± 8 (sit) -10 ± 8 (std)	0.4 ± 14.1 (sit) -1 ± 16 (std)		57	11.5
Frishman et al.[88]	GITS	30-90 [10]	31	H,B [56 ± 9]	-16 ± 11 (sit) -15 ± 13 (std)	-10 ± 6 (sit) -9 ± 6 (std)	-1 ± 10 (sit) -2 ± 11 (std)			
Gavras et al.[92]	GITS	30-60 [10]	33	H,B [53 ± 13]	-12 (sit) -10.9 (std)	-10.4 (sit) -10.8 (std)	+0.8			

Frohlich et al.[93]	GITS	30-150 [8]	10	H,B [39-71]	-20 (sit) -20 (std)	-11 (sit) -13 (std)	+4 (sit) +8 (std)	66		
Krakoff et al.[94]	GITS	30-180 [12]	1132	H,B [54 ± 12]	-17 (sit) -18 (std)	-13 (sit) -13 (std)	+1 (sit) +2 (std)		14.4	10.9
Murphy et al.[95]	GITS	60-180 [12]	8	H [54 ± 10]	-32 (sit)	-22 (sit)	+1 (sit)			
Lewis et al.[96]	GITS	30 [10]	≤ 60	H	-11.8 (sit) -12.0 (std)	-9.9 (sit) -9.0 (std)	+1.4 (sit) + 0.8 (std)			
Lewis et al.[96]	GITS	90 [10]	≤ 60	H	-14.2 (sit) -14.4 (std)	-11.0 (sit) -11.8 (std)	+1.4 (sit) +2.4 (std)			
Lewis et al.[96]	GITS	120 [10]	≤ 60	H	-19.9 (sit) -21.2 (std)	-12.7 (sit) -12.7 (std)	+0.8 (sit) +1.3 (std)			
Houston et al.[97]	GITS	30-150 [21]	23	H,B [18-70]	-18.5 (sit) -21.3 (std)	-11.7 (sit) -10.8 (std)	-0.4 (sit) -1.1 (std)			
Zanchetti et al.[108]	GITS	30 [4]	25	H	-17 ± 2 (amb)	-11 ± 2 (amb)		76	20	
Zanchetti et al.[108]	GITS	60 [4]	28	H	-16 ± 2 (amb)	-10 ± 1 (amb)		98.6	43	
Vertrovec et al.[101]	GITS	30-120 [12]	91	A,B [46-81]					18	6[a]

[a] Includes other vasodilator related effects.

Abbreviations: GITS= gastrointestinal therapeutic system; CC= Coat Core; INDAS= insoluble drug absorption system; ΔSBP= change in systolic blood pressure; ΔDBP= change in diastolic blood pressure; ΔHR= change in heart rate; T/P= trough/peak; N= normotensive; H= hypertensive (mild-to-severe); A= angina; M= males; B= both sexes; sit= sitting; std= standing; amb= ambulatory monitoring.

Studies with the nifedipine GITS have reported T/P ratios between 66% and 98.6%.[93,108] Hence, the nifedipine GITS appears to meet or exceed the current standards suggested for antihypertensive agents. Similarly, studies with 2 other types of once–daily oral nifedipine formulations, nifedipine coat–core (CC) and insoluble drug absorption system (INDAS), have reported T/P ratios ranging from 49% to 78% (see Table 2-3).[103,106] In placebo–controlled clinical trials both the nifedipine GITS and the nifedipine CC have been shown to safely and effectively lower BP,[115] and in a comparative study using 228 patients (with mild–to–moderate diastolic hypertension) they were found to be equivalent with respect to dose, BP reduction and adverse reactions (see Table 2-3).[107]

4.4 Nifedipine Concentration–Effect Relationships: Input Rate Dependency

A direct relationship appears to exist between plasma nifedipine concentrations and some of its pharmacological effects. However, conflicting data and opinions appear in the literature probably due to confounding factors such as: large interindividual variability; differences in the administration route and dose; differences in the haemodynamic parameters measured; induction of compensatory physiological responses; and differences in the magnitude of the haemodynamic response between normotensive and hypertensive study groups.[8,16,35,84,112,116,117] Additionally, the identification of a concentration–effect relationship is more easily demonstrated in those studies where the data for study participants are considered on an individual basis, and when repeated PK/PD parameter measurements are made throughout the dose interval.[112,118]

Stimulation of compensatory homeostatic mechanisms typically occurs shortly after rapid nifedipine administration. Thus, the characteristics of an observed effect such as BP reduction can be modulated by the rate of nifedipine input.[9,63] For example, Kleinbloesem et al.[9] demonstrated that slow intravenous nifedipine infusion had little or no effect on HR while enhancing the BP–lowering effect; whereas, rapid intravenous infusion of nifedipine apparently induces development of tachycardia. Similarly, as tachycardia is commonly observed after oral doses of IR nifedipine but not with the nifedipine GITS, it follows that these 2 formulations should not be considered pharmacodynamically equivalent.

Nifedipine administration results in BP reduction *via* direct vasodilation; therefore, compensatory homeostasis probably results from sympathetic outflow. However, modifying the clinical effect(s) of a drug as a result of manipulating oral absorption rate has only

recently received attention.[63,119] Castañeda-Hernández et al.[63] describe four potential types of response modulation that can result from slow versus rapid drug input: (1) simple response (the measured effect simply increases in the same fashion for both conventional- and slow-release formulations as the drug concentration rises); (2) complex response type A (slow-release formulations produce a relatively greater effect at a given concentration due to diminished compensatory responses); (3) complex response type B (the measured response is the outcome of direct drug effects and also compensatory responses which are initially less for the slow-release formulation but over time are the same for both types of formulations); and (4) development of tolerance (the measured response is subject to tolerance that develops with prolonged drug exposure).

With regard to nifedipine, input-rate differences produce a complex response type A relationship.[63] Hence, slow gradual intravenous input of nifedipine gives rise to plasma concentrations producing a greater BP response than similar plasma concentrations after rapid input. The most likely mechanism proposed to explain this phenomenon is that slow nifedipine infusion results in the gradual reduction of BP, 'fooling' the baroreceptor-initiated feedback regulation that would normally trigger a compensatory response.[120] Evidence supporting nifedipine input-rate dependent effects after oral administration is demonstrated in studies showing a similar magnitude of BP reduction after IR, PA and CR nifedipine administration, but with plasma nifedipine concentrations that are much less with the PA and CR formulations.[63,64,121]

To date, dose-ranging studies conducted with the IR forms of drugs are typically used to support new CR formulation applications to various drug regulatory bodies. Unfortunately, studies comparing the PK/PD profiles of drugs from IR and CR formulations are lacking.[63] Therefore, optimized release of orally administered nifedipine and drugs such as prazosin, furosemide, organic nitrates, etc. may not be utilized and the potential benefits of rate controlled drug delivery can be overlooked with newly developed agents.[63,119]

4.5 Adverse Effect Profile of the Nifedipine GITS

Typical adverse events in patients with hypertension or angina associated with the use of different nifedipine formulations, including the nifedipine GITS, are vasodilator-related and include: headache, flushing, increased HR and peripheral edema. It appears that the incidence of adverse effects of nifedipine is both dose and input-rate related.[1,8,122] In fact,

because of dose–limiting adverse effects, relatively low doses of IR nifedipine formulations are used in the treatment of ischaemia even though higher doses may improve anti–ischaemic efficacy.[123]

Vasodilator–related adverse effects are experienced in as many as 25% of patients taking conventional nifedipine capsules for angina. The reported incidence of adverse effects observed after oral nifedipine administration (all formulations) ranges from 14.4% to 63% (see Table 2-3). Unfortunately, most clinical studies of nifedipine done to date provide few details about the potential cause and severity of the reported adverse effects. Thus it is difficult to assess interstudy and interformulation differences with respect to the incidence of adverse events without a large, controlled, double–blind study directly comparing all nifedipine formulations.

The nifedipine GITS produces relatively low plasma nifedipine concentrations giving rise to nifedipine levels below the threshold required to induce reflex tachycardia; thus, this characteristic may represent an important pharmacodynamic advantage for the GITS formulation. This is illustrated in a study reporting the changes in the clinical response of patients switched from CR back to IR calcium channel blockers (verapamil, diltiazem and nifedipine).[89] The results show that in 41 hypertensive patients the nifedipine GITS is significantly better tolerated than IR nifedipine (incidence of adverse effects 32% versus 58%, respectively) (see Table 2-3). BP control is also better controlled when patients are using the nifedipine GITS. Interestingly, no advantages for CR verapamil or CR diltiazem are noted over their respective IR formulations.[89]

In contrast to β–blockers, diuretics, α–adrenoceptor antagonists, centrally–acting agents and ACE inhibitors, calcium channel blockers appear to be associated with fewer effects on metabolic parameters potentially reducing the incidence of cardiovascular morbidity and mortality.[99,124] Results of the MATH trial confirmed that the nifedipine GITS has little or minor effects on serum potassium, plasma glucose, serum total cholesterol, low density lipoprotein and high density lipoprotein fractions, or renal filtration function.[94,99,100] Consequently, the nifedipine GITS is recommended for use in a wide spectrum of patients.

The incidence of peripheral oedema is common in patients receiving nifedipine, and appears related to the dose and to long term or persistent nifedipine plasma concentrations rather than to the rate of drug absorption. Therefore, peripheral oedema might occur more frequently in patients prescribed the nifedipine GITS.[123] Results from the MATH study did

indeed find peripheral oedema to be the most common adverse effect (10.9%) of nifedipine GITS therapy.[94,99,100] However, results from several clinical trials (see Table 2-3) do not show evidence of an increase in the incidence of peripheral oedema with the nifedipine GITS, compared with any other nifedipine formulation.

As stated previously, only a controlled, double-blinded clinical trial comparing the different nifedipine formulations can definitively identify significant differences in the incidence of a particular adverse effect. It is worth noting, however, that although peripheral oedema resulting from administration of nifedipine GITS occurs without evidence of fluid retention,[100] a potential problem with congestive heart failure patients receiving this formulation may be that they could be mislead into believing their heart failure is worsening.

Verapamil, and to a lesser extent diltiazem, are known to elicit cardiodepressant effects, while the 1,4-dihydropyridines are deemed relatively vascular-selective agents. However, different members of the 1,4-dihydropyridine class exhibit various degrees of vascular selectivity. Nifedipine is noted to slightly decrease myocardial contractility,[125] and indeed isolated incidents of severe hypotension and decreased cardiac output after nifedipine administration have been reported.[77] In fact, nifedipine may cause unpredictable, variable and sometimes depressant effects; leading to aggravation or decompensation in a subset of patients with cardiac failure.[3]

Typically, the intrinsic, negative inotropic effect of the 1,4-dihydropyridine calcium channel blockers is either nullified or reversed by their reflex effects (i.e., due to increased sympathetic reflex activity and cardiac output secondary to decreased peripheral vascular resistance). Nonetheless, if the reflex effects are attenuated or abolished by disease or drugs (e.g., β-blockers[126]) then a depressant effect may emerge.[2] Also of some concern is that the cardiodepressant effects of nifedipine may be exposed and become clinically relevant, since administration of CR nifedipine formulations does not produce a large sympathetic reflex response. Thus, *a priori*, the use of nifedipine GITS in patients with congestive heart failure should be exercised with caution, and is, in fact, contraindicated.

Some clinicians have expressed concern regarding the possibility of excessive nocturnal BP reduction after nifedipine GITS administration. Reduction in BP by an antihypertensive agent may potentially induce myocardial ischaemia or produce symptomatic postural hypotension. However, reports in hypertensive patients have shown that the nifedipine GITS produces consistent but not excessive reductions in BP throughout the 24 hour dose

interval.[108,127] Thus, although the diurnal pattern of BP reduction is retained with the nifedipine GITS, the baseline pressures are only decreased to values within the 'normal' range using typical doses.

4.6 Acquired Nifedipine Tolerance

The dependency of the BP–lowering effect of nifedipine on its input rate (as discussed in section 4.4) should not be confused with the development of acquired drug tolerance. In the former case, a reduction in the drug input rate leads to an enhanced clinical effect (i.e., increased BP lowering), whereas the latter case typically describes a diminished clinical effect (i.e., decreased BP lowering) occurring with repeated doses and long term exposure to a drug, which necessitates an increase in the dose to maintain the desired clinical effect. Nonetheless, CR drug preparations may contribute to the development of drug tolerance (e.g., as seen in nitrate therapy) because therapeutic plasma drug concentrations are continuously maintained. However, nifedipine tolerance (with regards to diminishing BP reduction effects) does not appear to be a serious concern because it has not been reported to develop in most long term trials conducted with either the nifedipine GITS or IR nifedipine formulations.[101,128]

Interestingly, Martsevich and co–workers[129] reported that tolerance with respect to anti–ischaemic, anti–anginal and circulatory effects occurred within 2 months of implementing IR nifedipine therapy. Unfortunately, this study was conducted in only 15 patients and the results obtained with nifedipine were not compared with those of other anti–anginal agents. However, all 14 of the patients who completed the study showed a marked and significant reduction in the duration of the anti–ischaemic effect (5.4 ± 0.3 hours versus 3.6 ± 0.4 hours for short and long term therapy, respectively) — measured by a reduction in the maximal ST segment depression during exercise after drug administration. In addition, a decrease in the extent and duration of systolic BP reduction was also claimed. However, with respect to the extent of systolic BP reduction with nifedipine, a significant decrease was only found at 3 hours postdose (–14 ± 3% versus –8 ± 2% for short and long term administration, respectively); no significant differences were observed at 1, 2, 5 and 6 hours.

Similar findings to those of the preceding report have not been observed in other nifedipine studies; therefore, further studies are needed to confirm these results.

Nevertheless, the potential for development of tolerance after long term therapy with the nifedipine GITS may warrant further investigation.

5. Treatment Compliance

Salvetti and Di Venanzio,[130] identified 3 mechanisms by which CR calcium channel blockers can improve treatment compliance: (1) by offering a more favorable dosage schedule (i.e., reducing the number of daily doses and allowing greater choice in the timing of doses), (2) by allowing more efficient control of BP which may provide 'positive reinforcement' for patients actively monitoring their own condition, and (3) by lowering the incidence and severity of adverse effects.

Although the latter 2 points require further validation in well–controlled studies, many clinicians and researchers think that patient compliance is improved by decreasing the frequency of dosing or by increasing the tolerability of a medication, especially when 3 or more daily doses are involved. For example, one report found patient compliance to increase from 59% to 74% to 84% after 3–times–daily, twice–daily and once–daily dosage regimens, respectively.[131] In another study,[18] compliance rates of 39%, 77%, 81% and 87% with 4–times–daily, 3–times–daily, twice–daily and once–daily dosage regimens, respectively, are reported.

However, enhancement of treatment compliance with CR formulations is a complex issue, and has generated much debate and controversy. For example, Elliott and Meredith[132] point out that consideration must be given to the clinical implications of rapid loss of efficacy in poorly compliant patients receiving the nifedipine GITS. The problem they refer to is that once drug delivery is exhausted then the 'natural' pharmacokinetic properties of nifedipine are exposed, leading to rapid clearance from the blood and subsequent loss of antihypertensive efficacy.

Unfortunately, an important minority of chronic treatment patients exhibit suboptimal compliance with their medications, even when taken once daily.[133] However, drugs that possess long 'intrinsic' elimination half lives may be of less concern since drug concentrations could still be in the therapeutic range during the interval of the omitted dose. Thus, it is important to realize that although simple regimens may assist compliance they do not necessarily ensure therapeutic success. In some patients it may be desirable to prescribe drug,

formulations requiring more than 1 or 2 daily doses or, alternatively, to prescribe 'naturally' long–acting agents.

6. Conclusions

This chapter has outlined some of the important pharmaceutical, pharmacokinetic and pharmacological factors that should be considered when evaluating the nifedipine GITS and determining its therapeutic role. The choice of an appropriate calcium channel antagonist, and the dose and the type of formulation should be based on the requirements of the individual patient and on the pharmacology, efficacy and adverse effect profile of a particular drug.

The evidence for the nifedipine GITS, to date, indicates that this formulation enhances the therapeutic properties of nifedipine, therefore making available to clinicians a rational alternative to other nifedipine formulations and the 'second-generation' 1,4–dihydropyridines. However, as previously stated, any conclusions reached must be tempered by the paucity of large–scale, blinded comparative studies between these agents.

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Chapter 3

SENSITIVE HIGH–PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR NIFEDIPINE IN HUMAN PLASMA UTILIZING UV DETECTION◆

Introduction

Nifedipine, 1,4–dihydro–2,6–dimethyl–4–(2–nitrophenyl)–3,5–pyridinedicarboxylic acid dimethyl ester (Figure 3-1A) — parent compound of the dihydropyridine class of calcium channel antagonists — is widely used for the treatment of hypertension, angina pectoris and other cardiovascular disorders due to its selective dilation of arteries.[1] In humans, nifedipine is rapidly metabolized by oxidative mechanisms to dehydronifedipine (Figure 3-1B), which is further metabolized to more polar compounds.[2-10] Nifedipine is highly sensitive to chemical oxidation, forming dehydronifedipine upon ultraviolet light exposure and the nitroso–analogue of dehydronifedipine upon visible light exposure (i.e., daylight).[4,7,10,11] Hence, analytical methods for detection of nifedipine in various matrices must adequately resolve nifedipine from its metabolites or photodegradation products. Additionally, the assay procedure must be done under subdued lighting or sodium lamps.[6,7]

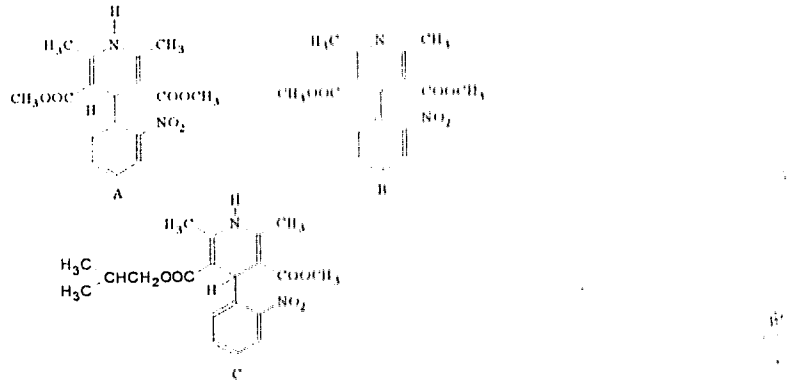

Figure 3-1. Structures of (A) Nifedipine, (B) Dehydronifedipine and (C) Nisoldipine.

Most reports of high–performance liquid chromatographic (HPLC) methods for measuring nifedipine concentrations in biological fluids give the precision of calibration data;

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however, accuracy results are frequently omitted especially for nifedipine concentrations below 10 ng mL^{-1}.[1,2,3,5,7,10,12,16] Hence, although detection limits below 10 ng mL^{-1} are often stated (usually 1 to 5 ng mL^{-1}) the minimum quantifiable concentrations are seldom reported. A summary report[17] describing analytical method validation — sponsored by the U.S. Food and Drug Administration (FDA) and the Canadian Health Protection Branch (HPB) — outlines the acceptable standards and procedures that should be used for development of valid analytical methods. Many current nifedipine HPLC methods do not meet all of the criteria suggested by this report, particularly methods using ultraviolet detection. However, the study of nifedipine pharmacokinetics in humans requires a completely validated assay that can measure concentrations below 10 ng mL^{-1}, since clinical samples may contain low plasma nifedipine concentrations.[9,18]

In this chapter a rapid, sensitive, and selective reversed–phase HPLC assay is described which meets or exceeds generally accepted criteria for analytical method validation, and which is suitable for rapidly processing nifedipine plasma samples taken during clinical studies.

Materials and Methods

Chemicals

Nifedipine was purchased from Sigma Chemical Company (St. Louis, MO, USA). Internal standard (nisoldipine, IS, Figure 3-1C) and nifedipine metabolites or photodegradation products were kindly provided by Miles Canada (Etobicoke, ON, Canada). Methanol and water (HPLC grade) and analytical grade acetic acid and triethylamine (TEA) were obtained from Mallinckrodt (Paris, KY, USA). Iso–octane and methyl–t–butyl ether (MTBE) were purchased from BDH (Toronto, ON, Canada) and were HPLC grade.

Chromatography

This assay is a modification of a previously reported HPLC method for nifedipine.[14] Samples were vortex–mixed with a Genie 2 mixer (Fisher Scientific, Edmonton, AB., Canada) and centrifuged with a Dynac II centrifuge (Becton–Dickinson, Parsippany, NJ, USA). Evaporation of the sample solvents was done using a Model SC 100 Savant Speed Vac concentrator–evaporator (Emerston Instruments, Scarborough, ON., Canada). The

HPLC system consisted of a Model 600E solvent delivery system, a Model 717 autosampler, a 486 tunable ultraviolet/visible absorbance detector set at 350 nm and a NEC Powermate 486/33i computer with Millennium 2010 chromatography manager v1.1 software (Waters, Mississauga, ON., Canada). Compounds were separated using a Nova–Pak 8 x 100 mm radial pack column containing 4 µm C8 packing material (Waters, Mississauga, ON., Canada).

The mobile phase consisted of methanol:water (65:35, v/v) adjusted to approximately pH 4.0 with acetic acid and TEA as 1% and 0.03% final concentration, respectively. The flow rate used was 1.1 mL min^{-1}. Sample preparation and analysis were conducted at room temperature under sodium lamps.

Standard solutions

Stock solutions of both nifedipine and of IS were prepared in methanol, 100 µg mL^{-1} (as base). Both solutions were protected from light by covering with aluminum foil, stored at 4° C and remained stable for at least 3 months. A 1 µg mL^{-1} solution of nifedipine in methanol (solution 1) was prepared daily from the nifedipine stock solution. A 100 ng mL^{-1} nifedipine solution in methanol (solution 2) was prepared daily from solution 1. A 10 µg mL^{-1} solution of IS in methanol (solution 3) was prepared daily from the stock IS solution.

Sample preparation

To 1.0 mL of drug–free human plasma, in disposable glass 16 x 125 mm culture tubes, was added nifedipine using either solutions 1 or 2, to yield final concentrations of 5, 10, 15, 25, 50, 100 and 250 ng mL^{-1}. After addition of 500 ng of IS (solution 3) and 100 µL of 1.0 M sodium hydroxide, samples were vortex–mixed for 3 seconds and 5 mL of MTBE:iso-octane (75:25, v/v) was then added. The resultant mixture was vortex–mixed for 30 seconds and centrifuged at 3000 rpm (1850 g) for 5 minutes. The upper organic layers were transferred to clean dry 13 x 100 mm glass tubes and evaporated to dryness (no heat was applied) using the Speed Vac concentrator–evaporator. To each of the resulting residues was added 200 µL of mobile phase and the solutions were vortex–mixed for 15 seconds. Aliquots of 150 µL of each sample were injected onto the HPLC column.

Extraction yield

Solutions 1 or 2 were used to add 10, 50 and 250 ng of nifedipine (n = 5 replicates) into disposable glass 16 x 125 mm culture tubes containing 1.0 mL of blank human plasma.

Nifedipine was extracted as previously described except that exactly 3.5 mL of each of the organic layers was transferred to clean dry 13 x 100 mm glass tubes and evaporated to dryness. To compare extracted versus unextracted samples, an equivalent amount of nifedipine (using solutions 1 and 2) was added to another set of 16 x 125 mm culture tubes containing only 5 mL extraction solvent (n = 5 replicates), of which 3.5 mL was transferred to clean dry 13 x 100 mm culture tubes and evaporated to dryness. The peak areas of extracted and unextracted nifedipine samples were compared under identical chromatographic conditions.

Quantitation

Calibration curves were constructed by plotting the peak/area ratios (nifedipine/IS) versus their corresponding added plasma concentrations. A weighted least–squares regression analysis was done to generate a best–fit regression line ($1/x$ weighting, where x is the concentration of nifedipine). Data are presented as mean ± s.

Mass spectrometry

To confirm the identity of the nifedipine peak, the corresponding peak was collected from the HPLC eluent and subjected to high–resolution electron impact mass spectral analysis (AEI, MS50, Manchester, U.K.) via direct insertion probe at 70 eV ionizing potential.

Other compounds tested

The following nifedipine metabolites or photodegradation products were tested for peak interference using the chromatographic conditions previously described: (1) nitropyridine metabolite (2,6–dimethyl–4–(2–nitrophenyl)–3,5–pyridinedicarboxylic acid dimethyl ester, Bay b 4759); (2) nitrosopyridine photodegradation product (2,6–dimethyl–4–(2–nitrosophenyl)–3,5–pyridinedicarboxylic acid dimethyl ester); and (3) carboxylic acid metabolite (2,6–dimethyl–4–(2–nitrophenyl)–3,5–pyridinedicarboxylic acid monomethyl ester, Bay o 2820).

Results and Discussion

Measurement of drug concentrations in biological samples (blood, plasma, serum, urine, etc.) requires a method having satisfactory precision, accuracy, sensitivity and specificity. This

is because errors in drug concentration measurements can significantly affect the accuracy of estimated pharmacokinetic parameters calculated from clinical studies.

Most published nifedipine HPLC methods report the precision of calibration data; however, accuracy results are either missing or reported for nifedipine concentrations ≥ 10 ng mL^{-1}.[1,2,3,5,7,10,12,16] Omitted accuracy data for low nifedipine concentrations (< 10 ng mL^{-1}) may arise from technological limitations (e.g., detector sensitivity), or possibly because acceptable accuracy is unattainable with some of these methods. In contrast, several published gas chromatographic (GC) assay methods present both accuracy and precision data over the entire concentration range reported.[4,9,19]

Interestingly, Patrick et al.[19] tried unsuccessfully to develop and validate a sensitive and specific HPLC nifedipine assay method but ultimately chose to develop a GC based method for the analysis of plasma samples — despite the fact that they also examined several previously published HPLC methods. It should be noted, however, that even GC methods can give poor accuracy because of potential problems with nifedipine thermodegradation.[7,9,10,19] On the other hand, Suzuki et al.[10]describe an HPLC method using electrochemical detection that gives excellent precision and accuracy, but again accuracy for low drug concentrations (less than 9.6 ng mL^{-1}) is not reported. Furthermore, electrochemical detection lacks the ability to detect nifedipine metabolites and in the opinion of some researchers is not a robust technique.[7]

Besides accuracy and precision considerations, the known metabolites and degradation products of nifedipine must be chromatographed to ensure that none of these compounds co–elute with nifedipine (i.e., the specificity of the method must be confirmed). The specificity's of several published nifedipine assays were not tested using all the known major nifedipine metabolites or photodegradation products.[3,7,11,14,16] Unfortunately, a non–specific nifedipine assay may result in the overestimation of nifedipine concentrations in plasma. However, the assay presented here provided the required sensitivity and resolution of nifedipine, without interference from tested metabolites, photodegradation products and the IS (nisoldipine). In fact, complete baseline resolution of these components was observed; however, the method was not optimized for detection and quantitation of nifedipine metabolites in clinical samples, because known nifedipine metabolites are inactive.[1,6]

In the current method, peaks for nifedipine and IS were eluted at about 6.5 minutes and 16 minutes, respectively (Figure 3-2B-D). The chromatograms presented in Figure 3-2 were

scaled for the highest peaks eluted (either IS or an unknown component coextracted from plasma) by the chromatography software and not scaled to emphasize the nifedipine peak. Therefore, the nifedipine peaks appear to be relatively small even for the high concentrations used and despite a signal–to–noise ratio greater than 5 for the 5 ng mL^{-1} spiked plasma samples (Figure 3-2B). The identity of the nifedipine peak was confirmed using electron impact high resolution mass spectrometry. Blank plasma samples did not show any potentially interfering peaks (Figure 3-2A). The lower limit of quantitation (LLQ) of nifedipine was determined as 5 ng mL^{-1}. However, the lower limit of detection (LLD) of nifedipine was 1 ng mL^{-1}.

The calibration curve fitted to the mean nifedipine data (combination of all runs) was described by $y = 0.00003 + 0.0025x$; where y corresponds to the peak–area ratio of nifedipine to IS and x to the added nifedipine concentration. Excellent linearity was observed for all individual calibration curves ($r^2 > 0.997$). A weighting factor ($1/x$) was applied in all the regression calculations of the calibration curves. Weighting was necessary in this analysis to achieve accuracy within 15% of the actual values, especially for the lowest calibration points. Unweighted least–squares regression analysis tends to bias the upper points on the calibration curve to minimize residual error; therefore, calculating a line of best–fit optimized for the highest concentrations. Hence, the fit of the lower points on the curve may be unsatisfactory since their contribution to residual error is usually small; therefore, accuracy of low concentrations may be compromised. On the other hand, the $1/x$ weighting factor used in this analysis shifted the bias to the lower nifedipine concentrations but still provided acceptable accuracy for the higher concentrations on the calibration curve (within ~10% of the actual value). A $1/x^2$ weighting factor was also evaluated, but unacceptable bias was observed for low calibration points resulting in poor accuracy for the higher concentrations. The results in Table 3-1 describe accuracy (% error) and precision (coefficient of variation, CV) of the method. The error in both accuracy and precision values throughout the concentration range (5 to 250 ng mL^{-1}) was acceptable (i.e., ≤ 15%).

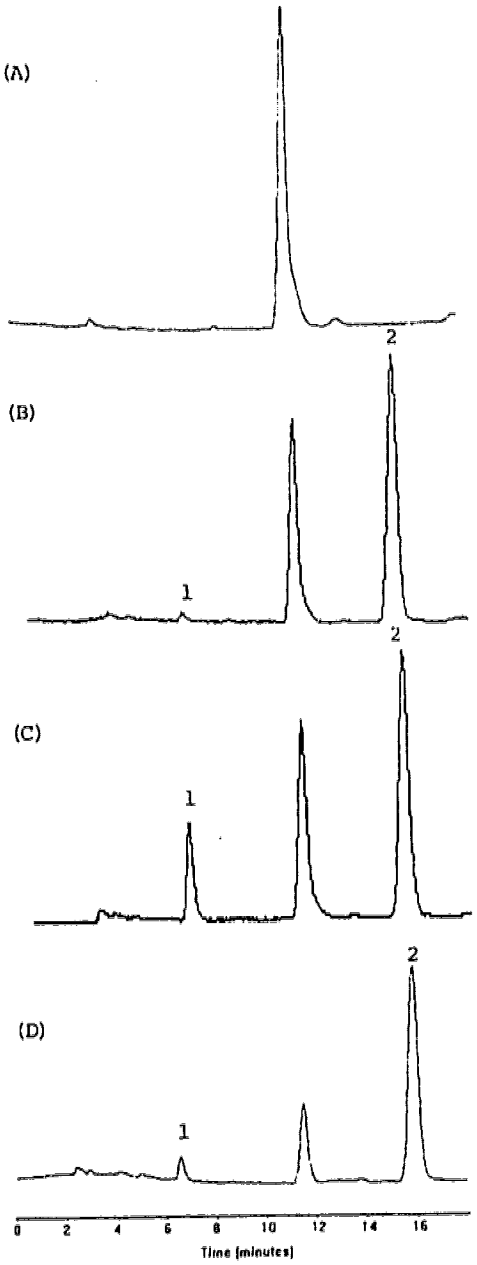

Figure 3-2. Chromatograms of (A) blank human plasma, (B) human plasma spiked with 5 ng mL^{-1} of nifedipine, (C) human plasma spiked with 100 ng mL^{-1} of nifedipine, and (D) human plasma sample taken 24 hours after oral administration of a single 30 mg dose of a controlled–release nifedipine formulation (osmotic pump). The concentration of nifedipine corresponds to 26 ng mL^{-1}. Peak identification: 1) nifedipine; 2) IS (nisoldipine).

Table 3-1. Accuracy and Precision of the Method.

Concentration Added (ng mL^{-1})	Measured Concentration[a] (ng mL^{-1})	Accuracy, error (%)	Precision, C.V.(%)
5	5.72 ± 0.68	+14.40	11.89
10	11.14 ± 1.23	+11.40	11.04
15	15.16 ± 1.10	+1.07	7.26
25	23.62 ± 0.82	-5.52	3.47
50	48.64 ± 5.14	-2.72	10.57
100	92.90 ± 6.18	-7.10	6.65
250	221.46 ± 12.31	-11.42	5.56

[a] Reported as mean ± *s* (n = 11, three sets on day 1, four sets on days 2 and 3).

After comparing the extraction efficiencies of various solvents (including chloroform and ethyl acetate), it was found that a mixture of MTBE and iso–octane (75:25, v/v) provided the best extraction yield while minimizing interfering peaks and baseline noise. The extraction yield of nifedipine from plasma was constant over the entire calibration range with a mean value of 89.87 ± 5.36% (extractions of 10, 50 and 250 ng mL^{-1} concentrations, n = 5 replicates). Although the mean extraction yield was less than 100% the method still provided adequate sensitivity to allow the processing of clinical samples.

During the development of this assay a problem was found achieving an acceptable signal–to–noise ratio of the nifedipine peak using detector wavelengths in the 200 to 280 nm range. Ultraviolet detection at 254 nm or less is commonly used in many nifedipine assay methods, and is advantageous for optimized detection of nifedipine metabolites and photodegradation products.[2,3,5-7,11] However, significant baseline noise can be seen in the published chromatograms from several of these methods, which could compromise accuracy at low nifedipine concentrations.[5-7] Therefore, ultraviolet detection at 350 nm was selected for the current method.

In addition, a mobile phase containing only methanol and water was evaluated during method development, but problems with peak tailing and a shortened column life needed to be overcome. Although replacement of water with phosphate buffer was considered — as previously applied by Snedden et al.[14]— the mobile phase mixture giving the best results was methanol and water adjusted to pH 4 with acetic acid and TEA.

Figure 3-2D depicts a chromatogram (scaled to show all the eluted peaks) of an extracted plasma sample, that was taken from a healthy human subject 24 hours after oral administration of a single controlled–release nifedipine formulation (Nifedipine Gastrointestinal Therapeutic System, 30 mg tablet). The nifedipine peak in the chromatogram corresponds to 26 ng mL^{-1}; therefore, the applicability of the method to the analysis of plasma samples obtained from clinical studies was demonstrated.

In conclusion, a rapid, sensitive and selective reversed–phase HPLC method using ultraviolet detection for the analysis of nifedipine in plasma samples was described. This method improved upon previously reported HPLC techniques for precise and accurate measurement of low nifedipine concentrations. Furthermore, the relatively short preparation and analysis times of plasma samples enhance the clinical suitability of this method.

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Chapter 4

PHOTOSTABILITY DETERMINATION OF COMMERCIALLY AVAILABLE NIFEDIPINE ORAL DOSAGE FORMULATIONS◆

Introduction

Nifedipine (Figure 4-1A) is highly sensitive to photo–oxidation, especially a solution of the compound which changes appearance from yellow to brown upon exposure to light.[1] Exposure of nifedipine to ultraviolet (UV) light results in the formation of DNIF (Figure 4-1B); whereas, nifedipine exposure to UV–visible light (i.e., daylight) generates the nitroso–analog of dehydronifedipine (NDNIF, Figure 4-1C).[2] Unfortunately, nifedipine photodegradation products have little or no pharmacologic activity.[3,4] Matsuda et al.[5] show that nifedipine, in fact, can undergo degradation to four products after light exposure (using fluorescent and mercury vapor light sources), however three of these (including DNIF) are produced in minor quantities only, whereas the fourth compound (NDNIF) is identified as the sole major photodecomposition product. Therefore, quantitative photodegradation analysis of nifedipine formulations requires accurate detection and quantitation of both nifedipine and NDNIF.

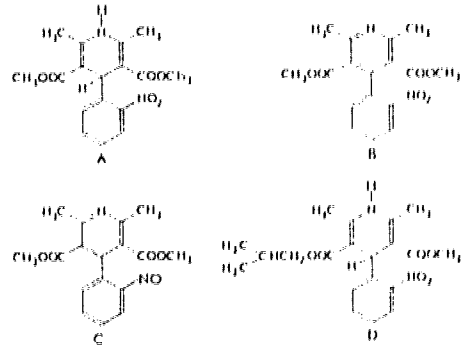

Figure 4-1. Structures of (A) nifedipine, (B) dehydronifedipine (DNIF), (C) nitroso–analog of dehydronifedipine (NDNIF), and (D) nisoldipine (IS).

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Official USP guidelines for nifedipine capsules state that the measured amount of nifedipine in a commercial product must be within 90% to 110% of the labeled quantity.[6] Hence, manufacturers of nifedipine products use light resistant coatings or packaging to minimize photodegradation of oral nifedipine preparations from inadvertent light exposure. Nonetheless, few quantitative studies of the light transmissive properties for different light protective tablet film coatings or protective packaging have been done.[7] Thus, the degree of light protection may differ between brands or types of nifedipine formulations.

It is possible that long term exposure (several weeks or longer) to direct sunlight may occur if nifedipine formulations are improperly stored by patients. Thus, inadequate storage conditions may potentially contribute to a decrease in clinical efficacy of light sensitive nifedipine products. Consequently, if differences in photostability between nifedipine products are present, then product substitution may not be warranted.

Several studies have investigated the photostability of nifedipine in solution and in the solid state, including the photostability of pulverized nifedipine tablet powders.[5,8-11] However, the study described in this chapter is one of the few to compare nifedipine stability in intact commercial oral nifedipine formulations[12,13]and is the first to report the photostability of prolonged–action (PA) and gastrointestinal therapeutic system (GITS) nifedipine products.

The extent of nifedipine photodegradation is reported for 10 different nifedipine formulations after long–term exposure to an artificial sunlight source, using a specific and sensitive HPLC assay to detect the nifedipine and NDNIF content. This study also compared the photodegradation of authentic nifedipine powder and a methanolic nifedipine solution after artificial sunlight exposure.

Materials and Methods

Chemicals

Nifedipine was purchased from Sigma Chemical Company (St. Louis, MO, USA). Internal standard (nisoldipine, IS, Figure 4-1D), NDNIF, DNIF (Bay b 4759), and the carboxylic acid metabolite (Bay o 2820) were kindly provided by Miles Canada Inc. (Etobicoke, ON, Canada). Methanol, water and triethylamine (TEA) were obtained from Mallinckrodt (Paris, Kentucky, USA). Iso–octane, methyl–t–butyl ether (MTBE), sodium hydroxide and glacial acetic acid were purchased from BDH (Toronto, ON, Canada).

Chloroform was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All reagents and chemicals were either analytical or HPLC grade.

Standard solutions

Stock solutions, 100 μg mL^{-1} (as base) of both nifedipine and NDNIF and 50 μg mL^{-1} (as base) of IS, were prepared in methanol. Solutions were kept protected from light with aluminum foil, stored at 4°C and were found to maintain their original concentrations of nifedipine or NDNIF, respectively, for a period of at least 3 months. Solutions containing 10 μg mL^{-1} of nifedipine and NDNIF in methanol (solutions 1 and 2, respectively) were prepared daily from the respective stock solutions.

Formulations

Ten nifedipine formulations obtained from several manufacturers in North America and Germany were studied. Formulations tested included: Six prolonged–action (PA) 20 mg tablets [Adalat PA, Miles (Canada); Nifelat 20 retard, TAD (Germany); Nifedipine–Wolff 20 retard, Vinces (Germany); Nifical tablinen retard, Sanorania (Germany); Nifehexal retard, Hexal Pharma (Germany); and Pidilat retard, Giulini Pharma (Germany)]; one 20 mg PA capsule [Nifedipin AL 20, Aliud Pharma (Germany)]; two immediate–release (IR) 10 mg capsules [Apo–Nifed, Apotex (Canada); Novo–Nifedin, Novopharm (Canada)]; and one gastrointestinal therapeutic system (GITS) 60 mg tablet [Adalat XL, Miles (Canada)].

Irradiation Test

The irradiation test used a full–spectrum lamp (20 Watt, 900 lumens, vacuum tube length 59 cm, Commercial Lighting Products Ltd., Edmonton, Alberta, Canada) placed 22 cm from the nifedipine samples. Samples were placed on aluminum foil to allow for more uniform irradiation. Irradiation was conducted inside a cabinet to protect the samples from extraneous light. Each formulation was irradiated (24 hours per day) by the artificial sunlight source for a period ranging from 0 to 12 weeks. Samples were taken at 0, 1, 2, 4, 6, 8, 10 and 12 weeks (n = 6 replicate samples per interval). In addition, a total of eleven 1 mL methanolic nifedipine solution samples (100 μg mL^{-1}), placed in 5 ml clear glass vials, were irradiated for a period of 0 to 360 minutes (aliquots for analysis were taken at 0, 5, 10, 15, 20, 30, 45, 60, 120, 240 and 360 minutes, n = 3 replicates). A total of seven 10 mg nifedipine powder samples, placed in 5 mL clear glass vials, were also irradiated for a period of 0 to 12

days (aliquots for analysis were taken at 0, 1, 2, 3, 7, 10 and 12 days, n = 3 replicates). Irradiation of samples was conducted at ambient temperature.

Sample preparation

(1) Prolonged–Action (PA) Tablet and Capsule–Pellet Formulations:

Each PA tablet (or contents of a PA capsule) was crushed into fine particles in a disposable 20 mL paper cup using a small glass pestle. The crushed particles were placed in a small microcentrifuge tube and 1 mL of chloroform was added. The resulting mixture was vortexed for 15 seconds and centrifuged for 5 minutes. Vortexing was done with a Genie 2 mixer (Fisher Scientific, Edmonton, Canada) and centrifugation was done with a microcentrifuge (Microfuge E, Beckman, Palo Alto, California, USA). From the supernatant, 25 µL of solution was placed in a small glass vial, diluted to a final volume of 1.5 mL with chloroform and vortexed for 15 seconds. A 30 µL volume of the diluted solution was added to a clean dry 13 x 100 mm glass test tube containing 10 µg (200 µL) of IS. The sample was then evaporated to dryness (low heat) in a concentrator–evaporator (Model SC 100 Savant Speed Vac concentrator–evaporator, Emerston Instruments, Scarborough, ON, Canada). To the resulting residue was added 200 µL of mobile phase and the solution was vortex–mixed for 15 seconds. Aliquots of 10 µL were injected onto the HPLC column.

(2) Liquid–Filled Immediate–Release Capsules:

A 100 µL volume of solution containing nifedipine was withdrawn from each nifedipine capsule using a 21–gauge needle and a 1 mL syringe and diluted 100 times with chloroform. A 25 µL aliquot of this solution was placed in a small glass vial, diluted to a final volume of 1.5 mL with chloroform and vortexed for 15 seconds. From this dilute solution, 30 µL was added to a clean dry 13 x 100 mm glass test tube containing 10 µg (200 µL) of IS. Sample preparation and injection was as described per PA tablets and PA capsules.

(3) Gastrointestinal Therapeutic System (GITS) Tablets:

Single 60 mg GITS tablets were added to 50 mL chloroform in a stainless steel blender (Model 702CR, Rotor Electric Co. Ltd.) and homogenized for 10 minutes. A 100 µL aliquot of the resulting mixture was placed in a small glass vial and diluted to a final volume of 1 mL with chloroform. A total of 83 µL (containing 10 µg of nifedipine) of the diluted mixture

was placed in a clean dry 13 x 100 mm glass test tube containing 10 µg (200 µL) of IS. Sample preparation and injection was as described above.

(4) Methanolic Nifedipine Solutions:

Three 10 µg (100 µL) samples were withdrawn from each vial at the sampling times previously described. Each of these samples was placed in a clean dry 13 x 100 mm glass test tube containing 10 µg (200 µL) of IS. Sample preparation and injection was as described above.

(5) Nifedipine Powder Samples:

Each irradiated nifedipine powder sample was diluted with 1 mL methanol (10 mg mL^{-1}), then further diluted with methanol to 100 µg mL^{-1}. Three 10 µg (100 µL) samples from each time point were placed in a clean dry 13 x 100 mm glass test tube containing 10 µg (200 µL) of IS. Sample preparation and injection was as described above.

Chromatography and Instrumentation

Determination of nifedipine and NDNIF content was done by modifying a previously reported HPLC method.[14] Briefly, the HPLC system consisted of a Model 600E solvent delivery system, a Model 717 autosampler and a 486 tunable UV/VIS absorbance detector set at 350 nm. Compounds were separated using a Nova–Pak 8 x 100 mm radial pack column containing 4 µm C8 packing material (Waters, Mississauga, ON, Canada). Mobile phase flow rate was 1.1 mL min^{-1} and consisted of methanol:water (65:35 v/v) adjusted to approximately pH 4 with acetic acid and TEA as 1% and 0.03% final concentration, respectively. The mobile phase was continuously degassed with helium (100 mL min^{-1}). Sample preparation and analysis were conducted at room temperature under sodium lamps. Sample spectra were recorded using either a Waters 745B integrator or Millennium 2010 chromatography manager software installed on an NEC 486/33i computer (Waters, Mississauga, ON, Canada).

Quantitation

Calibration curves were constructed by plotting the peak:area ratios (nifedipine/IS or NDNIF/IS) versus their corresponding added concentrations. Calibration curve concentration ranges used in testing of irradiated nifedipine formulations were 0 to 35 µg and 0 to 750 ng for nifedipine and NDNIF, respectively. Calibration curve concentration ranges used in testing of methanolic nifedipine solution and nifedipine powder were 0 to 35 µg and

0 to 15 μg for nifedipine and NDNIF, respectively. An unweighted least-squares linear regression analysis was done to generate a best-fit regression line for each compound. Calculation of nifedipine powder and solution $t_{1/2}$ were determined from the slope of the log % nifedipine–time plots (i.e., $t_{1/2} = 0.693/k$). Data are presented as mean ± *s*.

Mass spectrometry

Authentic nifedipine and NDNIF reference standards, and NDNIF prepared from exposure of a methanolic nifedipine solution (2 mg mL^{-1}) to artificial sunlight for 4.5 hours, were chromatographed and the eluent corresponding to the assumed nifedipine and NDNIF peaks were collected for analysis. Samples were evaporated using a Speed Vac evaporator–concentrator and the residues subjected to high–resolution electron impact mass spectral analysis (AEI, MS9, Manchester, UK.) via direct insertion probe at 70 eV ionizing potential.

Other compounds tested

Two additional nifedipine metabolites or photodegradation products were tested for peak interference using the chromatographic method employed for this study: (1) DNIF (Bay b 4759) and (2) the carboxylic acid metabolite (CAM, Bay o 2820).

Results and Discussion

In the present study, a measure of the percentage NDNIF content (w/w initial nifedipine content) in intact commercial nifedipine formulations was determined after continuous exposure to artificial sunlight. Up to 12 weeks of continuous artificial sunlight irradiation was evaluated for each commercial nifedipine formulation tested; since natural sunlight exposure could potentially be of this magnitude when these products are stored incorrectly. The use of artificial sunlight in this study overcomes the potential problems inherent when using natural sunlight, such as: daytime, regional, weather and seasonal variability.

Figure 4-2 depicts typical chromatograms of: a blank sample (Figure 4-2a); a sample spiked with authentic nifedipine and NDNIF (Figure 4-2b); a sample of nifedipine methanolic solution irradiated for 45 minutes (Figure 4-2c); and a commercial 20 mg nifedipine PA capsule irradiated for 12 weeks (Figure 4-2d) — note: each sample including the blank were spiked with 10 μg IS. UV absorption of all compounds was measured at a wavelength of 350 nm — as greater accuracy at this wavelength has been reported for the determination of both nifedipine and NDNIF compared to lower wavelength ultraviolet light.[11,15]

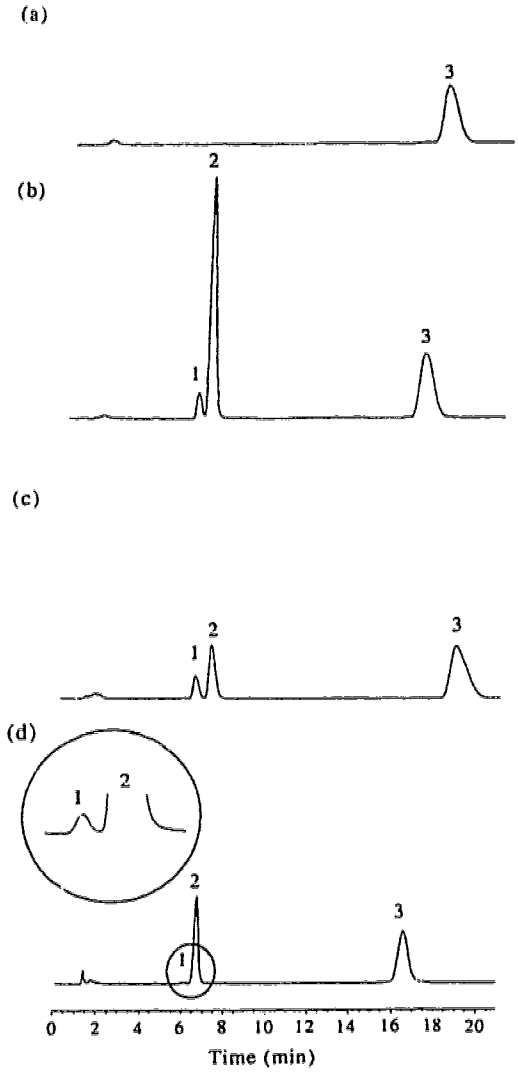

Figure 4-2. Chromatograms of: (A) blank sample containing 10 µg IS, (B) authentic nifedipine, NDNIF and IS (15, 5 and 10 µg, respectively), (C) methanolic nifedipine solution (100 µg mL^{-1}) irradiated by the artificial sunlight lamp for 45 minutes; NDNIF, nifedipine, and IS peaks correspond to 5.7, 4.3, and 10 µg respectively, and (D) a commercial 20 mg nifedipine PA capsule irradiated for 12 weeks (enlarged view shows the small NDNIF peak). Peak identification: 1) NDNIF; 2) nifedipine and 3) IS

Using the assay method previously described, a peak corresponding to nifedipine typically eluted at about 7.0 minutes, NDNIF eluted at about 6.0 minutes and the IS at about 17 minutes. Satisfactory chromatographic resolution ($R > 1.5$) of the three peaks of interest was observed. Calibration curves were linear over 1 to 35 µg, 50 to 750 ng and 0.5 to 15 µg for nifedipine (all tests), NDNIF (formulation tests) and NDNIF (solution or powder tests), respectively. Observed r^2 values were > 0.99 for all calibration curves. Additionally, two

known human nifedipine metabolites, DNIF (also a product of nifedipine photodegradation after UV light exposure) and the carboxylic acid metabolite (CAM) were tested for interference with the compounds of interest under identical HPLC conditions. No interference was observed between any of the compounds tested (peaks were obtained at ~2 and ~5 minutes for CAM and DNIF, respectively, chromatograms not shown). DNIF and CAM peaks were not detected in any nifedipine formulation, solution or raw powder analyses conducted in this study.

Mass spectra of both nifedipine and NDNIF residues (collected from evaporated peak eluents) supported the assignment of peak identity. Mass spectra of authentic nifedipine produced major fragments at m/z 346 (10%, base peak), 329 (100%), and 284 (62%). Mass spectra of authentic NDNIF produced major fragments at 328 (47%), 298 (11%) and 269 (100%). Nifedipine obtained from the assumed nifedipine peak eluent from an extracted nifedipine tablet, and NDNIF obtained from the assumed NDNIF peak eluent of a methanolic nifedipine solution previously exposed to artificial sunlight (> 4 hours), produced mass spectra consistent with those found for authentic nifedipine and NDNIF, respectively.

Figure 4-3 shows photodegradation plots of nifedipine powder (Figure 4-3A) and nifedipine methanolic solution (Figure 4-3B) after artificial sunlight irradiation. Photodegradation of nifedipine powder, measured as percent (%) loss of nifedipine, exceeded 10% in 24 hours ($t_{1/2}$ = 7.7 days). Photodegradation of the nifedipine methanolic solution exceeded 10% in about 5 to 10 minutes and was essentially complete within 4 hours ($t_{1/2}$ = 31 minutes). These results are consistent with those of previously published nifedipine powder and alcoholic solution degradation studies.[2,5,11] Berson and Brown[1] report that nifedipine photodecomposition appears to follow zero–order kinetics until the reaction is about 60% complete, after which the rate decreases in a first–order fashion. This is attributed to inhibition of the nifedipine degradation reaction by NDNIF. Other studies use first order kinetics or more complex models to describe nifedipine degradation.[7,16] However, no attempt was made in this analysis to further define nifedipine degradation kinetics, as the primary objective was to compare nifedipine stability in intact commercially available formulations. Nevertheless, apparent first–order degradation was observed for nifedipine degradation kinetics.

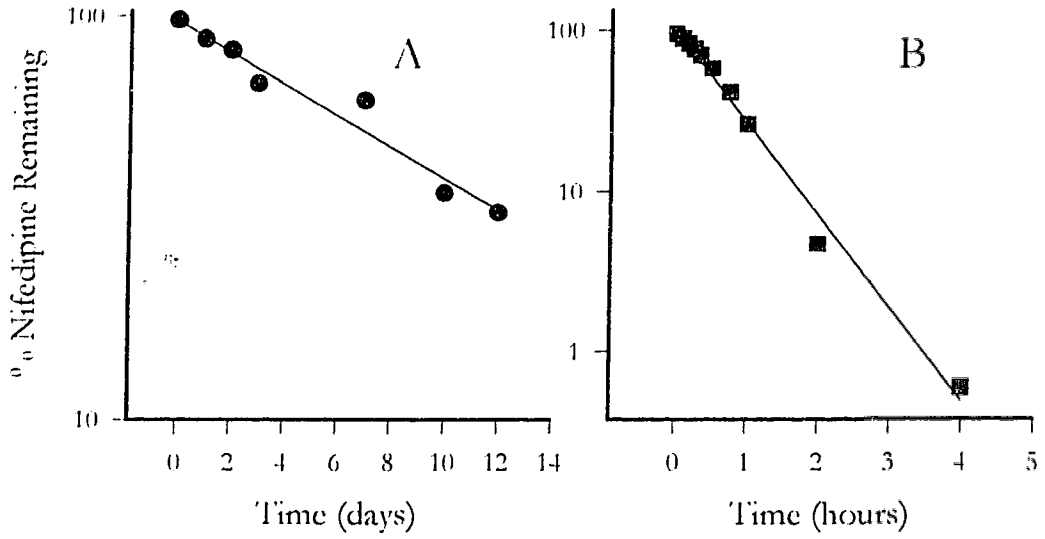

Figure 4-3. Nifedipine photodegradation curves. (A) nifedipine powder, (B) methanolic solution (100 μg mL^{-1}).

Exposure of the nifedipine methanolic solution to either natural sunlight (using mid–day sun) or the artificial sunlight source used in this study, yielded 100% decomposition of nifedipine after 4 hours in both cases. It appears reasonable, therefore, to conclude that the artificial sunlight lamp adequately approximated natural sunlight conditions for the purpose of this study.

The percentage of NDNIF (w/w initial nifedipine content) found in all 10 tested formulations, was very low (about 1% to 2%) even after irradiation for 12 weeks with artificial sunlight. Table 4-1 summarizes photodegradation data for each formulation after 0, 2, and 12 weeks of artificial sunlight exposure. The average percentage of NDNIF found in the formulations was 1.1%, 1.29% and 1.54% for 0, 2 and 12 weeks respectively. The intraformulation standard deviation of percent NDNIF (n = 6 samples per period) was always < 0.5%. The maximum recorded percentage of NDNIF was 2.64% ± 0.45%, found in the nifedipine 20 mg PA capsule after 12 weeks of continuous irradiation. Interformulation photodegradation differences were not determined since these variations, although possibly statistically significant, are likely clinically insignificant. Rates of nifedipine degradation for individual formulations were not calculated because of the relatively small degree of nifedipine decomposition observed in this study.

Table 4-1. Nifedipine Formulation Photodegradation Data

Formulation	Dosage Form	Strength	Percent NDNIF (w/w initial nifedipine content) 0 Weeks	2 Weeks	12 Weeks
Adalat XL	GITS tablet	60mg	1.45 ± 0.28	N/A	1.18 ± 0.09
Adalat PA	PA tablet	20mg	1.0[a]	1.09 ± 0.08	1.28 ± 0.17
Nifelat 20 retard	PA tablet	20mg	1.0[a]	1.07 ± 0.04	1.77 ± 0.14
Nife-Wolffe 20	PA tablet	20mg	1.0[a]	1.18 ± 0.13	0.81 ± 0.10
Pidilat retard	PA tablet	20mg	1.0[a]	1.0[a]	1.0[a]
Nifehexal retard	PA tablet	20mg	1.0[a]	1.40 ± 0.09	2.04 ± 0.33
Nifical retard	PA tablet	20mg	1.0[a]	1.0[a]	1.0[a]
Nifedipin AL 20	PA capsule	20mg	1.45 ± 0.12	1.81 ± 0.20	2.64 ± 0.45
Apo-Nifed	IR capsule	10mg	1.0[a]	1.20 ± 0.08	1.21 ± 0.09
Novo-Nifedin	IR capsule	10mg	1.0[a]	1.86 ± 0.12	2.51 ± 0.24

N/A: data not available

[a] Insufficient photodegradation product content to accurately report % NDNIF, however values were estimated as approximately 1.0 ± 0.5%.

Results obtained in this analysis indicated that all the tested nifedipine formulations did not undergo appreciable nifedipine decomposition (> 10%) even after 12 weeks of continuous artificial sunlight exposure. In addition, all the nifedipine formulations tested exceeded USP requirements. No clinically significant difference regarding the degree of light protection was found with any of the nifedipine formulations tested in this study.

In conclusion, if bioequivalency differences, therapeutic failures or pharmacodynamic differences of the tested nifedipine formulations are observed, then it is unlikely that a photostability difference would be a primary contributory factor.

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Chapter 5

OBSERVATION OF TIME–DEPENDENT AND VARIABLE SUBJECT KINETICS IN A NIFEDIPINE GASTROINTESTINAL THERAPEUTIC SYSTEM BIOEQUIVALENCY STUDY◆

Introduction

It has long been recognized that many human physiological functions vary according to endogenous circadian rhythms, (e.g., cardiovascular, pulmonary, hepatic, and renal function).[1,2] However, only relatively recently has it been recognized that daily variations in physiological functions can influence drug absorption, distribution, metabolism and elimination — for which the term 'chronopharmacokinetics' has been coined.[1,3] Several classes of drugs have been investigated for potential chronopharmacokinetic properties, including: antiasthmatics, antineoplastics, H_2–antagonists, non–steroidal anti–inflammatory drugs and cardiovascular agents.[1,4-9] Examples of cardiovascular agents found to display time–dependent kinetic properties include: digoxin, enalapril, propranolol, verapamil and nifedipine.[1,5,6,8-11]

The results of a single–dose study using an oral immediate–release (IR) nifedipine formulation[6,8,9] show a higher peak concentration (C_{max}), shorter time–to–peak (t_{max}) and increased bioavailability (AUC) when the formulation is given to human subjects in the morning. Interestingly, however, the results of 1 hour intravenous nifedipine infusions[6] do not display chronopharmacokinetics — suggesting that circadian variations occur primarily with the oral absorption or presystemic metabolism of nifedipine.

The nifedipine gastrointestinal therapeutic system (GITS) is an osmotic delivery device displaying controlled–release properties.[12] When taken perorally this formulation releases nifedipine at a preprogrammed zero–order delivery rate — after an initial 2 hour hydration period — that is independent of gastrointestinal pH and motility and of sufficient duration

◆ A version of this chapter has been accepted for publication:
J.S. Grundy, R.Z. Lewanczuk, D.W. Grace, R.T. Foster. *J. Contr. Rel.* (1996).

to permit once–daily dosing.[12,13] Several literature reports describe the single–dose pharmacokinetics of the nifedipine GITS in human beings[14-17] and, typically, plasma nifedipine concentration–time profiles consistent with zero–order drug absorption are observed. Nevertheless, chronopharmacokinetic properties of nifedipine might be observed following single–dose administration of the GITS formulation by comparison of evening plasma concentrations with those of the following morning (e.g., 12 hours versus 24 hours postdosing, respectively). Indeed, such a phenomenon may be seen in the mean nifedipine plasma concentration–time profiles shown in two previously published papers on the nifedipine GITS.[15,18]

Hence, indications of time–dependent kinetics and the degree of inter– and intrasubject variability were assessed in a bioequivalency study that was conducted with 4 commercially available nifedipine GITS formulations (2 lots of both 30 mg and 60 mg strengths obtained from the same manufacturer) given in single–dose fashion to healthy subjects.

Materials and Methods

Patients

Twelve human subjects (6 of each gender) were recruited for this study (mean age 34.2 ± 7.7 years, mean weight 76.2 ± 15.8 kg, and mean height 168.5 ± 13.4 cm). All were healthy volunteers with no pre–existing medical conditions and were not, at the time of the study, using systemic medications (including OTC preparations) which may have altered the disposition of nifedipine.

Bioequivalency Study Protocol

The protocol on the study days was the same on each occasion. At about 8 AM on each study day, subjects received — according to a randomized 4 x 4 Latin Square cross–over study design — one of the 4 nifedipine GITS formulations tested [two 30 mg lots (labeled A and B); two 60 mg lots (labeled C and D); Adalat® XL, Miles Canada Inc. (Etobicoke, Ontario, Canada)]. A minimum washout period (1 week) was observed between administration of each lot. No dietary restrictions were imposed except that food was withheld from midnight the day before each dosing session until 2 hours after administration.

Blood samples (about 6 ml) were withdrawn from an antecubital vein at 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hours postdosing. A blood sample (labeled 0 hours) was also

collected immediately before dosing. Blood collection was made into 10 ml Vacutainers® containing heparin as an anticoagulant and the Vacutainers® were immediately wrapped in aluminum foil to avoid exposure of the blood samples to light. Blood samples were centrifuged within 10 minutes of collection, and the resulting plasma samples were protected from light and stored frozen at $-18 \pm 3°C$ until needed for analysis.

Drug Analysis and Calculation of Pharmacokinetic Parameters

Plasma concentrations of nifedipine were measured with an established reversed-phase HPLC method (lower limit of quantitation 5 ng mL^{-1}).[19] The maximal plasma concentration (C_{max}, ng mL^{-1}) and time of peak plasma concentration (t_{max}, h) for nifedipine were apparent values observed from the plasma nifedipine concentration--time profiles obtained from each subject with each lot of the nifedipine GITS tested. Although plasma concentrations were generally in decline 36 hours postdosing, the individual terminal first–order elimination rate constants (k_{el}, h^{-1}) were not determined since, typically, only 1 or 2 blood samples decreased in each individual profile. The cumulative area under the plasma nifedipine concentration–time curve to the time of the last quantifiable concentration or the time of last sample taken [AUC(0-t), ng h mL^{-1}] was estimated using a noncompartmental approach (i.e., linear trapezoidal rule).

Statistical Analysis

Typical descriptive analysis [mean, standard deviation (s) or standard error ($s_{\bar{x}}$) and coefficient of variation (CV)] was applied to the data collected in this study. Comparisons of the standard pharmacokinetic measures obtained were carried out using an analysis of variance (ANOVA) suitable for a repeated measures study design in which subject, treatment, and order were accounted for.[20] Values of $P < 0.05$ were taken to indicate statistically significant differences. A post hoc test (Duncan's multiple range test) was used to determine where, if any, differences occurred. The 90% confidence limits of respective C_{max} and AUC(0-t) ratios were calculated on datum transformed into their respective natural logarithms. Intersubject variability (between individuals) for each standard pharmacokinetic measure of interest was calculated as the arithmetic mean ($\pm s$) of the CV's within each of the 4 different nifedipine GITS lots tested. Intrasubject variability (within individuals) in each standard pharmacokinetic measure of interest was calculated as the arithmetic mean ($\pm s$) of

the CV's found between all 4 nifedipine GITS lots tested for each of the 12 subjects. The method used for evaluating goodness of fit for t_{max} (comparing observed versus expected frequencies) was the Chi–Square test.

Results and Discussion

The mean ($\pm s_{\bar{x}}$) plasma nifedipine concentration–time profiles obtained in this study are shown in Figure 5-1. The initial phase of each mean profile is consistent with the required 2 hour lag–time before the start of nifedipine release from the GITS. However, quantifiable nifedipine concentrations were not detected until about 3 to 4 hours postdosing suggesting a delay in drug dissolution or absorption; a phenomenon first described in a previously published study.[14] All 4 mean profiles showed gradually increasing nifedipine concentrations with peaks at 24 hours; thereafter levels declined. Despite the apparent rise in nifedipine concentrations up to 24 hours, no significant differences (ANOVA, $p > 0.05$) were found between the mean nifedipine plasma concentrations at 6, 8, 12 and 24 hours postdosing for each nifedipine GITS lot tested. This was probably due to the small differences detected and the large intersubject variability observed (Table 5-1).

Standard pharmacokinetic measures calculated from the data in this study are shown in Table 5-2 and significant differences, where found, are noted. As expected, no statistically significant differences (ANOVA, $P > 0.05$) were observed between nifedipine GITS formulations of the same strength with respect to C_{max} and AUC(0-t). Furthermore, it was noted that both C_{max} and AUC(0-t) values of the 60 mg dosage forms were about double those of the 30 mg strengths, suggesting a linear dose versus concentration relationship. However, the AUC(0-t) values obtained in this study must be evaluated with caution since in many of the subjects the plasma nifedipine concentrations had not declined appreciably even by 36 hours; thus, terminal rate constants and total AUC could not be determined.

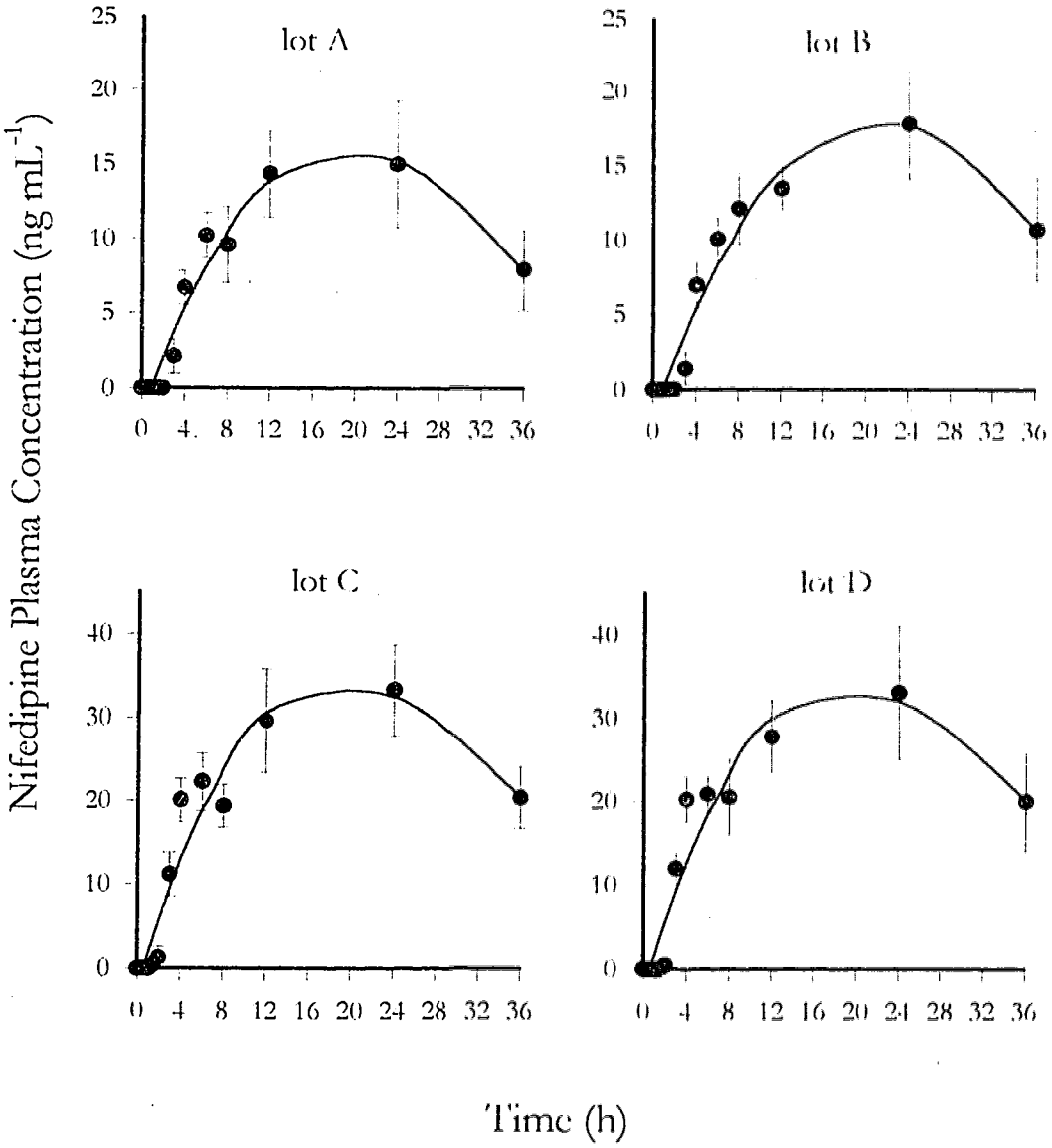

Figure 5-1. Plasma nifedipine concentration–time profiles obtained after single-dose administration of 4 commercially available nifedipine GITS formulations [2 lots of both 30 mg (lots A and B) and 60 mg (lots C and D) strengths obtained from the same manufacturer] to healthy volunteers. Data are presented as arithmetic mean values ± $s_{\bar{x}}$ (n = 12).

Table 5-1. Inter– and Intrasubject Variability of the Observed Standard Pharmacokinetic Measures Expressed as Coefficient of Variation (CV).[a]

Pharmacokinetic Measures	Intersubject Variability (CV, %)	Intrasubject Variability (CV, %)
C_{max}	57 ± 11	41 ± 16
t_{max}	47 ± 3	30 ± 23
AUC(0-t)	58 ± 8	44 ± 19

[a] Data are expressed as aritlmetic means ± *s*.

Table 5-2. Standard Pharmacokinetic Measures Describing the Absorption and Disposition of Nifedipine from 4 Different Lots of the Nifedipine Gastrointestinal Therapeutic System (n = 12 study participants).[a]

Pharmacokinetic Measures	*Nifedipine GITS* lot A (30 mg)		lot B (30 mg)		lot C (60 mg)		lot D (60 mg)	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
C_{max} (ng mL^{-1})	19.8[b]	68.4	22.3[b,c]	46.1	36.9[c]	48.0	40.9[c]	64.7
t_{max} (h)	16.5[d]	49.6	22.3[d]	47.4	20.8[d]	43.2	18.3[d]	48.8
AUC(0-t) (ng h mL^{-1})	418.9[e]	69.2	468.2[e]	50.5	914.7[f]	54.5	877.3[f]	57.3

[a] Data are expressed as aritlmetic means and the corresponding coefficient of variation (CV, n = 12).
[b-f] Identical superscript characters identify non–significant differences ($P > 0.05$).

The latter observation is interesting since in vitro dissolution tests conducted with the nifedipine GITS indicate that nifedipine release rates rapidly decline 18 to 20 hours after hydration (90% of the dose is released by 24 hours),[13,21] suggesting that in vivo drug absorption should not continue much beyond 24 hours. However, our results appear to show that the in vivo duration of drug absorption is longer than that predicted using traditional in vitro tests, perhaps because dissolution of the nifedipine suspension released from the GITS may be a rate–limiting factor for drug absorption, particularly in the lower gastrointestinal tract — note: in vitro dissolution tests conducted to date with the nifedipine GITS (modified USP, differential (ALZA) and flow–through methods[13,21]) solely measure the rate of release of the finely divided drug suspension.

No statistically significant differences in the measurement of the apparent t_{max} were observed between any of the 4 products tested (Table 5-2). Interestingly, t_{max} was observed at 24 hours in most of the individual profiles (56%, Table 5-3) although it should be noted that no other measurements were taken between 12 hours and 24 hours, which may somewhat limit data interpretation.

A Chi–Square test for goodness of fit was used to compare the expected frequencies of finding t_{max} at particular time points within the plateau phase (i.e., similar frequencies would be expected) with the observed frequencies, and showed a statistically significant difference ($\chi^2 = 31.16$, $P < 0.001$, Table 5-3). A similar finding (results not shown) was also obtained using other measures of goodness of fit including the Log–Likelihood Ratio, Kolmogorov–Smirnov Goodness of Fit for Discrete Data, and Friedman's Two Way ANOVA by Ranks for Dependent or Correlated Samples.[22]

Table 5-3. Chi–Square Test For Goodness Of Fit (n = 48).[a] Comparison of the Observed Frequency of t_{max} Values with the Expected Frequency at Sampling Times within the Nifedipine GITS Plateau Interval.

	Sampling Time			
	6 hours	**8 hours**	**12 hours**	**24 hours**
Observed Frequency (f_o)	4	3	14	27
[% of total]	[8.3]	[6.3]	[29.2]	[56.3]
Expected Frequency (f_e)	12	12	12	12
[% of total]	[25]	[25]	[25]	[25]

[a]Calculated $\chi^2 = 31.16$ ($P < 0.001$).

Analysis of the results by gender was not possible due to the relatively small number of subjects in each group; however, apparent gender differences in the data were not observed. No significant differences with respect to the administration sequence were also found, although statistically significant intersubject differences were noted ($P < 0.05$). Inter– and intrasubject variability's were determined for each standard pharmacokinetic measure of interest and are shown in Table 5-1.

For the purpose of evaluating bioequivalence of the equipotent nifedipine GITS lots in this study, lots A and C were designated reference compounds for the 30 mg and 60 mg strengths, respectively. The geometric mean ratios of C_{max} and AUC (including their respective 90% confidence intervals) are typically required to be between 80% to 125% to demonstrate bioequivalence. For both strengths the 90% confidence limits of C_{max} and AUC(0-t) geometric ratios fell outside the bioequivalence range (Table 5-4). The C_{max} ratios for both strengths and the AUC(0-t) ratio for the 60 mg strength were, however, between this range; but, the AUC(0-t) ratio for the 30 mg strength was outside the bioequivalence range (Table 5-4). A relatively small number of subjects participated in this study; nevertheless, considering that the nifedipine GITS products tested came from the same manufacturer (Bayer Canada Inc.), these results and the large inter– and intrasubject variability found (Table 5-1) may have considerable ramifications for those attempting to develop a product that is bioequivalent to Adalat® XL. Especially given existing regulatory requirements.

Table 5-4. Calculation of Geometric Mean Ratios for Comparison with Standards for Bioequivalence

Pharmacokinetic Measures	*Geometric Mean*				*Ratio (%)*[a] *[90 % Confidence Limit]*	
	lot A	lot B	lot C	lot D	lot B/lot A	lot D/lot C
C_{max} (ng mL^{-1})	16.2	20.0	33.5	35.5	123.6 [94.3-161.9]	106.1 [81.0-139.0]
AUC(0-t) (ng h mL^{-1})	305.8	412.9	805.3	775.2	135.0 [91.5-199.2]	96.3 [65.3-142.0]

[a] Lot A was used as the 30 mg reference standard; Lot C was used as the 60 mg reference standard

Several published papers or abstracts have characterized the single–dose pharmacokinetics of the nifedipine GITS.[14-17,23,24] The first paper was authored in 1987 by Chung et al.[14] In this study — involving healthy volunteers given single 30 mg or 60 mg doses of the nifedipine

GITS — the mean plasma nifedipine concentration–time profiles show a relatively stable 'plateau phase' between 6 and 24 hours, demonstrating zero–order release and absorption characteristics for the GITS formulations tested. Schneider et al.[16] show similar findings with a 60 mg nifedipine GITS tablet administered to normal and renally impaired subjects. However, Crome et al.[15]— comparing single–dose pharmacokinetics of nifedipine GITS (60 mg) given to 23 young and 24 elderly volunteers — show mean plasma nifedipine profiles exhibiting an apparent t_{max} of 24 hours in both groups.

Surprisingly, the authors of the latter report did not specifically comment on the results showing 24 hours as the apparent t_{max}, perhaps because the mean peak plasma nifedipine concentrations at 24 hours could be attributed to assay or intersubject variability. However, our group had also observed a similar trend in two studies involving administration of single–doses of the nifedipine GITS (30 mg) to young or elderly volunteers (see Figure 5-2).[23,24] Similarly, in a paper by Melia et al.[18] the mean nifedipine concentration–time profiles, obtained from 17 healthy volunteers receiving 60 mg nifedipine GITS tablets coadministered with placebo or orlistat (a lipase inhibitor), show the same trend in both cases. As well, the results of the current study (Figure 5-1) provide further support for this phenomenon.

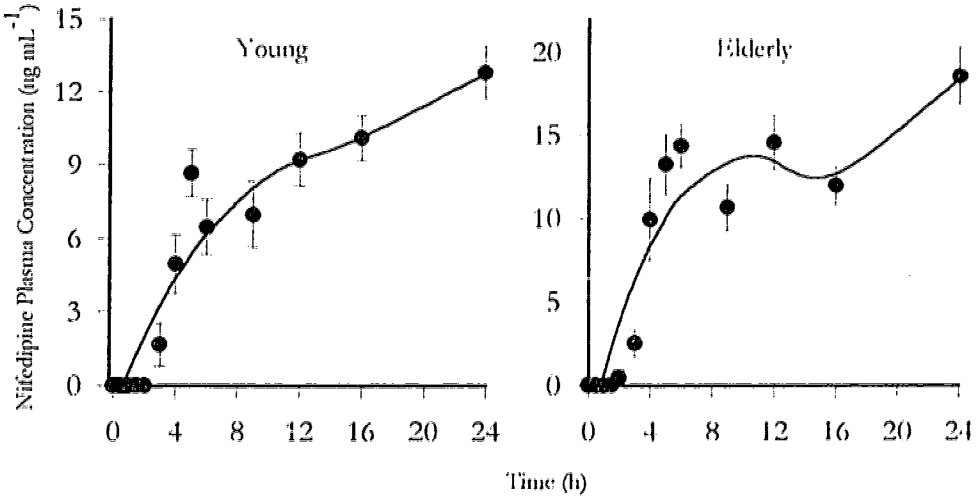

Figure 5-2 Plasma nifedipine concentration–time profiles obtained after single–dose administration of a commercially available nifedipine GITS formulation (30 mg) to young healthy males subjects, and elderly healthy male or female subjects [from data presented, in part, in 2 published abstracts[23,24]]. Data are arithmetic mean values ± $s_{\bar{x}}$ (n = 18).

This phenomenon is particularly interesting given that a concern with GITS, OROS and other single unit dosage forms designed for once daily administration is the possibility of incomplete drug release and absorption, as mouth–to–anus transit can be less than 24 hours in some subjects. For example, total GI transit times range from 6 to 32 hours (mean 24.7 hours) in a study with 6 fasted healthy male volunteers administered an oxprenolol OROS formulation.[25] Another study — investigating GI transit times of single–unit tablets in 10 ileostomy subjects — reports variable transit times between subjects ranging from 5 to 40 hours.[26] Thus, a reasonable expectation is a tendency for the mean nifedipine plasma concentrations to be below peak values 24 hours after administration of a single GITS tablet — since some individuals may have already passed the formulation into their stools, and because nifedipine has a relatively short intrinsic half–life (1 to 2 hours[27-29]).

Chronopharmacokinetic properties have been reported after dosing with IR nifedipine capsules,[1,5-9] and some of the observations in this study might be explained as a chronopharmacokinetic phenomenon. Lemmer et al.[6,8,9] showed that both C_{max} and AUC decrease (about 44% and 35%, respectively) after IR nifedipine dosing in the evening compared to the morning. Similarly, for each of the nifedipine GITS lots tested here, peak plasma levels were produced at 24 hours corresponding to 8 AM the day after dosing. However, because no other blood samples were taken between 12 and 24 hours, interpretation of the data as a chronopharmacokinetic phenomenon could be potentially misleading. Nonetheless, in other studies showing a similar effect and in which 16 or 20 hour samples were taken[15,23,24] it can be seen that 24 hour samples typically give the highest drug concentrations (also see Figure 5-2). However, data obtained from administration of the nifedipine GITS in the evening would be helpful in determining if time–dependent kinetics are indeed a factor but, to our knowledge, a study of this type has not been published to date.

It is worth noting, that in a group of hypertensive patients a sustained–release formulation of nifedipine did not display significant daily variation in its pharmacokinetic profile.[6,8,9] This has led to speculation that the galenic formulation of a drug may influence temporal variations in pharmacokinetics.[1,30] However, to date, no explanation for the potential mechanism(s) of this effect has been proposed.

Temporal physiological changes can be involved at each of the phases describing a drugs pharmacokinetic profile — i.e., absorption, distribution, metabolism and elimination.[1,2] The mechanism(s) considered responsible for temporal variations in IR nifedipine

pharmacokinetics include changes in absorption or presystemic metabolism.[1] The available evidence for these potential mechanisms includes the fact that no changes occur in the AUC ratio of nifedipine and its nitropyridine metabolite, as well as the absence of chronopharmacokinetics when short iv nifedipine infusions are given at different times of the day.[6,8,9]

Factors potentially affecting drug absorption (assuming passive diffusion) include but are not limited to: the drugs physico–chemical properties, the nature of the drug formulation, gastric emptying time, pH, and GI motility and blood flow. However, nifedipine has been shown to be well absorbed from both the upper and lower GI tract (>90%).[27,31,33] Thus, in our opinion, it is unlikely that a reduction in the extent of absorption is responsible for the temporal changes in drug bioavailability observed with IR nifedipine, or for the observations made with nifedipine GITS here. Nonetheless, changes in GI blood flow might be a factor in explaining the increase in nifedipine plasma concentrations found at 24 hours.

Temporal variations in drug metabolism have also been reported.[30] Nifedipine is metabolized mainly by the liver, although gut wall metabolism (small intestine) may contribute to the presystemic metabolism of this drug ($0.40 \leq F \leq 0.68$).[28,29,31,34-36] Temporal changes are reported in GI and hepatic blood flow (human studies) or enzyme activity (animal studies),[30] and could have potentially affected nifedipine bioavailability from the GITS.

An alternative mechanism to chronopharmacokinetics is that the data in this study may indicate a change in presystemic metabolism related to the location of the GITS tablet in the GI tract. As previously stated, presystemic metabolism of nifedipine within the wall of the small intestine but not the colon has been hypothesized,[31,36] which could lead to detectable differences in nifedipine bioavailability along the GI tract. However, the position of the GITS tablet would be expected to be somewhere in the colon within only a few hours after dosing, particularly in fasted individuals.[25,37,38] Another possible explanation could be increased nifedipine bioavailability following entry of the GITS dosage form into the rectum. Improved bioavailability of some drugs given by the rectal route has been demonstrated due to absorption directly into the vena cava via the lower (inferior and middle) hemorrhoidal veins[3]— i.e., bypassing hepatic 'first-pass' metabolism. A pharmacokinetic and pharmacodynamic study with a nifedipine osmotic system administered rectally shows that nifedipine is readily released and absorbed in this region.[33] The arrival and rectal residency

times of orally administered nifedipine GITS tablets would be expected to have high inter– and intrasubject variability, but this could also account for some of the variability seen in this study.

Conclusion

Similar standard pharmacokinetic measures were found between equipotent lots of the nifedipine GITS; however, typical standards of bioequivalence were not met because of large inter– and intrasubject variability that is only partially explained by the relatively small number of study participants. Hence, the latter finding could indicate that potential difficulties may be encountered by those attempting to develop bioequivalent products which comply with typical bioequivalence guidelines.

The observation of t_{max} at 24 hours in the majority of the individual nifedipine plasma concentration–time profiles in this study suggests that nifedipine in a GITS formulation displays chronopharmacokinetic properties (as had been reported earlier with an IR nifedipine formulation). However, comparison of the results from single–doses of the nifedipine GITS given in the morning versus the evening would help to further confirm our suspicions. Alternatively, the results in this current study could suggest that the location of the GITS tablet in the GI tract affects nifedipine bioavailability.

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Chapter 6

STUDIES ON DISSOLUTION TESTING OF THE NIFEDIPINE GASTROINTESTINAL THERAPEUTIC SYSTEM. I. DESCRIPTION OF A TWO–PHASE IN VITRO DISSOLUTION TEST◆

Introduction

The testing of pharmaceutical dosage forms for in vitro drug release and dissolution characteristics is very important for ensuring batch–to–batch quality control and to optimize formulations during product development.[1] The ideal system for evaluating the drug release characteristics of poorly water–soluble drug formulations should be relatively simple and inexpensive, and should mirror the physiological environment found in the human gastrointestinal (GI) tract (i.e., dissolution conditions should attempt to duplicate, as closely as possible, the composition, temperature and pH of the intestinal contents, as well as mimicking mixing of intestinal contents). Another ideal characteristic of dissolution systems for testing drug formulations is to maintain 'sink conditions', i.e., the maximum concentration expected in an experiment should not exceed 15% of the solubility of the analyte in the dissolution medium used,[2] which can be a problem with poorly water–soluble drugs, i.e., nifedipine (≤ 10 μg mL^{-1}).[3-5] These factors are particularly important in dissolution testing of controlled–release drug formulations such as the nifedipine gastrointestinal therapeutic system (GITS), even though the mode of drug release from the GITS is considered to be independent of the physiological environment.[4,6]

Maintaining 'sink conditions' throughout the testing of poorly water–soluble drugs can be difficult in single–phase dissolution systems. Techniques used to resolve this problem include: (1) using relatively large volumes of dissolution medium or frequently

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replacing the medium (e.g., the differential (ALZA) method),[6] (2) altering the pH of the dissolution medium, (3) adding co–solvents to the medium (e.g., different alcohols, propylene glycol, glycerin, polyethylene glycol, sorbitol), and (4) adding nonionic, cationic or anionic surfactants to the medium to enhance analyte solubility.[2] However, these approaches frequently give non–physiologic dissolution environments.[1] Flow–through dissolution systems (e.g., USP XXII apparatus 4) can be used to overcome solubility limitations; but, the dissolution medium flow rates used may not be physiologically relevant. As well, for all these tests potentially rate–limiting dissolution of the suspended drug particles may be masked. Hence, in vitro results obtained from a single–phase dissolution system may not reflect in vivo drug release and dissolution characteristics of formulations of poorly water–soluble drugs.

The nifedipine gastrointestinal therapeutic system (GITS) is administered perorally and uses a push–pull osmotic process designed to deliver a finely–divided nifedipine suspension — in a zero–order fashion — within the GI tract over a period of time long enough to permit once daily dosing.[4,6]. Dissolution tests which have typically been used for this formulation include: a modified classical dissolution test (USP XXII apparatus 2),[6] a flow–through method[1] and a differential (ALZA) method.[6] All of these methods adequately characterize the in vitro release rate of suspended nifedipine particles from different strengths of nifedipine GITS; however, the results do not appear to correlate satisfactorily with in vivo drug absorption rates.

Qureshi et al.[1] state that compared to classical dissolution systems a "flow–through system more closely resembles the physiological environment of the gastrointestinal tract, with continuous extraction (removal) of drug from the dissolution vessel mimicking absorption into the systemic circulation." However, the authors fail to consider that for a poorly water–soluble drug the dissolution of a particle suspension, following tablet disintegration or release from a non–disintegrating formulation (e.g., the nifedipine GITS), can be a factor affecting the absorption of a drug. In fact, in the original pharmacokinetic study of the nifedipine GITS[7] it is speculated that the longer lag–time noted before the start of the in vivo zero–order absorption phase versus the in vitro zero–order release phase is probably due to a delay in the dissolution of drug in the GI tract, as well as transport of the drug out of the gut lumen, across the gut wall and to the systemic circulation.

This paper reports on the applicability of using a two–phase dissolution system — consisting of simulated intestinal fluid, USP, without pancreatin (SIF*) and a n–octanol phase — to determine the release and dissolution profile of nifedipine from two different strengths of the nifedipine GTTS.

Materials and Methods

Materials and Chemicals

The drug products tested were commercially available and obtained from a local pharmacy. The formulations evaluated were 30 mg and 60 mg strengths of the nifedipine GTTS (Adalat® XL, Bayer Canada Inc., Etobicoke, ON, Canada).

Nifedipine powder was purchased from Sigma Chemical Company (St. Louis, MO, USA), a methanolic solution of which (100 μg mL^{-1}) was used as the reference standard for generating all the calibration curves in the study. Nisoldipine (Bayer Canada Inc., Etobicoke, ON, Canada) dissolved in methanol (100 μg mL^{-1}) was used as the internal standard in the HPLC assay of nifedipine. n-octanol was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were either reagent or HPLC grade. SIF* was prepared with 6.8 g KH_2PO_4, 190 mL 0.2N NaOH in water (double distilled) to make 1000 mL and adjusted to pH 7.4 ± 0.1 with 0.2N NaOH solution.

Two-Phase Dissolution Test

The two–phase dissolution test (Figure 6-1) was a modification of USP XXII apparatus 2 (Erweka® DT6, Erweka® GmbH, Heusenstamm, Germany). A single stainless steel cylindrical basket (40 x 40 mesh) was attached near the base of the stainless steel paddle using two thin wire metal straps, to form a basket–paddle hybrid stirrer (Figure 6-1). The dissolution medium consisted of 750 mL SIF* (lower phase) and 250 mL n–octanol (upper phase). The n–octanol was pre–equilibrated with SIF* at 37 °C (to ensure that the upper phase volume did not change during the experiment). A temperature of 37 ± 0.5 °C was maintained throughout and the basket–paddle hybrid stirrer was rotated at 100 ± 4 rpm. During dissolution and sample collection, dimmed laboratory lighting and an opaque blanket which completely covered the apparatus were used to prevent nifedipine photodegradation.[8]

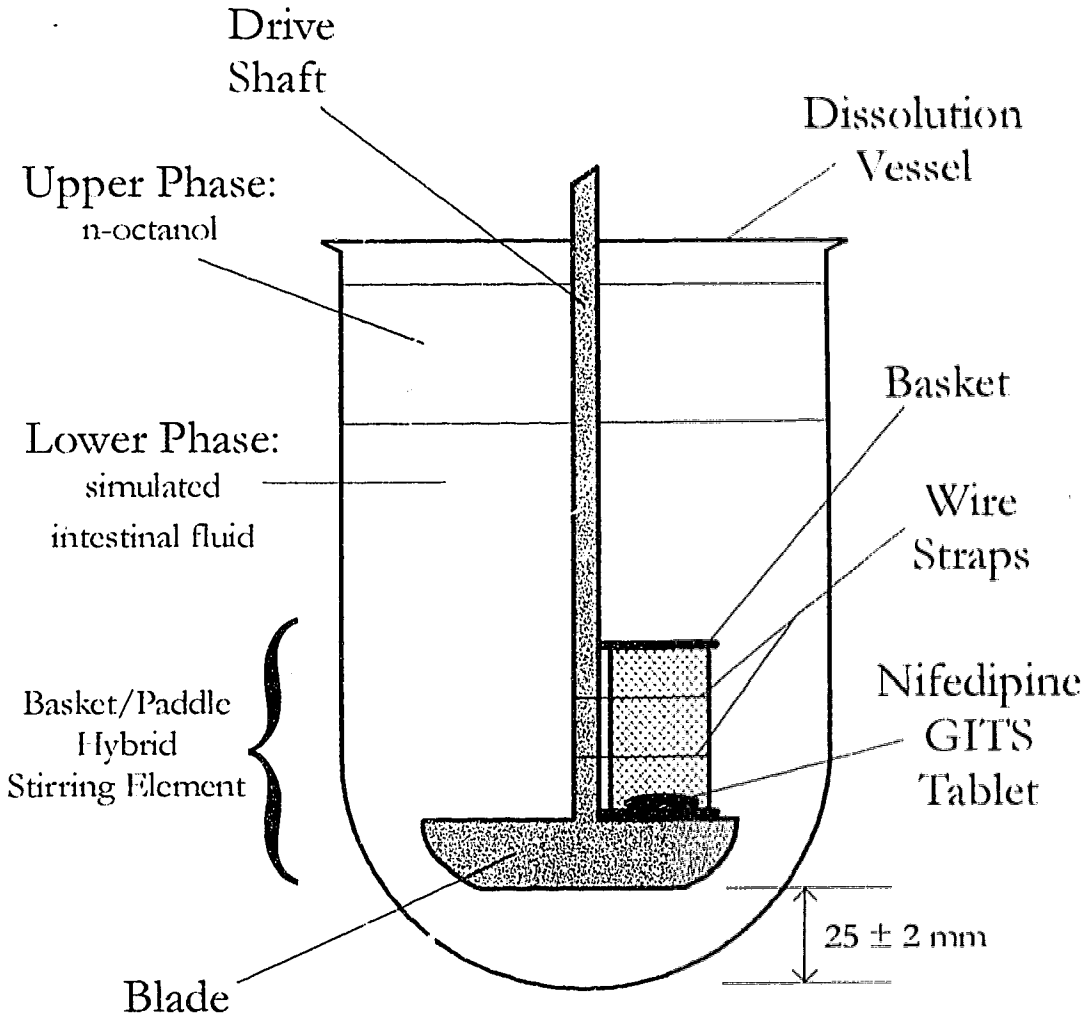

Figure 6-1. A schematic diagram of the basket-paddle hybrid stirrer formed from a stainless steel paddle (comprised of the blade and drive shaft, USP XXII apparatus 2) and a stainless steel cylindrical basket (40 x 40 mesh, USP XXII apparatus 1), the latter holding a single nifedipine GITS tablet, placed in a dissolution vessel containing two immiscible phases (n-octanol, 250 mL; simulated intestinal fluid, USP, without pancreatin, 750 mL).

Method

Six nifedipine GITS tablets of each strength were tested for 30 hours. Individual nifedipine GITS tablets were placed into the baskets attached to the paddle and lowered into the aqueous SIF* in the glass dissolution vessels. The SIF* saturated n-octanol was then slowly layered on the aqueous phase (being careful to prevent n-octanol contact with the test formulation) and stirring was begun at the specified rotation speed. A 2 mL aliquot of the n-octanol phase was removed at 2 hour intervals and each was replaced with 2 mL of n-

octanol phase. Each aliquot was placed in a clean, glass scintillation vial, protected from light by wrapping in aluminum foil, and stored at room temperature until assayed (no longer than 2 to 3 days). A 5 ml aliquot of the aqueous SIF[c] phase was removed after 30 hours and similarly stored at room temperature (about 1 week) until assayed. After 30 hours the used tablets were each crushed and the remaining contents were dissolved in 100 ml of methanol, wrapped in aluminum foil and stored at room temperature until assayed (within a week).

Ultraviolet Analysis

The absorbances of appropriate dilutions of the n–octanol phase samples were measured in a 1 cm cell at 235 nm using a double–beam spectrophotometer (Beckman Model 25 Spectrophotometer, Beckman Instruments Inc., Irvine, CA, USA). SIF[c] equilibrated n–octanol was used as the blank, and the absorbance of each sample was converted to concentrations using a calibration curve (0.5 to 20 μg mL^{-1}, $r^2 > 0.999$) obtained from appropriate dilutions of a stock solution (10 mg nifedipine in 100 mL of SIF[c] equilibrated n–octanol).

High-Performance Liquid Chromatographic Method

Nifedipine concentrations of samples prepared from methanolic solutions of the nifedipine GITS tablet residuals (0.1 mL samples diluted to 1.0 mL with HPLC grade water), and the 30 hour aqueous phase aliquots (1 mL samples) were determined by an established HPLC method (lower limit of quantitation 5 ng mL^{-1}).[9] Calibration curve standards were prepared by adding a known amount of the drug to 1.0 mL of HPLC–grade water. A calibration curve (50 to 10,000 ng of nifedipine) was determined from the best–fit regression line calculated using a $1/x$ weighting factor (where x corresponds to the amount of nifedipine added; $r^2 > 0.999$). HPLC instrumentation included: a Model 600E solvent delivery system, a Model 717 autosampler; a Model 486 tunable UV–VIS absorbance detector (set to 350 nm); and an NEC 486–33 MHz computer running Millennium 2010 chromatography manager software Version 1.1 (Waters, Mississauga, ON, Canada).

Results and Discussion

Both the 30 mg and 60 mg strengths of the nifedipine GITS contain, in addition to the labeled dose, a 10% overage of drug which is not released from the dosage form,[4,6] i.e., the tablet formulations contain 33 mg and 66 mg nifedipine, respectively. The total recovery of

nifedipine from each of the GITS tablets tested using the two-phase dissolution system are shown in Table 6-1, and are in excellent agreement with the labeled quantities including the 10% overage (mean recovery was about 97%). Hence, no significant loss or degradation of nifedipine occurred during the collection, storage and analysis of the samples. For the nifedipine GITS 30 mg and 60 mg formulations tested, on average about 85% and 93% of the 'releasable' nifedipine dose, respectively, was 'transferred' to the n-octanol phase within 30 hours (Table 6-1 and Figure 6-2). The 'releasable' dose was calculated from the total amount of nifedipine recovered (last column of Table 6-1) multiplied by 0.9 (i.e., analogous to the total dose less the 10% overage amount). Nifedipine 'transfer' is used in this and the next chapter to describe a process dependent on release of the drug suspension from the GITS tablet, dissolution of the particles in the aqueous phase and drug partitioning in the organic phase.

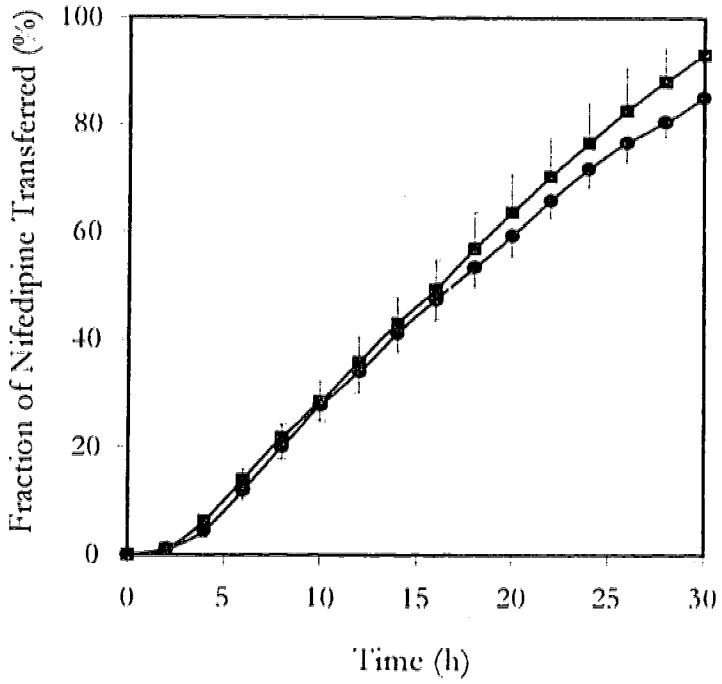

Figure 6-2. Comparison of in vitro mean nifedipine transfer rate-time profiles using a two-phase dissolution system for nifedipine GITS 30 mg (●) and 60 mg (■) tablets. Means ± *s* (n = 6).

Table 6-1. Total Recovery of Nifedipine from each Nifedipine GITS Tablet at 30 hours.[a]

Nifedipine GITS Formulation	Amount of Nifedipine Transferred[b] (mg) [% of releasable dose][c]	Amount of Nifedipine Remaining in the Tablet[d] (mg)	Amount of Nifedipine in the Aqueous Phase[e] (mg)	Total Recovery (mg) [% of theoretical][f]
30 mg Strength				
Tab 1	25.90 [88.8]	6.13	0.37	32.40 [98.2]
Tab 2	24.87 [84.6]	7.51	0.30	32.68 [99.0]
Tab 3	24.07 [84.6]	7.23	0.31	31.61 [95.8]
Tab 4	24.34 [84.2]	7.34	0.43	32.11 [97.3]
Tab 5	23.24 [82.4]	7.72	0.38	31.34 [95.0]
Tab 6	25.40 [86.4]	6.92	0.36	32.68 [99.0]
mean ± s =	24.64 ± 0.96 [85.2 ± 2.2]	7.14 ± 0.56	0.36 ± 0.05	32.14 ± 0.56 [97.4 ± 1.7]
60 mg Strength				
Tab 1	52.46 [93.4]	8.93	1.00	62.39 [94.5]
Tab 2	53.87 [97.3]	6.88	0.786	61.54 [93.2]
Tab 3	50.45 [85.5]	14.59	0.54	65.58 [99.4]
Tab 4	53.46 [89.2]	11.78	1.38	66.62 [100.9]
Tab 5	53.51 [93.8]	8.85	1.05	63.41 [96.1]
Tab 6	57.43 [100]	5.03	1.02	63.48 [96.2]
mean ± s =	53.53 ± 2.28 [93.2 ± 5.3]	9.34 ± 3.42	0.96 ± 0.28	63.84 ± 1.92 [96.7 ± 2.9]

[a] Mean data are presented as arithmetic means ± standard deviation (s).

[b] Amount of nifedipine transferred from the aqueous phase to the organic phase in 30 hours.

[c] Calculated from the amount of nifedipine transferred divided by 90% of the total amount of nifedipine recovered (see text).

[d] Amount of nifedipine remaining or unreleased from the nifedipine GITS tablet by 30 hours.

[e] Amount of nifedipine in the aqueous phase at 30 hours.

[f] Based on the labeled quantity of nifedipine plus 10% overage as claimed by the manufacturer.

The mean rates of nifedipine transferred per unit time are shown in Figure 6-3 for both nifedipine GITS 30 mg and 60 mg tablets. The mean maximum drug transfer rate for the 60 mg dose (2.23 mg h^{-1}) was found to be about twice that of the 30 mg dose (1.16 mg h^{-1}), which was expected given the design parameters programmed for each form of the nifedipine GITS.[6] However, these rates are considerably less than the average zero–order delivery rate values reported by Swanson et al.,[6] which correspond to the manufacturers formulation design parameters: 3.4 and 1.7 mg h^{-1} for the 60 mg and 30 mg nifedipine GITS tablets, respectively. These investigators used both differential (ALZA) and modified USP XXII dissolution methods, which measure the total amount of nifedipine released from the tablet but do not distinguish between the amount of nifedipine in suspension and in solution.

Hence, any differences found between the two–phase method and the single–phase methods could be attributed to a lag in nifedipine particle dissolution. This could also account for the differences seen in the apparent duration of drug release and drug transfer. For example, single–phase methods indicate that drug release (zero– and then first–order delivery) is essentially complete within 24 to 30 hours (mean cumulative amount released ≥ 90% in 24 hours); whereas, the two–phase system showed that drug transfer was incomplete even after 30 hours (mean cumulative amount transferred was only about 72% to 77% in 24 hours). In addition, a delay in dissolution of the nifedipine suspension would explain the finding by Chung et al.[7] that the time–lag before the start of in vivo zero–order absorption is somewhat longer than that reported before the start of in vitro zero–order release (i.e., results from single-phase methods) for the nifedipine GITS.

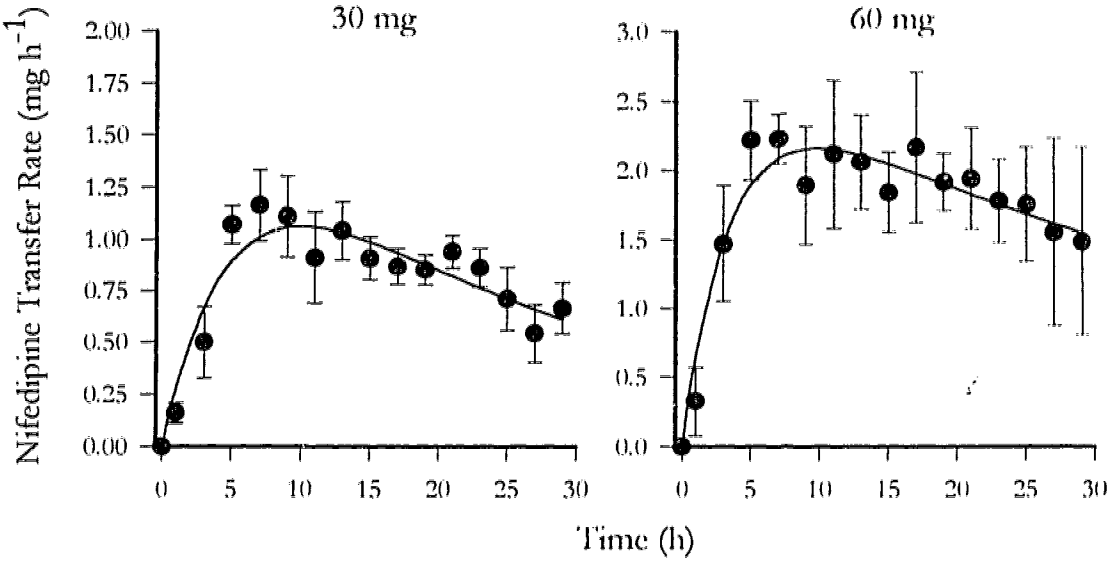

Figure 6-3. Comparison of in vitro mean fraction of nifedipine transferred–time profiles for nifedipine GITS 30 mg and 60 mg tablets using a two-phase dissolution system. Means ± *s* (n = 6).

Prior to developing the two–phase dissolution test, a modified USP XXII dissolution test (a single–phase method, apparatus 1) was done with the same nifedipine GITS products to compare the results with published reports. Unpublished results from this study (Figure 6-4) corroborated results reported by Swanson et al.[6] and Qureshi et al.[1] regarding the duration of nifedipine release from the nifedipine GITS. Briefly, the method used involved moving each nifedipine GITS tablet (placed in a stainless steel 40 x 40 mesh basket) at 2 hour intervals to a

clean dissolution vessel filled with 'fresh' SIF over a period of 30 hours in order that nifedipine concentrations did not exceed the limit of solubility, although sink conditions were not maintained. Aliquots of the dissolution media were collected at the end of each 2 hour interval and then assayed for nifedipine content using an HPLC method.[9] Unfortunately, problems were encountered obtaining mass balance, since, on average, only about 86% of the total amount contained in the tablets could be accounted for, the deficiency possibly due to drug adsorption as a result of multiple transfers between dissolution vessels. This may account, in part, for the lower mean release rates found (Figure 6-4) compared to the manufacturers design parameters.[6] Nonetheless, the shape of the release rate profiles in Figure 6-4 illustrate that drug release (zero– and then first–order delivery) was essentially complete after 25 to 30 hours for the 30 mg and 60 mg nifedipine GITS tablets (mean cumulative amount released ≥ 90% in 24 hours).

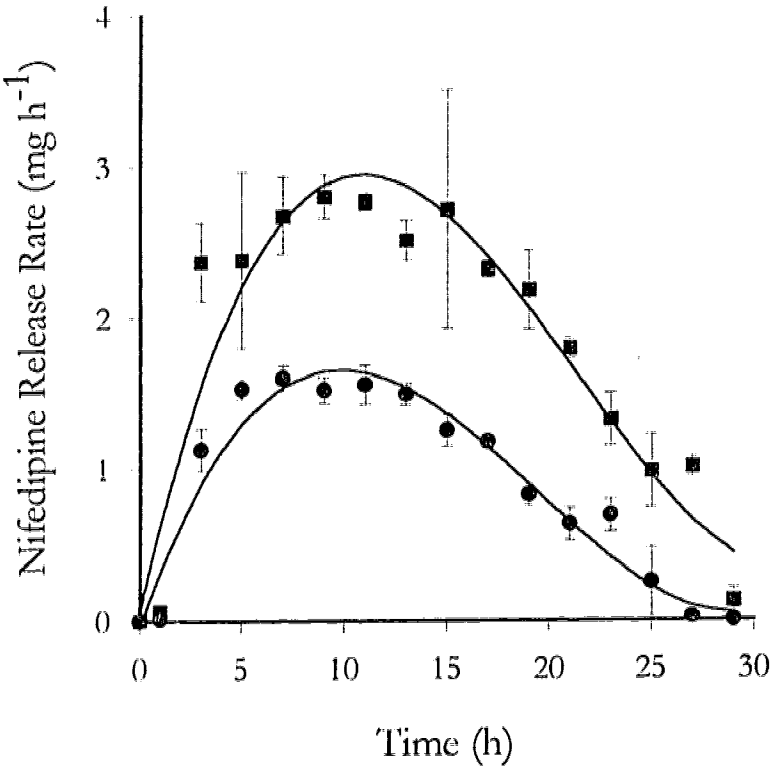

Figure 6-4. Previously unreported observations comparing the in vitro mean nifedipine release rate–time profiles for nifedipine GITS 30 mg (●) and 60 mg (■) tablets using a modified USP XXII method apparatus 1. Means ± *s* (n = 3).

A two–phase dissolution system possesses two major advantages over single–phase systems. First, 'sink conditions' are maintained at all times (assuming drug partitioning is faster than drug dissolution). Second, the transfer of drug between the two phases mimics the entire process of drug release and dissolution within the GI tract, and drug absorption from the GI tract (assuming that drug partitions only when in the dissolved state).

Interestingly, a similar two–phase dissolution method (described as an acidic biphasic system) to that reported here was used for testing 2 different nifedipine sustained release formulations.[10] The method is a modification of USP XXII dissolution apparatus 2 and is carried out with a two–phase system consisting of simulated gastric fluid (500 mL) and n–octanol (400 mL). An additional blade is attached to the same shaft of the paddle to stir the interface of the two liquids. Results obtained using this method are reproducible and, as inferred by the authors, correlate well with data from a flow–through apparatus.[10] However, the authors experienced difficulties correlating the dissolution (transfer) profiles of the two products tested with the specifications provided by Bayer (Germany) for the reference product Adalat® SR (i.e., 40 to 70 % dissolution in 2 hours, minimum 65% in 6 hours[10]). Unfortunately, Adalat® SR itself was not tested and in vitro–in vivo correlation studies were not attempted.

Another two–phase system, described as an automatic system for the determination of dissolution of slightly soluble drug substances, has also been reported and successfully used for testing both nifedipine and nimodipine dissolution.[11] In this method, aqueous dissolution medium is pumped through a flow–through cell (of variable design) in which a drug becomes partially dissolved. In a separate extraction compartment, a chloroform phase is used to extract the dissolved drug and the two phases are subsequently separated. The chloroform serves as both a sink for a slightly soluble drug substance and is used for spectrophotometric determination of the compound of interest.

The choice of n–octanol — a solvent often used to calculate the partition coefficients of drugs — as the organic phase in the two–phase system described in this report was based on its desirable physical–chemical properties, including: (1) n–octanol is practically insoluble in water (0.05 g/100 g H_2O);[12] (2) n–octanol is less dense than water (specific gravity 0.825 at 20 °C),[12] permitting ease of sampling; (3) n–octanol possesses low volatility (b.p. = 195 °C),[12] hence n–octanol will not readily evaporate at 37 °C and thus a relatively constant upper phase volume can be maintained; and (4) nifedipine possesses a high n–octanol:water distribution

coefficient of about 10,000.[3,10] Substituting chloroform for n–octanol was also attempted (results not reported), however this solvent possesses less desirable physical–chemical properties [i.e., greater water solubility (0.742 g/100 g H_2O), and it is more dense than water (specific gravity 1.484)][13] and ultimately gave unsatisfactory results.

A basket–paddle hybrid stirrer was used in this study (Figure 6-1) primarily because it was observed that a paddle stirrer (USP XXII apparatus 2) did not displace, to our satisfaction, the nifedipine GITS tablet from its resting position at the bottom of the dissolution vessel. This caused concern that imbibition of water by the tablet may be inhibited (i.e., due to a reduction in exposed surface area of the tablet), or could lead to occlusion of the precision laser drilled hole through which the nifedipine suspension is released,[4,6,14] or that some of the released suspension could be entrapped beneath the tablet. It should be noted, however, that a separate experiment using the paddle stirrer alone was not conducted, thus the results of both methods could not be compared. On the assumption that a hybrid system of the type described here is required and the method was adopted routinely for the nifedipine GITS, a one–piece customized unit could be designed and developed.

Likewise, using the basket method (USP XXII apparatus 1) did not provide enough mixing at the interface between the organic and aqueous phases, or prevent the nifedipine suspension released from falling to the bottom of the dissolution vessel. On the other hand, the hybrid stirrer used in the final method enabled streamlined hydrodynamic flow of dissolution medium adjacent to all sides of the tablet, adequate stirring of the two phases, and the degree of mixing obtained did not allow nifedipine particles to settle at the bottom of the dissolution vessel. At the same time, the integrity of the planar oil/water interface was not altered at the stirring rate used, avoiding irregular mixing of the two phases. Hence, n–octanol did not come into contact with the nifedipine GITS tablet at any time ensuring that the partitioning process of nifedipine was not compromised.

There is a paucity of reports in the literature comparing in vitro nifedipine dissolution with in vivo absorption and efficacy with any of the nifedipine formulations currently available (e.g., immediate–release, prolonged–action, sustained–release, GITS, etc.).[15] Takahashi et al.[5] showed that the in vivo drug absorption pattern of nifedipine from a soft gelatin capsule (5 mg) was best simulated by the in vitro dissolution pattern obtained using the rotating dialysis cell (RDC) method, in which the cell contained a buffered solution paired with n–octanol as the dissolution medium. Alternatively, the advantages of the proposed two–phase system in

predicting in vivo drug absorption are the simplicity and flexibility of organic and aqueous phase ratios to accommodate different types of formulations and drugs.

Comparison of the nifedipine transfer rate–time profiles obtained in this study (Figure 6-2) with the plasma nifedipine concentration–time profiles obtained in several human clinical trials[4,7,16,17] appears to suggest an improved in vivo–in vitro correlation. Measurable plasma nifedipine concentrations have been reported for up to 36 hours in some cases.[4,7] This suggests that drug absorption extends to at least 30 hours using the GITS, given the fact that nifedipine has a relatively short plasma half-life in young healthy subjects (1 to 2 hours).[4,18,19] Thus, the shorter duration of drug release (< 30 hours) determined with single-phase dissolution methods does not correlate satisfactorily with in vivo drug absorption profiles (> 30 hours). The rate of in vivo drug absorption also appears to be considerably less than that predicted from the rate of in vitro drug release. Comparison of the data obtained with the two–phase dissolution method with pharmacokinetic data obtained from a human clinical trial with both 30 mg and 60 mg nifedipine GITS tablets is the subject of the next chapter.

In summary, the application of a relatively simple and inexpensive two–phase dissolution test for evaluating drug release and dissolution characteristics of the nifedipine GITS was demonstrated by slightly modifying existing dissolution apparatus. The modified dissolution test described here has potential applicability for improved prediction of in vivo performance of the nifedipine GITS. Also, it could be adapted for use with GITS or similar pharmaceutical formulations of other poorly water–soluble drugs.

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Chapter 7

STUDIES ON DISSOLUTION TESTING OF THE NIFEDIPINE GASTROINTESTINAL THERAPEUTIC SYSTEM. II. IMPROVED IN VITRO–IN VIVO CORRELATION USING A TWO–PHASE DISSOLUTION TEST◆

Introduction

To assess in vitro–in vivo correlations for immediate– and extended–release oral dosage forms, comparisons of in vitro dissolution curves to in vivo absorption curves are typically made. The in vitro dissolution curve is usually determined by a suitable dissolution test and the in vivo absorption curve is frequently determined by deconvolution using model–dependent (e.g., Wagner–Nelson or Loo–Riegelman) or model–independent (e.g., DeMonS) methods.[1,2] Developing an in vitro dissolution test that gives a 1:1 in vitro–in vivo correlation for a particular drug product is an important objective to facilitate product development and serves as a quality control procedure during product manufacture. Drug manufacturers typically use such tests to assess lot–to–lot variability, product shelf–life, and to predict in vivo performance (i.e., bioavailability) with reasonable assurance after conducting minor formulation and process changes (i.e., colour, size, shape, preservatives, flavour, coating procedure, amount and composition of materials, sources of inactive and active ingredients, and changes in equipment or site of manufacture).[3] In the absence of a suitable in vitro test that can be interpolated to changes in drug plasma concentration–time profiles, appropriate testing in humans may have to be carried out which can add much to the development costs of pharmaceutical formulations.[4]

Few studies have attempted to correlate in vitro dissolution with in vivo absorption of oral nifedipine formulations,[5] including immediate–release, prolonged–action, sustained–release, and the gastrointestinal therapeutic system (GITS). The latter is a controlled–release formulation which uses a push–pull osmotic process to deliver a finely–divided nifedipine

◆ A version of this chapter has been submitted for publication:
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suspension by zero–order kinetics within the gastrointestinal (GI) tract over a period sufficient to permit once daily dosing.[6,7]. Zero–order release of nifedipine suspension from this product lasts about 16 to 18 hours and is assumed to be independent of the physiological environment (i.e., pH, GI motility). Given that nifedipine is rapidly absorbed (when in solution)[8] and efficiently absorbed over the entire intestine,[9-12] then a 1:1 correlation between in vitro drug release and in vivo drug absorption is expected, assuming dissolution of the finely–divided nifedipine suspension is not rate–limiting. Nifedipine, however, is poorly water soluble ($\leq 10\ \mu g\ mL^{-1}$)[7,8,13] and its solubility in phosphate buffer is reported to decline as a function of increasing buffer concentration.[14,15] Additionally, the physical state of nifedipine in the GITS formulation could impact significantly on its dissolution characteristics (i.e., nifedipine can exist as amorphous and polymorphic crystalline forms). Unfortunately, the physical state(s) of nifedipine incorporated in the GITS has not been reported to date.

Chung et al.[16] state that good in vitro–in vivo correlations were achieved with several strengths of nifedipine GITS tablets, but do not describe these correlations in their report. However, their data indicates that zero–order drug absorption (calculated using the Wagner–Nelson method) persists for at least 24 hours postdosing (only about 70% of the 'available' dose was absorbed within 24 hours), whereas the authors state that zero–order drug release ends (becoming first–order) about 20 hours after initial hydration (in vitro dissolution, $\geq 90\%$ of the labeled dose released by 24 hours). In addition, the authors note that a time lag exists between the in vitro zero–order release phase (approximately 2 to 20 hours) and the in vivo zero–order absorption phase (approximately 6 to 24 hours).

In another report[6] it is shown that the in vivo rate of nifedipine release as suspension from the GITS in the GI tract of dogs is equal to the rate of release found for identical products in vitro (i.e., a 1:1 correlation). However, dissolution of the released suspension is not evaluated or considered as potentially rate–limiting for absorption. Nonetheless, it is stated[6] that nifedipine absorption rates in humans (administered GITS) indicate that cumulative amounts absorbed over 24 hours are proportional to the amounts released in vitro; but unfortunately no human data is given and few additional details which may have lead to this conclusion are provided.

Potential problems in correlations of bioavailability and dissolution data originate from the complexity of drug absorption or the weakness of the dissolution design.[17] For the

nifedipine GITS, as described in chapter 6, single–phase dissolution tests accurately measure drug suspension release but fail to account for drug dissolution of suspended particles. This may explain the discrepancies found with in vitro–in vivo correlations of the nifedipine GITS, as previously described.

Based on these considerations, data obtained from a two–phase in vitro dissolution test of 30 mg and 60 mg nifedipine GITS tablets (see chapter 6) were compared with in vivo data obtained in a previously conducted bioequivalency study[18] (see chapter 5) for the same two product strengths. Improved in vitro–in vivo correlations compared to those found using single–phase dissolution tests were anticipated.

Materials and Methods

Materials and Chemicals

The nifedipine GITS products tested were commercially available (Adalat® XL, 30 mg and 60 mg strengths, Bayer Canada Inc., Etobicoke, ON, Canada) and obtained from a local pharmacy. Nifedipine powder was purchased from Sigma Chemical Company (St. Louis, MO, USA), a methanolic solution of which (100 μg mL^{-1}) was used as the reference standard for generating all calibration curves in the study. Nisoldipine (Bayer Canada Inc., Etobicoke, ON, Canada) dissolved in methanol (100 μg mL^{-1}) was used as the internal standard in the HPLC assay for nifedipine. All other chemicals and solvents were either reagent or HPLC grade and obtained from commercial suppliers.

In Vitro Drug Transfer Study

The two–phase dissolution test system was described in detail in chapter 6. Briefly, 6 nifedipine GITS tablets of each strength were tested for 30 hours at 37 ± 0.5°C in a two–phase dissolution medium consisting of simulated intestinal fluid, USP, without pancreatin (SIF', 750 mL) and n–octanol (250 mL). The amount of nifedipine transferred from the lower aqueous SIF' phase (containing a single nifedipine GITS tablet) to the upper n–octanol phase was determined every 2 hours from absorbances of the n–octanol phase (2 mL samples) at 235 nm and comparison to a calibration curve. The residual amounts of nifedipine remaining in the tablet and aqueous phase after 30 hours were determined by HPLC.[19]

In Vivo Drug Absorption Study

Human data were taken from a previous study (see Chapter 5) which assessed the bioequivalency of nifedipine GITS tablets.[18] Briefly, twelve healthy human subjects were recruited (6 of each gender, mean age 34.2 ± 7.7 years, mean weight 76.2 ± 15.8 kg). The study participants had no pre–existing medical conditions and were not using systemic medications at the time of the study (including OTC preparations) which may have altered the disposition of nifedipine. The subjects received 4 different lots of nifedipine GITS on separate occasions [Adalat® XL, Miles Canada Inc. (Etobicoke, ON, Canada)]; however, the data from only 2 of these [designated lot A (30 mg) and lot C (60 mg)] were used for comparison with the in vitro data since the other 2 lots [lot B (30 mg) and lot D(60 mg)] gave very similar results (not shown). A minimum washout period (1 week) was observed between administration of each lot. Food was withheld from midnight the day before each dosing session until 2 hours after administration. Blood samples (6 mL) were withdrawn from an antecubital vein 5 minutes before dosing and 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hours postdosing. Blood samples were centrifuged within 10 minutes of collection, and the resulting plasma samples were protected from light then stored frozen at −18 ± 3°C until analyzed. Nifedipine plasma concentrations were measured using an established reversed–phase HPLC method (lower limit of quantitation in plasma was 5 ng mL^{-1}).[19]

Data Analysis

The rate of nifedipine absorption in human test subjects was determined from the plasma nifedipine concentration–time data and calculated by two different deconvolution techniques: (1) the Wagner–Nelson method[20] (model–dependent), and (2) a new numerical deconvolution method[21] (DeMonS, model–independent). For Wagner–Nelson calculations, the mean terminal elimination rate constant of nifedipine (k_{el}, 0.41 h^{-1}) was taken from previously reported data,[6,11,22,23] because rate constants could not be determined from each subjects nifedipine plasma concentration–time plots due to the presence of flip–flop kinetics[1] and because, typically, only 1 or 2 blood samples showed a decrease in each profile. Hence, the Wagner–Nelson method was applied only to the mean plasma concentration–time data. For numerical deconvolution calculations (DeMonS), the impulse response function typically calculated from data obtained after iv bolus injection in the study subjects (but not available) was, instead, approximated by a monoexponential function for each subject. One set of

disposition model parameters was used for the same subject for both treatments. In addition, lot C (60 mg) was designated the reference treatment (100% absorbed) and lot A (30 mg) was the test treatment for the percent absorbed calculations using DeMonS. Linear regression analysis was applied to time points between 6 and 24 hours in the fraction of nifedipine transferred/absorbed–time plots. The zero–order rates of nifedipine transfer or absorption were calculated by multiplying the slope of the regression lines with either the total dose recovered or the labeled quantity, respectively. Linear regression analysis was also applied to in vitro–in vivo correlation plots (r^2, slope and intercept values were determined).

Results and Discussion

Figure 7-1 shows the mean nifedipine plasma concentration–time profiles obtained with the two tested nifedipine GITS strengths in vivo. These profiles indicate that measurable drug concentrations can be observed up to at least 36 hours postdosing (in some subjects), hence drug absorption (zero– and first–order) must extend for at least 30 hours given that nifedipine has a relatively short intrinsic half–life (1 to 2 hours).[10,22,23] Similar profiles have been shown in other single–dose studies conducted with the nifedipine GITS.[16,24-26]

Figure 7-2 and Figure 7-3 show the fraction of nifedipine absorbed–time profiles for the nifedipine GITS strengths tested, calculated using either the Wagner–Nelson method or DeMonS, respectively. Figure 7-4 shows the fraction of nifedipine transferred–time profiles for the nifedipine GITS strengths tested, determined using the two–phase dissolution method. Figure 7-2, Figure 7-3 and Figure 7-4 have similar appearances, and this is translated to similar in vitro–in vivo correlations (Figure 7-5) displaying very good linearity ($r^2 > 0.993$) and slopes of about 1.0 (0.998 to 1.140). The y–intercept was reasonably close to zero for plots in which the Wagner–Nelson method was used (0.64 to 2.17), but a larger deviation was found from DeMonS plots (-9.72 to -11.04). This is probably due to the large inter– and intrasubject variability in the in vivo study,[18] as well as errors in the estimates of terminal elimination half–life in individual subjects for the model–independent calculations.

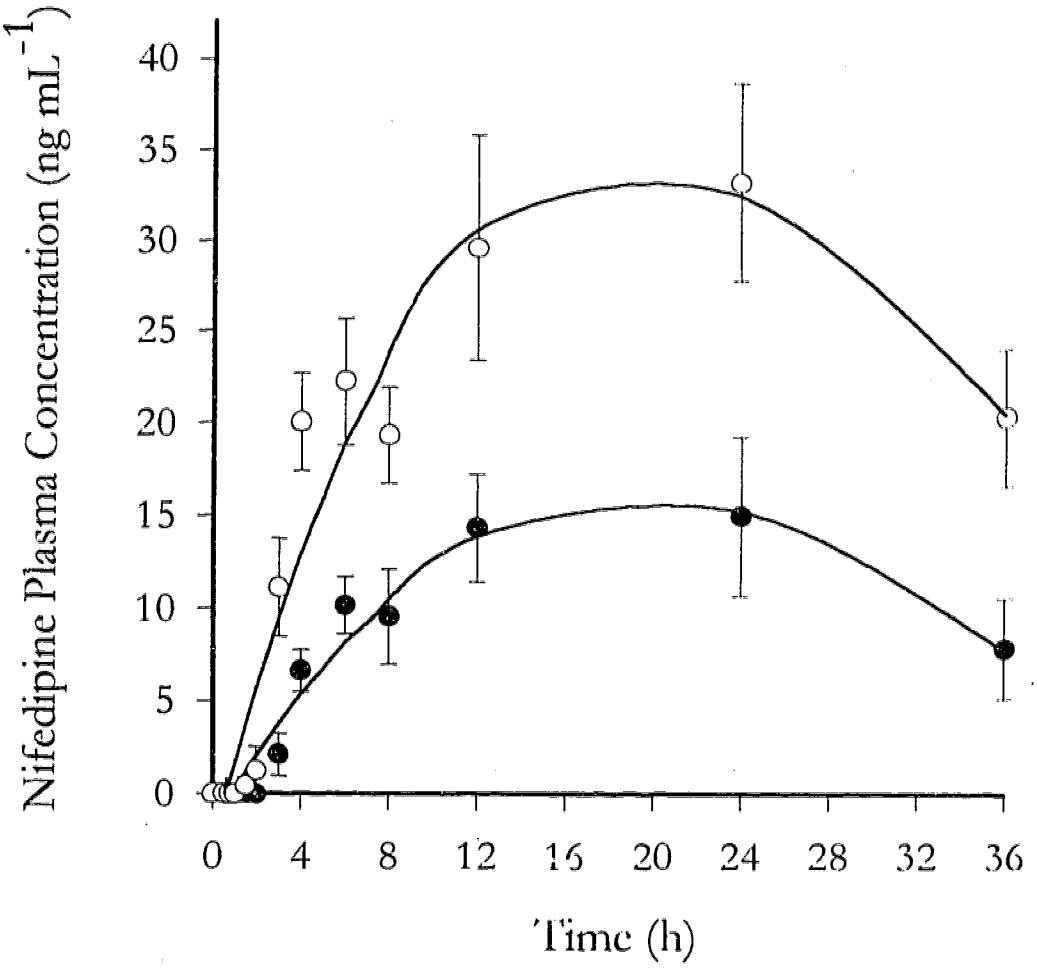

Figure 7-1. Plasma nifedipine concentration–time profiles obtained after single-dose administration of 2 different strengths [30 mg (●) and 60 mg(○)] of commercially available nifedipine GITS to healthy volunteers. Data are means ± standard error ($s_{\bar{x}}$, n = 12).

Nevertheless, the results in Figure 7-5 demonstrate a good correlation between in vitro nifedipine transfer and in vivo nifedipine absorption rates. In contrast, Figure 7-6 shows that the fraction of nifedipine released [determined using a modified USP XXII dissolution technique (apparatus 1, see chapter 6)] when plotted against the fraction of nifedipine absorbed (Wagner–Nelson method) gave less satisfactory correlations ($r^2 < 0.973$) — note: similar findings (not shown) were also obtained using the DeMonS results.

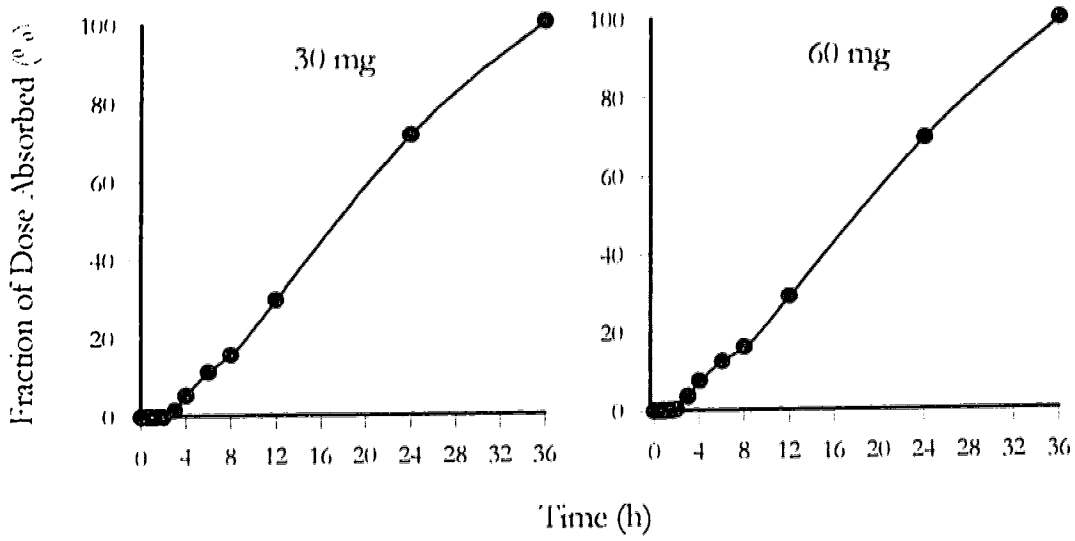

Figure 7-2. Model-dependent method (Wagner–Nelson): in vivo fraction (%) of nifedipine absorbed–time profiles for nifedipine GITS (30 mg and 60 mg) administered to 12 human subjects. *Note*: dose refers to the bioavailable dose.

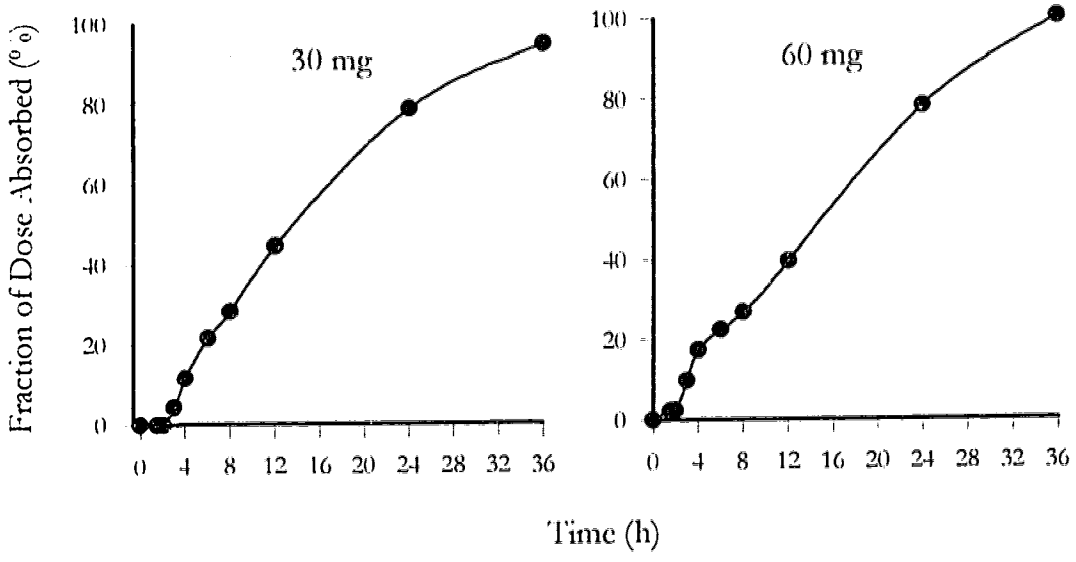

Figure 7-3. Model-independent method (DeMonS): in vivo fraction (%) of nifedipine absorbed–time profiles for nifedipine GITS (30 mg and 60 mg) administered to 12 human subjects. *Note*: dose refers to the bioavailable dose.

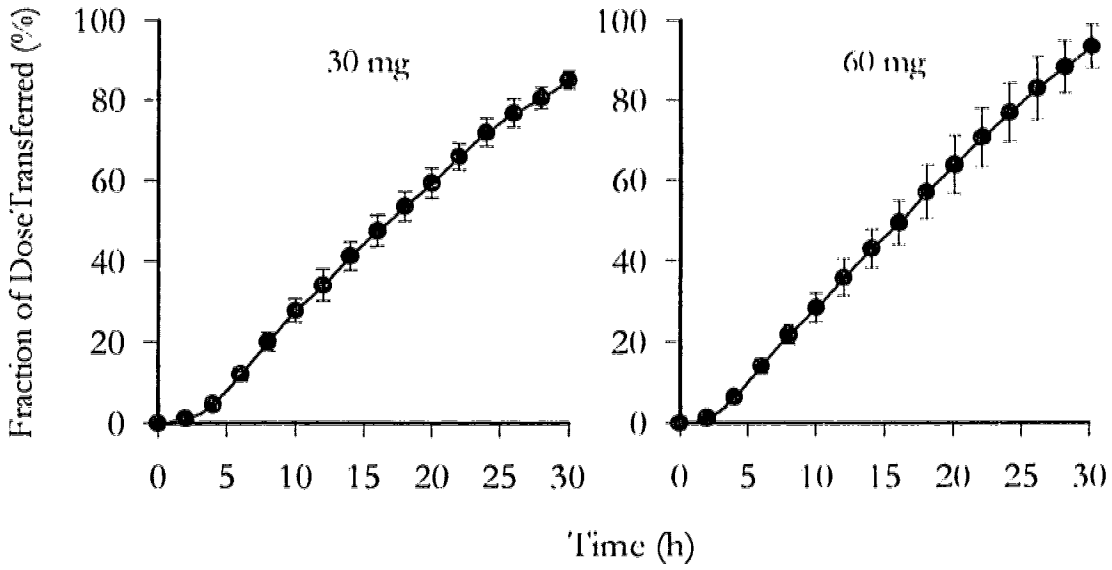

Figure 7-4. Comparison of in vitro mean fraction (%) of nifedipine transferred–time profiles using a two-phase dissolution system for nifedipine GITS, 30 mg and 60 mg. Means ± standard deviation (*s*, n = 6).

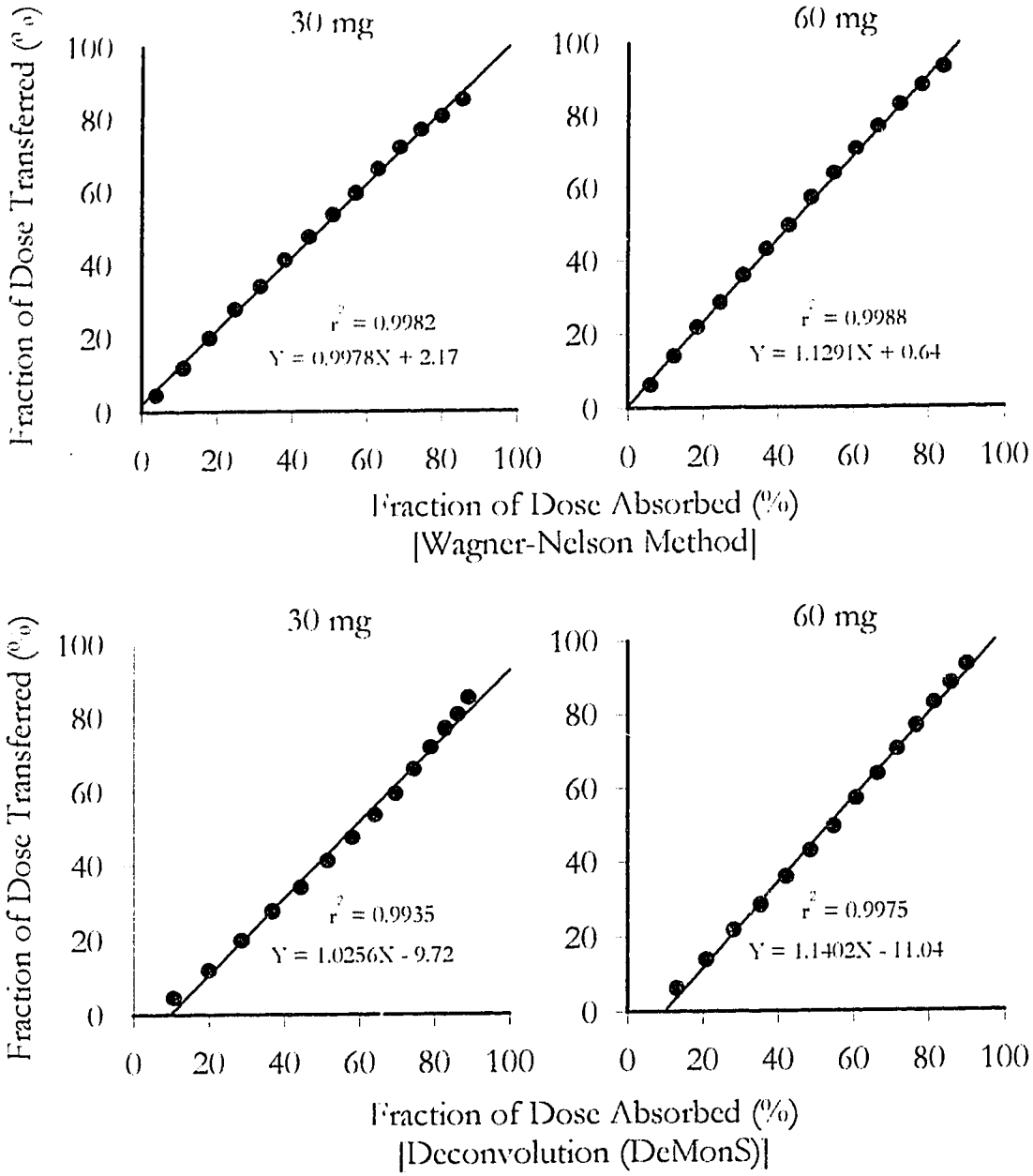

Figure 7-5. In vitro–in vivo correlation plots for nifedipine GITS, 30 mg and 60 mg. In vitro nifedipine transfer calculated using a two–phase dissolution test system. In vivo nifedipine absorption calculated using either model–dependent (Wagner–Nelson) or model–independent (DeMonS) methods.

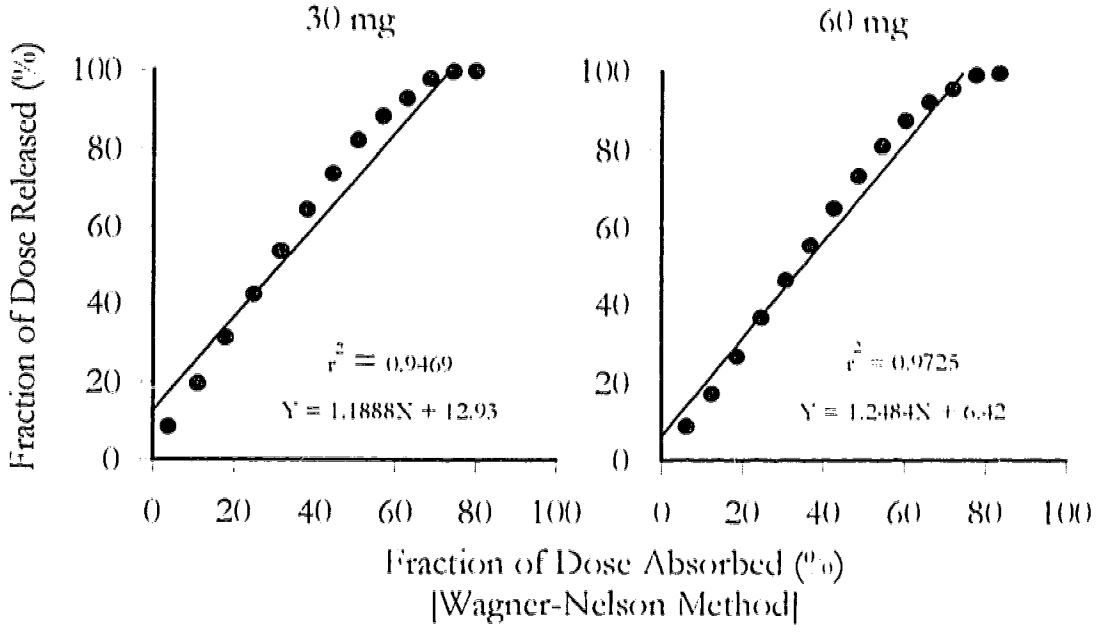

Figure 7-6. In vitro–in vivo correlation plots for nifedipine GITS, 30 mg and 60 mg. In vitro nifedipine release calculated using a single–phase dissolution test system.[27] In vivo nifedipine absorption calculated using a model-dependent method (Wagner–Nelson).

Table 7-1 compares the zero–order nifedipine transfer and absorption rates of the two nifedipine GITS strengths calculated between 6 and 24 hours. These results showed that zero–order in vivo release rates corresponded reasonably well with zero–order in vitro transfer rates found using the two–phase dissolution test. The zero–order in vitro transfer rates found are considerably less than the zero–order in vitro release rates calculated using single–phase dissolution methods or the manufacturers design specifications (1.7 and 3.4 mg h^{-1} for the 30 mg and 60 mg strengths, respectively).[6,27]

Table 7-1. Zero–order nifedipine transfer (in vitro) and absorption (in vivo) rates obtained using the nifedipine gastrointestinal therapeutic system. Calculated between 6 and 24 hours using linear regression.

	In Vitro Study		In Vivo (Human) Study			
	Two–Phase Dissolution Test		*Wagner–Nelson Method*		*DeMonS*	
	30 mg GITS	60 mg GITS	30 mg GITS	60 mg GITS	30 mg GITS	60 mg GITS
Transfer/Absorption Rate (6 to 24 hours, mg h^{-1})	0.96	2.02	1.03	1.94	0.95	1.88
r^2 value (linear regression fit)	0.9979	0.9996	0.9983	0.9971	0.9946	0.9989

Note. In vivo absorption rates calculated assuming 100% bioavailability.

Improved in vitro–in vivo correlations found in this study can be explained by the fact that the two–phase dissolution test simulates release and dissolution of the nifedipine suspension in the GI tract, as well as absorption from the gut lumen. Chung et al.[16] were the first to speculate that dissolution of the released nifedipine suspension may be a potentially rate–limiting factor in absorption since their results showed a time lag between the appearance of apparent zero–order in vivo absorption compared with the appearance of apparent zero–order in vitro release. Further evidence of rate–limiting dissolution of the nifedipine suspension may also be seen in their results[16] showing the effect of food on the performance of the nifedipine GITS. In this case, the rate of nifedipine absorption is found to be slightly altered by food (28% increase in the mean peak nifedipine concentration) but food does not influence the extent of drug availability compared to the fasting state. However, by design, drug release from the GITS is dependent on osmotic processes and is not dependent on erosion, disintegration, pH and gastric motility,[28,29] or presumably, the presence of food. In addition, studies with similar osmotic devices (e.g., OROS) show the release of specific markers[30] or various drugs[31,32] to be independent of the presence of food. Therefore, although food would not be expected to influence the rate of release of the nifedipine suspension, it could, however, influence the rate of drug dissolution which, in turn, would alter the rate of absorption. Indeed, Challenor et al.[33] speculate that food may have increased the rate of dissolution of a nifedipine biphasic tablet during retention in the stomach, and Ueno et al.[34] note that the C_{max} of a nifedipine sustained–release preparation is increased by food.

The USP Subcommittee on Biopharmaceutics (1988) defined in vitro–in vivo correlation as the establishment of a relationship between a biological property, or one or more pharmacokinetic parameters of a dosage form, and the physiochemical characteristics (e.g., in vitro dissolution mechanism) of the same dosage form.[2] This committee also introduced a classification system of correlation methods for extended–release dosage forms which specifies 4 levels designated A, B, C and D, listed in the order of descending quality. The preferred correlation for controlled–release delivery systems is Level A,[2,35] for which the in vitro dissolution profile is compared to the in vivo dissolution curve and should be essentially superimposable. Level A correlations are favoured because (1) each plasma concentration and dissolution time point generated is used and thus reflects the whole curve, (2) it is an excellent quality control procedure since in vivo performance of a particular dosage form can

be predicted by an in vitro test, and (3) because the boundaries of the in vitro dissolution curve can be justified on the basis of convolution or deconvolution.[36] Skelly et al.[2] state that a level A correlation is most applicable to controlled–release formulations that demonstrate in vitro drug release rates that are essentially independent of the dissolution medium. However, the results reported here with the nifedipine GITS may suggest a further caveat needs to be included with this statement, such that drug dissolution is also considered.

Given that controlled–release dosage forms are now quite common and are an increasingly important segment of newly–introduced products, various regulatory bodies — i.e., the FDA, HPB — are considering requiring the pharmaceutical industry to develop correlations for their products which can be used to support regulatory decisions.[36] Application of a two–phase dissolution test for the nifedipine GITS is one simple approach that can be used to obtain reasonable in vitro–in vivo correlations and can potentially aid future improvements in the design of the product, and benefit the development and testing of GITS or similar formulations of other poorly water–soluble drugs.

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Chapter 8

EXTRAHEPATIC FIRST–PASS METABOLISM OF NIFEDIPINE IN THE RAT◆

Introduction

The pharmacokinetics of the calcium channel antagonist nifedipine have not yet been fully characterized in rats or human beings. One reason for this being the difficulty measuring plasma nifedipine concentrations in the low nanogram range with sufficient specificity, sensitivity and accuracy to give good pharmacokinetic parameter estimates after nifedipine dosing. Although specific and sensitive analytical methods for nifedipine are now available — typically using high–performance liquid chromatography (HPLC) or gas chromatography[1-3] — several questions remain to be answered regarding the absorption and disposition of this drug, including: (1) Does the gastrointestinal (GI) tract contribute to the first–pass effect observed after peroral (po) dosing?; (2) What is the underlying cause of inter– and intraindividual variability in nifedipine systemic availability (F) after po dosing?; and (3) What mechanisms are involved in modifying F and clearance by various foods and drugs? — including the poorly understood interaction between grapefruit juice and nifedipine (and other 1,4-dihydropyridine calcium channel antagonists).

Nifedipine is well absorbed from the gut lumen in both rats and humans (≥ 90%).[4-10] After absorption, nifedipine is metabolized by oxidative mechanisms — involving cytochrome P450 (CYP) 3A isozymes — to a pharmacologically inactive nitropyridine analogue, which is subsequently metabolized to more polar compounds.[11-13] The liver is considered the principle organ responsible for systemic plasma clearance of nifedipine[14,15] and, hence, thought to be responsible for the pronounced 'first–pass' effect observed after po nifedipine administration in the rat ($0.45 \leq F \leq 0.58$)[16,17] and humans ($0.40 \leq F \leq 0.68$)[7,8,16,18]

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— although it has been suggested that the gut wall may also contribute to the presystemic elimination of nifedipine.[19-22] In human beings, hepatic plasma clearance of nifedipine is reported to account for only 65% of systemic plasma clearance suggesting extrahepatic sites of metabolism.[21] Interestingly, systemic plasma nifedipine clearance in the rat (about 2.4 to 10 mL min^{-1} kg^{-1})[16,17,22-24] is relatively low compared to hepatic blood flow (Q_h, about 55 to 80 mL min^{-1} kg^{-1})[25,26] suggesting a low extraction ratio through the liver. Review of the data from an isolated perfused rat liver experiment conducted by Scherling et al.,[5] shows that unchanged nifedipine can still be detected in the perfusate even after about 12 passes through the liver, further indicating low extraction by this organ. Thus, hepatic extraction alone cannot account for the relatively poor po bioavailability of nifedipine in the rat — suggesting that the rat may be a good model to investigate presystemic nifedipine gut wall biotransformation.

This study was undertaken to determine if intestinal metabolism contributes to the first–pass elimination of nifedipine in the male Sprague–Dawley rat. Standard pharmacokinetic parameters (including systemic availability) of nifedipine given by intravenous (iv), po, intracolonic (ic) and intraperitoneal (ip) routes were compared after dosing in adult male Sprague-Dawley rats. In addition, the whole blood–to–plasma concentration ratio of nifedipine (BP_r) in the rat was measured to calculate systemic blood clearance and therefore estimate the hepatic extraction ratio (E_h) using literature values of Q_h.

Materials and Methods

Chemicals

Nifedipine was purchased from the Sigma Chemical Company (St. Louis, MO, USA). Nisoldipine [Internal Standard (IS)] was kindly provided by Miles Canada Inc. (Etobicoke, ON, Canada). The following mobile phase solvents (HPLC grade) and modifiers, or extraction solvents (analytical grade) were used: Methanol and triethylamine were supplied by Fisher Scientific (Nepean, ON, Canada); water was obtained from Caledon Laboratories Ltd. (Georgetown, ON, Canada); and glacial acetic acid, iso–octane and methyl–tert–butyl ether were purchased from BDH Inc. (Toronto, ON, Canada). Other compounds used: heparin (Hepalean®, 1000 U.S.P. units mL^{-1}) was supplied by Organon Teknika Inc. (Toronto, ON, Canada); sodium pentobarbital (Somnotol®, 65 mg mL^{-1}) was purchased from MTC Pharmaceuticals (Cambridge, ON, Canada); and polyethylene glycol–300 and –400

(Carbowax® Sentry®, FCC grade) were obtained from Union Carbide Chemicals (Danbury, CT, USA).

Animals and Surgical Cannulation Procedure

Adult male Sprague–Dawley rats (initially weighing 240 to 300 g) were obtained from the Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada) and housed for at least 2 days in a clean room and fed food and water *ad libitum*. The day before dosing experiments were done, rats were moved to the laboratory and subjected to surgical cannulation. Under anesthesia induced by sodium pentobarbital (65 mg kg^{-1}) a small longitudinal incision was made in the skin of each rat over the right jugular vein, which was then made accessible by clearing the surrounding tissues. The vein was catheterized with Silastic® laboratory tubing (0.635 mm i.d., 1.194 mm o.d., Dow Corning Corp., Midland, MI, USA) containing heparinized (100 IU mL^{-1}) normal saline and fixed in place with two nonabsorbable surgical sutures (Surgical Suture USP, Cyanamid Canada Inc., Montreal, QC, Canada). Each cannula was terminated with a long piece of polyethylene tubing (PE-50, i.d. 0.58 mm, o.d. 0.965 mm, Clay Adams, Parsippany, NJ, USA) and the free end exteriorized to the dorsal side of the neck. The exposed areas were then closed using nonabsorbable surgical suture. The animal was allowed to recover for the next 16 to 20 hours in a metabolic cage and provided only drinking water for the remainder of the experiment (i.e., animals were fasted before and during each experiment). The implanted cannulas provided a site for iv administration of nifedipine (if necessary) and permitted frequent blood sampling from each rat.

Pharmacokinetic Study: Experimental Design

All drug preparation, dosing and collection of blood samples was done under sodium lamps to prevent nifedipine photodegradation.[27] A nifedipine dosing solution (5 mg mL^{-1} in polyethylene glycol–400) was prepared the day before each experiment — to allow sufficient time for drug solubilization — and wrapped in aluminum foil to protect the solution from light and stored at room temperature. Each rat received a single dose of nifedipine (6 mg kg^{-1}) by a predetermined route of administration (randomly assigned among the 4 groups), as described below. No adverse events or symptoms were observed in any of the rats used in the study, before or during nifedipine treatment. Blood draws (0.25 mL samples) were made at predetermined times using heparinized 1 mL syringes connected to the jugular cannula.

The blood volume drawn was immediately replaced with an equal volume of normal saline. Blood samples were centrifuged [Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA, USA)] at 15,000 rpm for 4 minutes and the plasma samples obtained were placed in 1.5 mL poly(propylene) microfuge tubes, stored in light resistant bags and kept at –20°C until needed for analysis.

Intravenous Administration

Six rats (249 to 300 g) received an iv dose of nifedipine through the jugular vein cannula (given over 4 minutes to prevent injury to the animal that could result from excessively high nifedipine plasma concentrations). The cannula was immediately rinsed with 0.5 mL of normal saline and blood samples were taken at –5, 5, 8, 10, 15, 20, 30, 45, 60, 90, 120, 180 and 240 minutes — the start of the 4 minute nifedipine infusion period was designated 0 minutes.

Intraperitoneal Administration

Six rats (264 to 293 g) received an ip dose of nifedipine given over about 10 seconds using a short 26G needle (length 12.7 mm) inserted into the left caudal area of the abdomen. Blood samples were drawn at –5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes.

Peroral Administration

Six rats (260 to 297 g) received a po dose of nifedipine given over about 10 seconds using an oral feeding tube. Blood samples were drawn at –5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes.

Intracolonic Administration

Seven rats (257 to 299 g) received an ic dose of nifedipine given over about 10 seconds through a 15 cm length of PE-100 tubing (i.d. 0.86 mm, o.d. 1.52 mm, Clay Adams, Parsippany, NJ, USA) -- the entire length of tubing was inserted rectally after lubricating the outer surface of the tube with polyethylene glycol–300. Blood samples were drawn at –5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes.

Whole Blood-to-Plasma Concentration Ratio: Experimental Design

Three stock solutions were prepared by dissolving known amounts of nifedipine in 100 mL of methanol (20 μg mL^{-1}, 500 μg mL^{-1}, and 2 mg mL^{-1} solutions, respectively). The BP,

was measured after adding nifedipine to freshly collected heparinized blood obtained from male Sprague–Dawley rats (280 to 311 g) — yielding low (200 ng mL^{-1}), medium (5 μg mL^{-1}) and high (20 μg mL^{-1}) concentrations (n = 5 samples per concentration). Methanol concentrations in the blood samples were limited to 1% (v/v) by using an appropriate stock solution concentration (described above). The blood samples were gently mixed and incubated at 37 °C for 30 minutes to allow for distribution of the drug into erythrocytes. The concentrations of nifedipine were determined in 0.05 to 0.1 mL samples of blood and plasma obtained after centrifugation using HPLC.[2]

Chromatography

Unknown nifedipine concentrations in rat plasma and blood were determined by means of a previously reported HPLC method (lower limit of quantitation 5 ng mL^{-1})[2] using 0.05 to 0.2 mL samples diluted to 1.0 mL with HPLC grade water. All analyses were conducted under sodium lamps to prevent photodegradation of nifedipine. Calibration curve standards (containing 5 to 2000 ng of nifedipine) were prepared by adding a known amount of the drug to 0.1 mL of blank rat plasma and diluting to 1.0 mL as described above. The calibration curve best–fit regression line was calculated using a $1/x^2$ weighting factor (where x corresponds to the amount of nifedipine added; r^2 values > 0.99 were always obtained; inter– and intraday variability < 10%). The analyses were done with the following instrumentation: a Model 600E solvent delivery system, a Model 717 autosampler; a Model 486 tunable UV/VIS absorbance detector (set at 350 nm); and an NEC 486–33 MHz computer running Millennium 2010 chromatography manager software Version 1.1 (Waters Ltd, Mississauga, ON, Canada).

Pharmacokinetic Analysis

Standard pharmacokinetic parameters obtained from each of the individual rat plasma concentration–time profiles were calculated by non–compartmental methods using the computer program: WinNonlin Standard Edition Version 1.0 (Scientific Consulting Inc., Apex, NC, USA) — area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule from time zero (t_0) to the time to reach peak concentration (t_{max}), and the logarithmic trapezoidal rule applied from t_{max} to the time of the last observable concentration (t_{last}), followed by extrapolation to infinity.

Statistical Analysis

All the calculated pharmacokinetic parameters — except t_{max} — were assumed to follow a log–normal distribution, and were log–transformed before analysis. The pharmacokinetic parameter estimates obtained were expressed as either geometric or arithmetic means with their corresponding 95% confidence intervals, as appropriate. The pharmacokinetic parameters obtained were further analyzed by independent measures one–way analysis of variance (ANOVA), and a post hoc test (Duncan's multiple range test) was used to determine where, if any, differences occurred. A value of $P \leq 0.05$ was considered statistically significant. The computer program SPSS for Windows Version 6.1 (SPSS Inc., Chicago, IL, USA) was used for all the statistical tests. For the purposes of generating plasma concentration–time profiles, plasma nifedipine concentrations were expressed as arithmetic means ± standard deviation (s).

Results and Discussion

The mean (± s) plasma nifedipine concentration–time profiles obtained in this study after iv, ip, po and ic dosing of nifedipine (6 mg kg^{-1}) to separate groups of rats are shown in Figure 8-1. The individual profiles obtained after iv dosing displayed a distributive phase consistent with a drug known to display multi-compartmental characteristics. Individual profiles from extravascular dosing, typically showed a rapid rise in plasma nifedipine concentrations after which levels declined monoexponentially. However, plasma concentration-time profiles obtained after po dosing were found to be more variable, perhaps due to rate–limiting absorption from the GI tract in some of the rats.

Standard pharmacokinetic parameters calculated from the data obtained in this study are shown in Table 8-1 and significant differences, where found, are noted. Interestingly, statistical analysis of the clearance values obtained after extravascular administration (i.e., po, ic, ip) showed that only po clearance was statistically different from systemic (iv) clearance. The values for mean absorption time and mean residence time (see Table 8-1) showed that nifedipine was rapidly absorbed and eliminated in this study. Nifedipine was found to have a relatively high volume of distribution at steady state (V_{ss}, see Table 8-1) indicating that, despite high plasma protein binding (about 99% in rat plasma, unpublished observations from our laboratory), the drug is very highly bound to extravascular tissues in the rat.

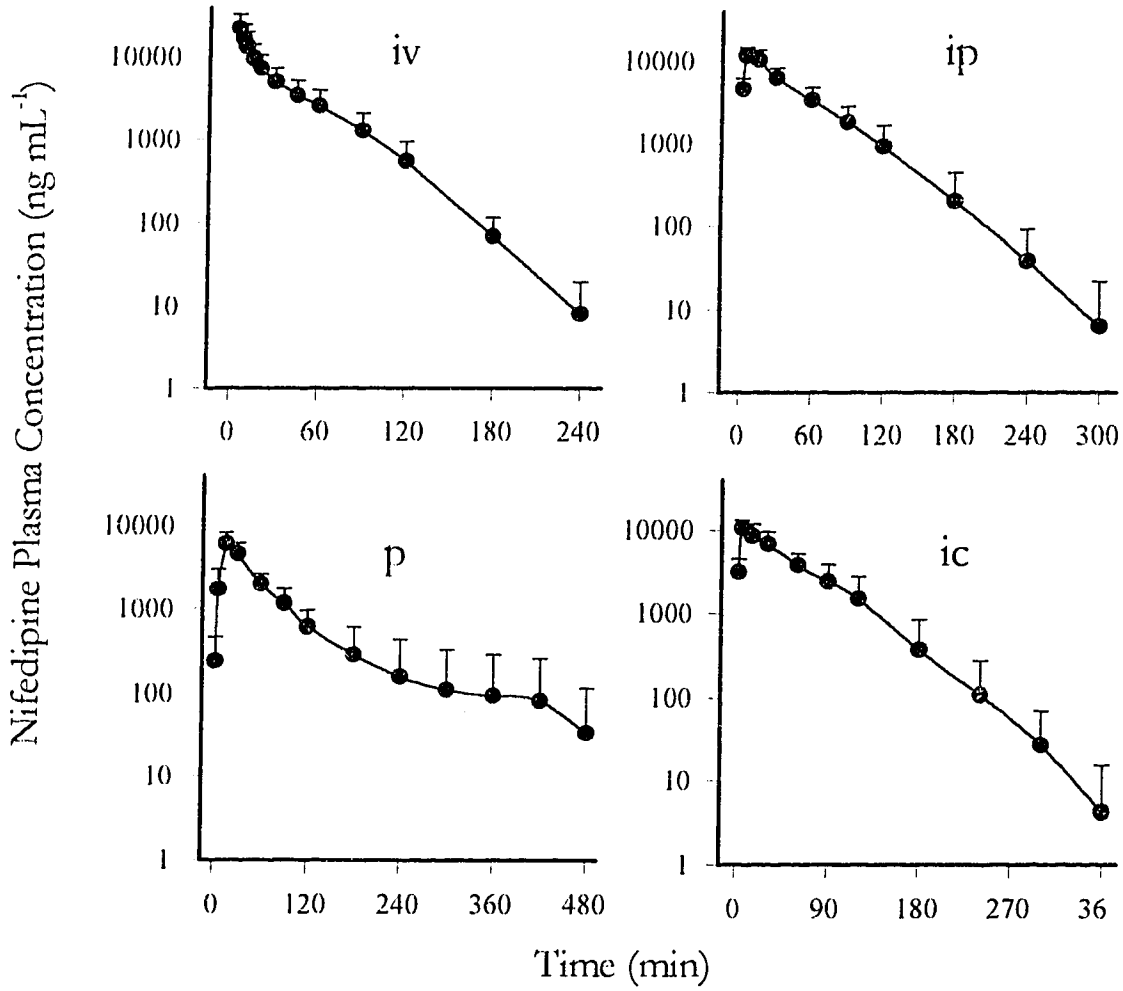

Figure 8-1. Single dose nifedipine (6 mg kg^{-1}) plasma concentration–time profiles in male Sprague-Dawley rats after: intravenous (iv), intraperitoneal (ip), peroral (po) and intracolonic (ic) administration. Data are presented as arithmetic mean values ± *s* (n = 6 or 7).

Table 8-1. Pharmacokinetic Parameters Describing the Absorption and Disposition of Nifedipine Given by Several Routes of Administration in the Rat.[a]

	Route of Administration			
Parameters	**iv**	**ip**	**po**	**ic**
AUC (μg mL^{-1} min)	584 (470 - 724)[b]	523 (354 - 772)[b]	354 (284 - 441)[c]	582 (451 - 751)[b]
C_{max} (ng mL^{-1})	25663 (20535 - 32070)[d]	13098 (10787 - 15907)[e]	5884 (3331 - 10397)[f]	12257 (10278 - 14615)[e]
t_{max} (min)	–	7.5 (4.6 - 10.4)[g]	16.7 (9.5 - 23.8)[h]	8.6 (6.3 - 10.8)[g]
$t_{1/2}$ (min)	21.4 (18.2 - 25.2)[i]	21.3 (14.4 - 31.5)[i]	29.6 (20.6 - 42.5)[i]	30.5 (21.0 - 44.4)[i]
MRT (min)	31.8 (26.4 - 38.2)[j]	41.4 (32.2 - 53.2)[jk]	62.9 (34.2 - 115.6)[k]	50.6 (40.5 - 63.2)[k]
MAT (min)	–	9.6	31.1	18.8
CL_p (mL min^{-1} kg^{-1})	10.3 (8.3 - 12.7)[l]	–	–	–
CL_p/F (mL min^{-1} kg^{-1})	–	11.5 (7.8 - 16.9)[l]	17.0 (12.9 - 22.4)[m]	10.3 (8.3 - 12.8)[l]
V_{ss} (mL kg^{-1})	326 (287 - 372)	–	–	–
F	1.0	0.90	0.61	1.0

[a] Where appropriate, data are expressed as geometric means with a corresponding 95% CI; except for t_{max} which is presented as the arithmetic mean with a 95% CI (n = 6 or 7 rats per group).

[b-m] Identical superscript characters identify non–significant differences ($P > 0.05$).

Abbreviations: AUC= area under the plasma drug concentration–time curve from zero to infinity; CL_p= systemic plasma clearance of drug; C_{max}= maximum (peak) plasma drug concentration; *F*= systemically available fraction of the dose of a drug; ic= intracolonic; ip= intraperitoneal; iv= intravenous; MAT= mean absorption time; MRT= mean residence time; po= peroral; t_{max}= time to reach the maximum (peak) drug concentration following extravascular drug administration; $t_{1/2}$= elimination half–life of the drug associated with the terminal slope of a semilogarithmic plasma drug concentration–time curve; V_{ss}= volume of distribution at steady–state.

Little or no unchanged nifedipine is found in the excreta (urine and bile) of rats;[5,16] hence, the drug is cleared from plasma via biotransformation, presumably by the liver. Therefore, systemic clearance can be assumed to equal hepatic clearance.[16] The mean systemic nifedipine clearance found in this study (Table 8-1) is similar to that given by Boje et al.,[24] who report a value of 9.1 ± 6.9 mL min^{-1} kg^{-1} (from a 2 mg kg^{-1} iv dose) in six fasted male Sprague–Dawley rats (200 to 350 g). Several other reports give values ranging from about 2.4 to 10 mL min^{-1} kg^{-1}.[16,17,22,23] Thus, although the reported systemic clearance of nifedipine in the rat is somewhat variable, nonetheless, clearance of the drug is low relative to literature values of Q_h for this animal (about 55 to 80 mL min^{-1} kg^{-1}).[25,26]

To calculate the nifedipine E_h from systemic plasma clearance — assuming the liver is the sole eliminating organ — it is necessary to estimate the hepatic blood clearance (Cl_{h_b}) of nifedipine using the BP_r (see Materials and Methods). Hence, a study to determine the BP_r of nifedipine in the rat was undertaken and the results are shown in Table 8-2. The BP_r measured at low, medium and high nifedipine concentrations (spanning the concentration range typically found in the rat plasma samples of the pharmacokinetic study) was relatively constant with a mean value of about 0.59. A low BP_r value (i.e., < 1.0) indicates that the drug has a high affinity for plasma — indeed nifedipine is highly bound to rat plasma proteins as stated previously — and given the value obtained very little, if any, nifedipine was present in the hematocrit.

Table 8-2. Whole Blood–to–Plasma Concentration Ratio of Nifedipine in the Rat.

Expected Blood Concentration	Actual Blood Concentration Measured[a]	Blood-to-Plasma Ratio (BP_r)[a]
200 ng mL^{-1}	245.6 ± 29.8 ng mL^{-1}	0.60 ± 0.05
5 μg mL^{-1}	4.9 ± 0.6 μg mL^{-1}	0.60 ± 0.06
20 μg mL^{-1}	18.6 ± 0.8 μg mL^{-1}	0.56 ± 0.05

[a] Data are expressed as means ± *s* (n = 5 samples).

Hepatic nifedipine blood clearance (Cl_{h_b}) was calculated to be about 17.5 mL min^{-1} kg^{-1}, using the expression:

$$Cl_{h_b} = Cl_{hp}\frac{[Plasma]}{[Blood]} = \frac{Cl_{hp}}{BP_r} \tag{8.1}$$

where, Cl_{hp} is the hepatic plasma clearance. The value of E_h can be derived from the expression:

$$E_h = \frac{Cl_{h_b}}{Q_h} \tag{8.2}$$

From equation 8.2, E_h in the rat was calculated to be in the range of 0.22 to 0.32 using Q_h values of 55 and 80 mL min^{-1} kg^{-1}, respectively.

The systemically available fraction (F) of a po dose is determined from the expression:

$$F = F_l \bullet F_g \bullet F_h \tag{8.3}$$

where, F_l is the fraction of intact (not decomposed) drug absorbed from the gut lumen; F_g is the fraction of the absorbed dose escaping destruction within the walls of the GI tract and passing into the portal vein; and F_h is the fraction of hepatically available drug escaping liver

extraction (see Figure 8-2).[28] In rat, the absorption of nifedipine from the GI tract is rapid and relatively complete (> 90%).[4] In addition, to our knowledge, nifedipine has not been shown to decompose in the lumen of the GI tract of any animal species. Hence, assuming F_l and F_g are unity, the predicted F of the drug was calculated from:

$$F = F_h = 1 - E_h \qquad (8.4)$$

which gives a range of values between 0.68 to 0.78 (using the values of E_h determined above). In this study, however, F was found to be lower (Table 8-1) than predicted, indicating that extrahepatic first–pass metabolism may be involved, although incomplete drug absorption from the GI tract cannot be completely ruled out. Similarly, Kondo and Sugimoto[16] report that nifedipine F after intraduodenal administration in male Wistar rats averaged between 0.52 and 0.57; whereas, systemic nifedipine clearance averaged only 5.2 mL. $min^{-1} kg^{-1}$ (using iv doses of 0.025 to 0.1 mg kg^{-1}) from which F values of 0.84 to 0.89 would be predicted.

Comparison of po and ip routes of administration of a drug allows an indirect method for estimating the relative contribution of the intestine to first–pass metabolism (see Figure 8-2).[29,30] Similarly, comparison of po and ic routes of administration allows an indirect method for estimating the relative contribution of the small intestine versus the colon to first–pass extraction of a drug.

Administration via the po route subjects a drug to potential first-pass elimination by the gut and liver; whereas, the majority (> 90%) of an ip dose of a drug is absorbed into the hepatoportal circulation and only undergoes hepatic extraction.[29,30] After ip dosing, the resulting value for F can be used to estimate E_h by rearrangement of equation 8.4, assuming that hepatic extraction is not saturated. In this study F was 0.90 after ip dosing (Table 8-1); hence, E_h was estimated as 0.10 which is lower than the range reported above using equation 8.2. However, the variability in systemic plasma clearance between rats introduces a degree of uncertainty in the estimation of E_h which probably accounts for the discrepancy found. Nevertheless, the results confirm that the poor po bioavailability of nifedipine cannot be entirely accounted for by hepatic extraction.

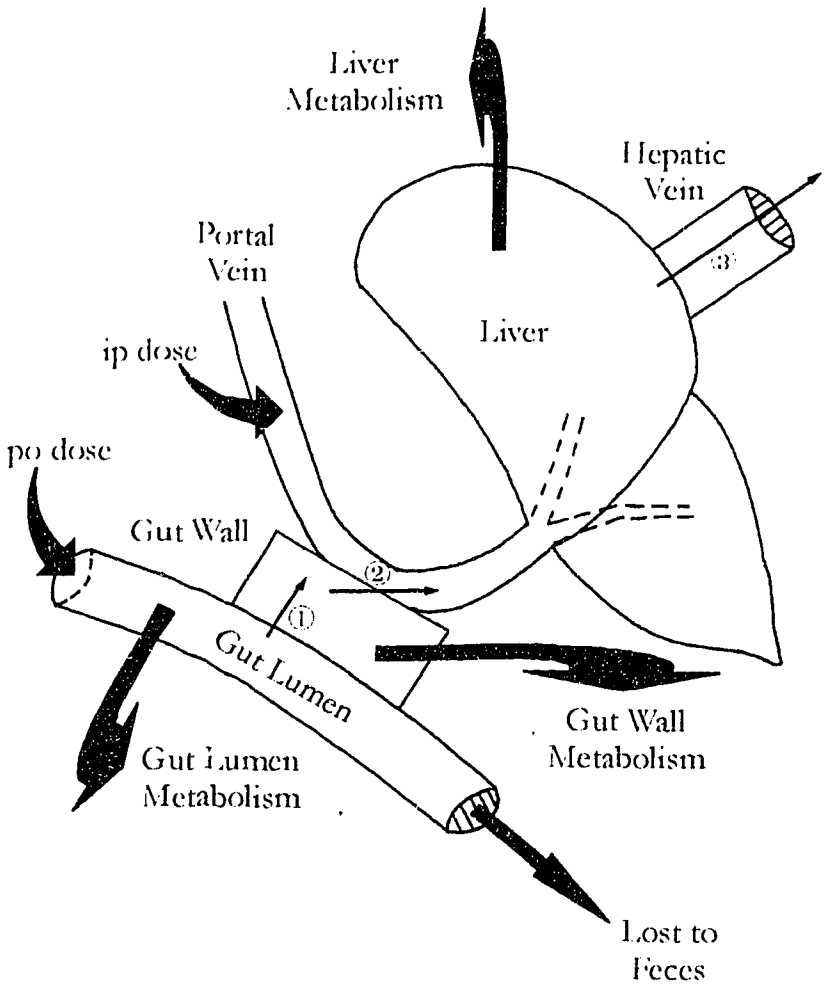

Figure 8-2. An illustration showing the potential sites of drug loss and drug metabolism that can limit the systemic availability of pharmaceutical compounds dosed by peroral (po) and intraperitoneal (ip) routes of administration. ① Fraction of the (intact) drug dose absorbed from the gut lumen (F_1); ② Fraction of the drug dose absorbed into the hepatoportal circulation ($F_1 \bullet F_2$), where F_g is the fraction of the absorbed dose that passes into the portal vein; ③ Fraction of the drug dose systemically available ($F_1 \bullet F_g \bullet F_h$), where F_h is the fraction of the hepatically available drug that passes into the hepatic vein.

The colon, including the caecum and rectum, in the adult white rat is about 21 to 27 cm in length.[31,32] Considering the total combined lengths of the proximal colon (5.5 to 6.5 cm), major flexure (3 to 3.5 cm), distal colon (4.5 to 6.5 cm) and rectum (3 to 3.5 cm);[31] therefore, a 15 cm length of PE–100 tubing was inserted rectally into adult male Sprague-Dawley rats to deliver nifedipine into the proximal colon (i.e., for the ic dosing experiments).

The majority of a nifedipine dose administered *via* the rat proximal colon (ic dosing) would be expected to be absorbed into the hepatoportal circulation and undergo hepatic

extraction; however, a significant portion of the dose could escape hepatic first–pass metabolism by spreading to the rectum where it could be absorbed directly into the general circulation.[16] In addition, metabolic activity of CYP isoenzymes within the gut wall is generally higher in the duodenum and jejunum than the ileum and colon of humans and rats.[19,33-35] Hence, nifedipine F after ic dosing would be expected to exceed F after po dosing, and be equal to or greater than F after ip dosing assuming complete absorption of intact drug. In fact, in this study F was about unity after ic dosing (see Table 8-1) suggesting no or little colonic wall metabolism (as expected). Additionally, either a large portion of the ic dose bypassed hepatic extraction, or *alternatively*, hepatic presystemic extraction was too small to be detected in this group of rats given the variability observed in nifedipine clearance. These results also suggest that none of the ic nifedipine dose was lost in the feces — i.e., nifedipine absorption from the colon was complete.

Until relatively recently it was believed that only the liver, and not the intestinal mucosa, contained a sufficient amount of CYP enzymes to affect drug bioavailability.[29,36] However, metabolic and pharmacokinetic studies with drugs such as cyclosporine, L-dopa and flurazepam show that significant biotransformation can occur within enterocytes of the intestinal wall of rats and humans.[33,37-40]

To date, conflicting evidence exists for gut wall metabolism of 1,4–dihydropyridine calcium channel antagonists in rats. Wang et al.,[30] showed that felodipine, a second–generation 1,4–dihydropyridine calcium channel antagonist, undergoes substantial first–pass elimination by the intestine of male Sprague-Dawley rats. However, Elinois et al.[41] showed that in vitro and in vivo metabolism of oxodipine, another 1,4–dihydropyridine calcium channel antagonist, was negligible in rats. Gut wall metabolism has also been speculated to contribute to the first–pass elimination of nifedipine in human beings.[21,42-47] Most of the available evidence comes from studies in which nifedipine is coadministered with grapefruit juice, showing increased bioavailability of nifedipine after po but not iv dosing.[43-47] Considering that the mechanism responsible for the interaction of grapefruit juice with 1,4–dihydropyridine compounds has not been fully established, the rat may be a useful model with which to study this effect.

The CYP catalyzed oxidation of nifedipine in the rat is mediated by the isozymes 3A and 2C in the rat (also referred to by their common names PCN–E and UT–A, respectively); whereas, in human beings nifedipine is oxidized solely by CYP 3A.[48] Hence, this and other

potential differences in the absorption and disposition of nifedipine mean the results of this study cannot be necessarily extrapolated to human beings. However, the intestinal mucosa in humans may partially contribute to the metabolism of nifedipine and may explain some of the inter– and intrasubject variability in systemic availability of this drug and could play a role in some observed food and drug interactions (e.g., grapefruit juice).

In conclusion, the results of this study showed that nifedipine F in the rat is affected by both gut wall (small intestine) and hepatic presystemic extraction after po administration. Relatively low extraction of nifedipine by the rat liver was confirmed and no evidence of first–pass metabolism within the wall of the proximal colon was observed after ic dosing. These findings suggest that the rat is a suitable model with which to investigate CYP mediated metabolism of drugs in the intestinal mucosa and to further study the mechanism(s) of various food and drug interactions.

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Chapter 9

GRAPEFRUIT JUICE AND ORANGE JUICE EFFECTS ON THE BIOAVAILABILITY OF NIFEDIPINE IN THE RAT*

Introduction

Grapefruit and its juice are common constituents of a healthy balanced human diet and are typically consumed at breakfast; however, grapefruit–drug interactions may occur since drugs are also often taken in the morning. Grapefruit juice has been shown to augment the bioavailability or plasma concentrations of many different classes of compounds in human beings and rats[1-13] — including the 1,4–dihydropyridine class of calcium channel antagonists (e.g., nifedipine, felodipine, nimodipine, nisoldipine, nitrendipine).[1,14-21] However, to date, the inhibitory substance(s) or mechanism(s) of action of grapefruit juice has not been conclusively identified and the effects are not duplicated by orange juice.[1]

It is speculated from the results of in vitro studies[9,25-28] that bioflavonoids (e.g., naririutin, hesperidin, naringin, neohesperidin, quercetin, kaempferol) could inhibit in vivo cytochrome P450 (CYP) isozyme mediated drug oxidation (including CYP3A4, CYP1A2 and CYP2A6) via the intestinal wall or the liver.[1,5] Bioflavonoids are naturally occurring phenolic substances with a bitter or non–bitter taste (depending on the structural arrangement of the sugars rhamnose and glucose[29]), and are found in many fruits and vegetables including the grapefruit and orange.[30] However, citrus fruits and their juice contain specific bioflavonoids that are relatively uncommon in the plant kingdom.[29] Naringin, a flavanone neohesperidoside, is the major bioflavanoid found in grapefruit juice and is responsible for this juices characteristic bitter taste, but interestingly naringin is not found in orange juice[1,29,31] — note: a review by Kühnau[30] lists naringin as a component of sweet orange and Drawert et al.[32] reports that naringin is found in orange juice; however, a more recent study by Rouseff et al.[31] using a sensitive HPLC method argues convincingly against these claims. Several in vivo

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studies[1,16,17,27] show no or little evidence of an interaction between individual flavonoids (including naringin, quercetin and kaempferol) and different 1,4–dihydropyridine compounds; nonetheless, naringenin, the aglycone and human metabolite of naringin, could be the inhibitory substance responsible for the 'effect' of grapefruit juice.[1,33] Cleavage of the sugar moiety, presumably by intestinal bacteria or possibly by gut wall or hepatic enzymes, is the first step in naringin metabolism to naringenin in vivo.[33] In vitro inhibition of nifedipine metabolism by naringenin in liver microsomes has been demonstrated, however greater inhibition was found in rat compared to human liver preparations.[28] The differences between CYP isozymes in rat and humans may explain these results. Alternatively, it has also been speculated that urodiolenone may play a role in grapefruit juice–drug interactions[34,35] — note: urodiolenone is not a bioflavonoid but a dihydroxy derivative of the sesquiterpene, nootkatone; both urodiolenone and nootkatone are constituents of grapefruit.

In both human beings and rats, nifedipine is well absorbed from the gut lumen (≥ 90%).[36-42] After absorption, nifedipine is metabolized by oxidative mechanisms — involving CYP 3A isozymes — to a pharmacologically inactive nitropyridine analogue, which is subsequently metabolized to more polar compounds.[43-45] The absolute bioavailability of nifedipine is similar in both humans and rats, ranging from about 0.40 to 0.68.[39,40,46-49] A previous study from this laboratory using male Sprague–Dawley rats (see Chapter 8) indicated that, in addition to hepatic extraction, nifedipine undergoes substantial extrahepatic first–pass elimination presumably within the wall of the small intestine.[49] Similarly in man, enzymes located both in the gut wall and liver are thought to be involved in the interaction of grapefruit juice with felodipine.[50] Miniscalco et al.[28] showed that naringenin, quercetin and kaempferol separately inhibited 1,4–dihydropyridine metabolism [nifedipine and (R)– and (S)–felodipine] in an in vitro study using rat liver microsomes. Hence, these observations suggest that the rat may be a good model species with which to further investigate the in vivo interaction of grapefruit juice with nifedipine. In fact, a preliminary in vivo study from our laboratory[14] indicates that grapefruit juice concentrate (GJC) delays nifedipine absorption and may increase nifedipine bioavailability in rats.

In man, grapefruit juice does not affect systemic nifedipine clearance;[24] assuming the same holds true for rats, the mechanism(s) by which GJC affects nifedipine absorption and bioavailability in the rat could involve: (1) delaying gastric emptying and subsequently inhibiting nifedipine first–pass metabolism in the small intestinal wall or liver (or both); or (2)

delaying absorption until the drug reaches the large intestine — where drug bioavailability may increase due to the absence of gut wall presystemic metabolism.[49] The mechanism(s) of action of GJC might be partially resolved by comparing GJC with orange juice concentrate (OJC) which has a similar consistency and sugar content but lacks inhibitory effects on nifedipine presystemic metabolism,[1] and also by administration of regular strength solutions of each juice, both of which should not influence gastric emptying to a significant extent.

Based on these considerations, a bioavailability study was conducted with different groups of adult male Sprague–Dawley rats comparing standard pharmacokinetic measures of nifedipine given by the peroral (po) route when coadministered with either GJC, OJC, grapefruit juice regular strength (GJRS), orange juice regular strength (OJRS) or (tap) water.

Materials and Methods

Chemicals

Nifedipine powder was purchased from Sigma Chemical Company (St. Louis, MO, USA). Nisoldipine powder [Internal Standard (IS)] was obtained from Miles Canada Inc. (Etobicoke, ON, Canada). Unsweetened grapefruit juice and orange juice pure frozen concentrates (sugars: 0.47 g mL^{-1} and 0.49 g mL^{-1}, respectively; Bel Air®, Lucerne Foods Ltd, Vancouver, BC, Canada) were purchased from a local supermarket and contained no artificial colours, flavours or preservatives. Other compounds: heparin (Hepalean®, 1000 U.S.P. units mL^{-1}) was supplied by Organon Teknika Inc. (Toronto, ON, Canada); sodium pentobarbital (Somnotol®, 65 mg mL^{-1}) was purchased from MTC Pharmaceuticals (Cambridge, ON, Canada); and polyethylene glycol–300 and –400 (Carbowax® Sentry®, FCC grade) was obtained from Union Carbide Chemicals (Danbury, CT, USA). All other chemicals and solvents used in this study were either reagent or HPLC grade and obtained from commercial suppliers.

Animals and Surgical Cannulation Procedure

From the Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada), adult male Sprague–Dawley rats (initial weight 260 to 310 g) were obtained and housed for at least 2 days in a clean room and fed food and water *ad libitum*. The day prior to dosing experiments, rats were moved to the laboratory and subjected to surgical cannulation. During anesthesia induced by sodium pentobarbital (65 mg kg^{-1}), a small longitudinal incision

was made in the skin of each rat over the right jugular vein, which was then made accessible by clearing the surrounding tissues. The vein was catheterized with Silastic® laboratory tubing (0.635 mm i.d., 1.194 mm o.d., Dow Corning Corp., Midland, MI, USA) that contained heparinized (100 IU mL^{-1}) normal saline and fixed in place with two nonabsorbable surgical sutures (Surgical Suture USP, Cyanamid Canada Inc., Montreal, QC, Canada). Each cannula was terminated with a suitable length of polyethylene tubing (PE-50, i.d. 0.58 mm, o.d. 0.965 mm, Clay Adams, Parsippany, NJ, USA) and the free end exteriorized to the dorsal side of the neck. The exposed areas were then closed using nonabsorbable surgical suture. Each rat was allowed to recover for the next 16 to 20 hours in a metabolic cage and provided only drinking water for the remainder of the experiment. The implanted cannulas permitted frequent blood sampling from each rat.

Pharmacokinetic Study: Experimental Design

All drug preparation, dosing and collection of blood samples was done under sodium lamps to prevent nifedipine photodegradation.[51] A nifedipine dosing solution (5 mg mL^{-1} in polyethylene glycol–400) was prepared the day before each experiment to allow sufficient time for drug solubilization, wrapped in aluminum foil to protect the solution from light, and stored at room temperature. On experimental days, cannulated rats received 6 mL of GJC, GJRS, OJC, OJRS or (tap) water by an oral feeding tube over approximately 15 seconds. Exactly 15 minutes later, each rat received a single po dose of nifedipine (6 mg kg^{-1}) also given by an oral feeding tube. About 120 minutes after the nifedipine dose, the rats received a further 3 mL of GJC, GJRS, OJC, OJRS or (tap) water, as described above. No adverse events or symptoms in the rats were observed, before or after nifedipine dosing or as a result of administration of any of the 5 treatments. Blood draws (0.25 mL samples) were made at predetermined times (see below) using heparinized 1 mL syringes connected to the jugular cannula. The blood volume drawn was immediately replaced with an equal volume of normal saline. Blood samples were centrifuged [Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA, USA)] at 15,000 rpm for 4 minutes and the plasma samples obtained were placed in 1.5 mL poly(propylene) microfuge tubes, stored in light resistant bags and kept at –20°C until needed for analysis.

(1) Grapefruit Juice Concentrate Administration:

Six rats (295.5 to 325.5 g) received both GJC (undiluted) and nifedipine perorally as previously described. Blood samples were drawn at –5, 15, 30, 60, 90, 120, 240, 360, 480, 600, 720, 840 and 1440 minutes — the time of nifedipine dosing was designated 0 minutes.

(2) Grapefruit Juice Regular Strength Administration:

Six rats (295.0 to 330.5 g) received both GJRS [GJC diluted 1 : 3 with (tap) water] and nifedipine perorally as previously described. Blood samples were drawn at –5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480 and 600 minutes — the time of nifedipine dosing was designated 0 minutes.

(3) Orange Juice Concentrate Administration:

Six rats (303.5 to 354.5 g) received both OJC (undiluted) and nifedipine perorally as previously described. Blood samples were drawn at –5, 15, 30, 60, 90, 120, 240, 360, 480, 600, 720, 840 and 1440 minutes — the time of nifedipine dosing was designated 0 minutes.

(4) Orange Juice Regular Strength Administration:

Six rats (302.5 to 339.0 g) received both OJRS [OJC diluted 1 : 3 with (tap) water] and nifedipine perorally as previously described. Blood samples were drawn at –5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480 and 600 minutes — the time of nifedipine dosing was designated 0 minutes.

(5) (Tap) Water Administration:

Six rats (315.5 to 334.5 g) received both (tap) water and nifedipine perorally as previously described. Blood samples were drawn at –5, 2, 5, 10, 15, 30, 60, 90, 120, 240, 300, 360, 480 and 600 minutes — the time of nifedipine dosing was designated 0 minutes.

Chromatography

Nifedipine concentrations in rat plasma samples were determined by means of an established HPLC method (lower limit of quantitation 5 ng mL^{-1})[52] using 0.1 to 0.2 mL samples diluted to 1.0 mL with HPLC–grade water. All analyses were conducted under sodium lamps to prevent photodegradation of nifedipine.[51] Calibration curve standards were prepared by adding a known amount of the drug to 0.1 mL of blank rat plasma and diluting to 1.0 mL as described above. The calibration curve (5 to 2000 ng of nifedipine) was

determined from the best–fit regression line calculated using a $1/x^2$ weighting factor (where x corresponds to the amount of nifedipine added; $r^2 > 0.99$; interday variability < 10%). HPLC instrumentation included: a Model 600E solvent delivery system, a Model 717 autosampler; a Model 486 tunable UV–VIS absorbance detector (set at 350 nm); and an NEC 486–33 MHz computer running Millennium 2010 chromatography manager software Version 1.1 (Waters Ltd., Mississauga, ON, Canada).

Pharmacokinetic Analysis

Standard pharmacokinetic measures from each of the individual rat plasma concentration–time profiles were calculated by non–compartmental methods using the computer program: WinNonlin Standard Edition Version 1.0 (Scientific Consulting Inc., Apex, NC, USA) — note: area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule from time zero (t_0) to the time of the last quantifiable concentration (t_{last}), followed by extrapolation to infinity. The apparent systemic bioavailability (F) after po nifedipine administration was determined according to the expression:

$$F = \frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = \frac{CL_{iv}}{CL_{po}} \qquad (9.1)$$

where, AUC_{iv} and AUC_{po} were the areas under the plasma concentration–time curve after iv and po dosing, respectively, $Dose_{iv}$ and $Dose_{po}$ were the respective iv and po doses given, and CL_{iv} and CL_{po} were systemic (iv) nifedipine plasma clearance and po nifedipine plasma clearance, respectively.

Statistical Analysis

All the calculated pharmacokinetic parameters — except t_{max} — were assumed to follow a log–normal distribution, and were log–transformed before statistical analysis. The pharmacokinetic parameter estimates obtained were expressed as either geometric or arithmetic means with their corresponding 95% confidence intervals, as appropriate. Pharmacokinetic data were analyzed using independent measures one–way analysis of variance (ANOVA) and a post hoc test (Duncan's multiple range test) was used to determine where, if any, significant differences occurred ($P \leq 0.05$). The computer program SPSS for Windows Version 6.1 (SPSS Inc., Chicago, IL, USA) was used for all the statistical tests. For

the purposes of generating plasma concentration–time profiles, plasma nifedipine concentrations were expressed as arithmetic means ± standard error ($s_{\bar{x}}$).

Results and Discussion

Administration of relatively large volumes of the treatment solutions [i.e., GJC, GJRS, OJC, OJRS and (tap) water], and the timing of the treatments in this study were done to maximize any potential inhibitory effects on nifedipine presystemic metabolism and to ensure their presence in the gut throughout the period of nifedipine absorption. The volumes that were used would not be expected to harm the animals as typical water consumption of rats is about 80 to 110 mL kg^{-1} day^{-1},[53] and a safe intragastric injection volume in rats (250 g) is reported to be about 5 mL (depending, however, upon the degree of stomach filling).[54] Additionally, it is reported that a 300 g rat can be tube fed 13 mL of water at a time and trained to take up to 26 mL.[53] With regard to the timing of treatment administration used in this study, Lundahl et al.[50] studied the relationship between the time of intake of grapefruit juice and the pharmacokinetic effect on felodipine in human subjects. The effect of grapefruit juice was apparently instantaneous with regard to increasing the AUC and C_{max} of felodipine, with similar and maximal effects seen when grapefruit juice was given 0 and 1 hour before felodipine dosing, and reduced but significant effects seen even when grapefruit juice was given 10 hours earlier compared to controls (note: felodipine C_{max} was also significantly increased 24 hours after grapefruit juice dosing). Thus, the administration schedule used in the current study should have allowed for near optimal effects.

The mean ($\pm s_{\bar{x}}$) plasma nifedipine concentration–time profiles obtained in the different groups of rats, given one of the 5 treatments previously described, are shown in Figure 9-1. Individual and mean profiles for the GJRS, OJRS and (tap) water groups displayed single–peaks, whereas the GJC and OJC groups displayed double–peaks. The first peak in the GJC group exhibited the lowest peak nifedipine concentration (all 6 rats), but the opposite was true in most rats of the OJC group (4 of 6 rats). Nonetheless, the area under curve of the first peaks were about 2.6% and 16.7% of the total areas under the mean curves for the GJC and OJC groups, respectively. This indicates that the majority of nifedipine absorption was significantly delayed in both GJC and OJC treatments, and the position of the second peak (Figure 9-1) showed that the delay was of about the same extent. Mean nifedipine plasma

concentration–time profiles also showed more variability in the GJC and OJC groups which was expected given the anticipated variability in gastric emptying or colonic arrival times.

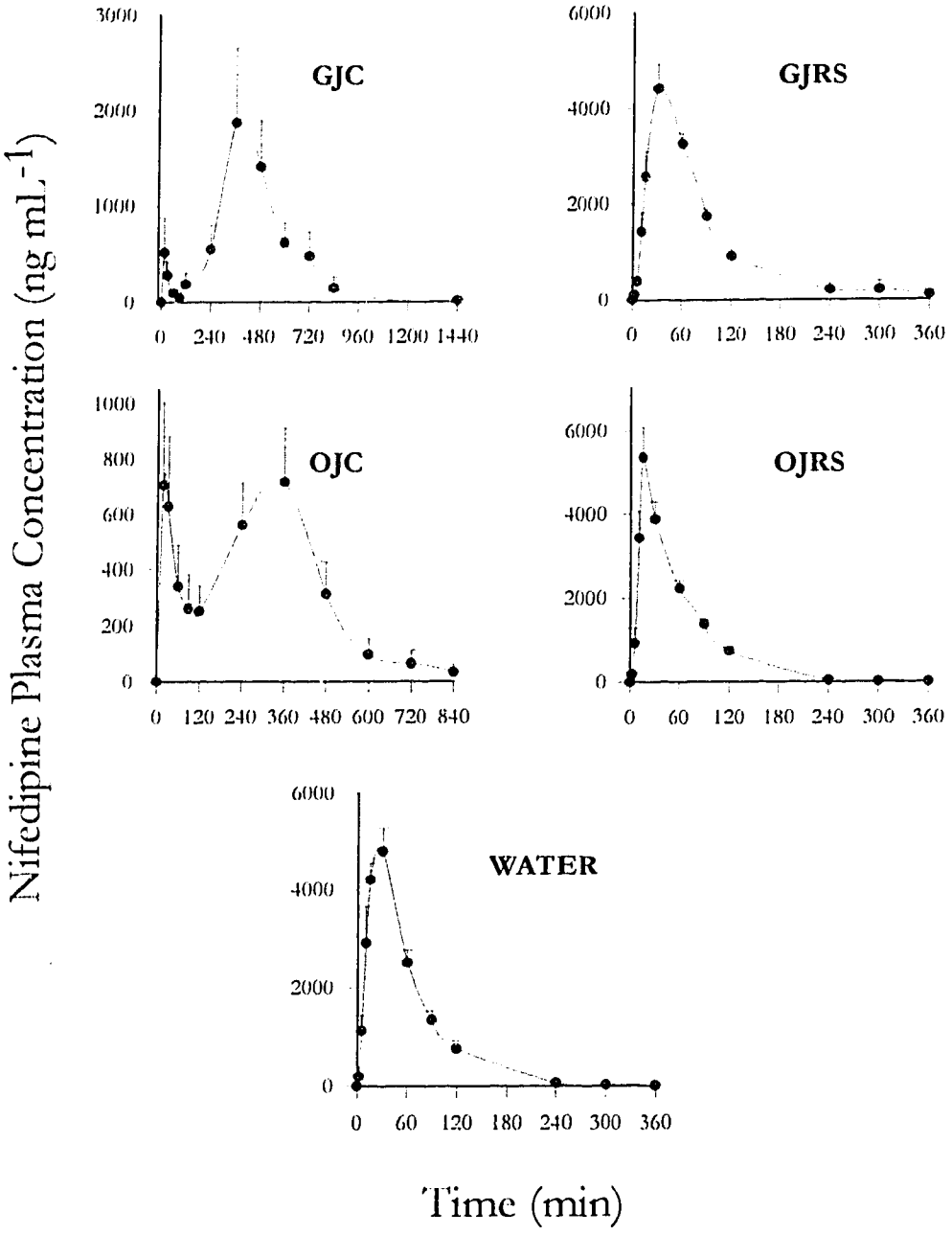

Figure 9-1. Plasma concentration-time profiles in male Sprague-Dawley rats after a single peroral dose of nifedipine (6 mg kg^{-1}) was given to rats in the following treatment groups: grapefruit juice concentrate (GJC), grapefruit juice regular strength (GJRS), orange juice concentrate (OJC), orange juice regular strength (OJRS), and (tap) water. Data are mean values $\pm$ $s_{\bar{x}}$ (n = 6 animals per group).

Standard pharmacokinetic measures of nifedipine bioavailability calculated from the data obtained from all 5 treatments are shown in Table 9-1 and significant differences, where found, are noted. Nifedipine oral clearance found in the (tap) water group (see Table 9-1) was similar to that found in a group of rats administered po nifedipine [(tap) water given *ad libitum*] from a previous study (i.e., 17.0 mL. min^{-1} kg^{-1}, n = 6).[49] Oral nifedipine clearance for the GJC group alone was significantly lower than the (tap) water group. In the GJRS group, oral clearance was not significantly decreased but the geometric mean value was lower than (tap) water, OJC and OJRS groups.

Table 9-1. Pharmacokinetic parameters obtained following peroral administration of nifedipine (6 mg kg^{-1}) to rats coadministered grapefruit juice, orange juice or (tap) water treatment regimens.[a,b]

	Treatment Regimen				
Parameters	**GJC**	**GJRS**	**OJC**	**OJRS**	**(Tap) Water**
AUC (μg mL^{-1} min)	700 (450 - 1088)[c]	393 (335 - 460)[d]	274 (213 - 354)[e]	327 (280 - 383)[d,e]	345 (283 - 419)[d,e]
C_{max} (ng mL^{-1})	2097 (1106 - 3978)[f]	4657 (3976 - 5453)[g]	1223 (899 - 1664)[h]	5563 (4260 - 7264)[g]	5230 (4548 - 6013)[g]
t_{max} (min)	443 (132 - 753)[i]	35 (22 - 48)[j]	126 (0 - 276)[j]	16 (8 - 24)[j]	23 (12 - 34)[j]
$t_{1/2}$ (min)	70 (39 - 127)[k]	37 (27 - 51)[k,m]	56 (30 - 106)[k,l]	31 (26 - 37)[m]	31 (25 - 38)[m]
Cl_p/F (mL. min^{-1} kg^{-1})	8.6 (5.5 - 13.3)[n]	15.3 (13.0 - 17.9)[o]	21.9 (17.0 - 28.2)[p]	18.3 (15.7 - 21.4)[o,p]	17.4 (14.3 - 21.2)[o,p]
F [relative to (tap) water][q]	2.02	1.14	0.79	0.95	1.0
F (relative to iv bolus)[r]	1.20	0.67	0.47	0.56	0.59

[a] Where appropriate, data are expressed as geometric means with corresponding 95% confidence intervals (CI, n = 6 animals per group).

[b] t_{max} is presented as arithmetic mean with 95% CI (n = 6 animals per group).

[c-p] Identical superscript characters identify non-significant differences ($P > 0.05$).

[q] Calculated by substituting Cl_p/F [(tap) water] for Cl_{iv} in equation (1).

[r] Calculated with equation (1) using a value of systemic nifedipine clearance calculated from a previous study [geometric mean (95% CI): 10.3 (8.3 - 12.7) mL. min^{-1} kg^{-1}, n = 6].[49]

Abbreviations: AUC= area under the plasma drug concentration-time curve from zero to infinity; Cl_p/F= oral plasma clearance of nifedipine; C_{max}= maximum (peak) plasma drug concentration; F= systemically available fraction of the dose of a drug; GJC= grapefruit juice concentrate; GJRS= grapefruit juice regular strength; iv= intravenous; OJC= orange juice concentrate; OJRS= orange juice regular strength; t_{max}= time to reach the maximum (peak) drug concentration following extravascular drug administration; $t_{1/2}$= elimination half-life of the drug associated with the terminal slope of a semilogarithmic plasma drug concentration–time curve.

Bioavailability of nifedipine in the GJC group was about double that of the (tap) water group. Absolute bioavailability of nifedipine coadministered with GJC exceeded 1.0. Although inhibition of systemic plasma nifedipine clearance cannot be ruled out, this result

was probably due to the wide confidence intervals of the clearance values used [equation (1)] and the fact that iv data was obtained from another study[19] (i.e., interstudy variability). The determination of relative and absolute bioavailabilities in this report assumes that nifedipine systemic clearance was unchanged by each treatment and was similar in both studies. Support for the former assumption, comes from human studies in which grapefruit juice did not alter the kinetics of an iv nifedipine dose but increased the AUC and bioavailability of nifedipine given po, and by the fact that the elimination half–life of the drug was unchanged.[24] Similarly, grapefruit juice did not effect the systemic clearance of cyclosporine or midazolam but did improve oral bioavailability of both agents suggesting that its mechanism of action is to increase absorption or reduce gut wall metabolism.[7,8] The high absolute bioavailability found suggests that both hepatic and extrahepatic presystemic metabolism were inhibited by GJC, or that a large portion of the dose bypassed presystemic metabolism [via absorption in the lower gastrointestinal (GI) tract]. Surprisingly, GJRS did not significantly increase nifedipine bioavailability in rat (although a small increase was found) as has been shown with regular or double–strength grapefruit juice in human beings,[1,19,22,24] indicating that a relatively large dose of the inhibitory substance(s) in grapefruit juice is required to achieve an effect in rat (i.e., GJC). This is in contrast to in vitro findings with naringenin which show that rat liver microsomal metabolism of nifedipine is inhibited more than in human preparations.[28]

In addition to its effect on nifedipine bioavailability, GJC significantly increased the t_{max} found for the drug compared to all other groups (Table 9-1), corroborating the findings of a previous study.[14] The apparent increase in nifedipine t_{max} observed for OJC was not significantly different than (tap) water or regular strength juice groups. However, this was a result of the double–peaking phenomenon previously described. Hence, if the first peak in the OJC rat profiles had been excluded when determining t_{max} then a significant difference would have been observed. The regular strength solutions of both juices did not significantly alter t_{max} from that of (tap) water, and these values were not statistically different from that reported in a previous study (see Chapter 8) for po dosing in a group of rats fed water *ad libitum* [t_{max} (95% CI) = 16.7 (9.5 - 23.8) minutes]. Interestingly, GJRS has been shown to postpone the peak time of po administered cyclosporine, midazolam, quinidine and triazolam in human subjects.[7,8,13,55] Variable effects on t_{max} of 1,4–dihydropyridines (nifedipine,

felodipine, nisoldipine) by regular or double–strength grapefruit juice have been reported in humans including reduced,[17] none[15,16,21,50] or increased[18,19,22] effects.

The presence of a double–peak phenomenon in the nifedipine plasma concentration–time profiles, observed with GJC and to a greater extent with OJC is interesting. The reason for the larger double–peak effect with OJC is not clear at present, as both juices are of similar consistency and composition (except for the differences previously noted). A double–peak phenomenon has been observed for several drugs administered orally such as cimetidine, L-dopa, morphine, pafenolol and others.[56] Several potential explanations for this phenomenon could include: enterohepatic circulation, pH–dependent solubility, different permeabilities or extents of presystemic metabolism in various regions of the GI tract, or interruption of gastric emptying. Lack of a double–peak phenomenon in the (tap) water or regular strength juice groups would tend to rule out the possibility of enterohepatic circulation. As well, nifedipine is rapidly, extensively and presumably irreversibly metabolized via oxidation in the rat, with the principle metabolite further metabolized to several other biotransformation products which themselves may undergo enterohepatic circulation.[36,37] Given that the pKa–value (basic) of nifedipine is –0.9,[57] the drug exists primarily in its unprotonated form at all pH conditions in the GI tract and thus pH–dependent solubility and absorption can be ruled out. The existence of specific sites of absorption ('absorption windows') for nifedipine can also be ruled out since the drug is well absorbed (> 90%) throughout the upper and lower intestine in both rats and man,[38,49,58] although differences in the extent of gut wall metabolism in different regions of the gut could contribute, in part, to the observed double–peak effect.[49] Therefore, it appears that the most likely explanation for the results found was an alteration of gastric emptying by both GJC and OJC.

Commonly used laboratory animals display a cyclic gastric motility pattern (i.e., the unfed state has several phases which repeat every 2 hours and empty the stomach contents), but in the fed state gastric retention can be several hours.[59] In the rat, gastric emptying of liquids is relatively rapid as shown by a study were gastric–emptying of ^{131}I–polyvinylpyrrolidone was 75% complete within 15 minutes.[59,60] Therefore, following coadministration of a nifedipine solution with (tap) water or regular strength juice, rapid absorption of drug from the small intestine can be expected (see Table 9-1). When a nifedipine solution is coadministered with grapefruit and orange juice concentrates (both relatively viscous solutions with high osmolarity), the effect on gastric emptying rate is more difficult to predict since the exact

mechanism of inhibition by liquid and solid meals is not yet known in rats or humans.[61] Human studies have shown gastric emptying to be delayed by hot meals, fatty foods, high protein or carbohydrate meals, and viscous solutions.[61] As well, citric acid is used to inhibit gastric motility in ^{13}C–urea breath tests for detection of Helicobacter pylori infection.[62,63] On the other hand, large fluid volumes can increase gastric emptying rate.[61]

If it is assumed that GJC and OJC did not significantly affect gastric emptying, then the delayed absorption of nifedipine observed would have to involve a physical effect, whereby nifedipine is incorporated into the concentrate and prevented from being absorbed until the concentrate is digested, presumably in the small intestine or colon. The transit time of material through the rat small intestine is reported to take about 88 minutes,[61] and in another report[65] 50% of ^{125}I–PVP given intraduodenally passed through the small intestine in 20 minutes. Small intestinal transit of a ^{14}C–PEG4000 saline solution (1 g L^{-1}) was found to be about 3 hours in unanesthetized rats.[56] Hence, under the conditions described and the kinetic profiles obtained in the GJC and OJC groups, one would expect the majority of the nifedipine dose to be absorbed from the colon in each case. Data from intracolonic and rectal nifedipine dosing in rats shows that bioavailability increases relative to po dosing.[46,49] However, the relative nifedipine bioavailability of the OJC group was less than the (tap) water group (Table 9-1), indicating that a physical effect by each of the concentrates on nifedipine absorption is unlikely (assuming nifedipine absorption was complete in all cases). This observation, taken with the finding of double–peaking, strongly indicates that both GJC and OJC affected the rate of gastric emptying in this study.

Interestingly, du Souich et al.[66] noted a lack of intestinal, hepatic or pulmonary presystemic metabolism of nifedipine in the rabbit, leading to speculation by the authors that nifedipine may be decomposed in the GI lumen of rats and humans to account for its poor oral bioavailability. The results presented here seem to contradict this theory, as nifedipine appears to be completely absorbed from the GI tract of rats when coadministered with GJC. This is despite the fact that GJC greatly delays the absorption of nifedipine which might be expected to increase any decomposition of the parent drug in the GI tract.

In conclusion, the results of this study show that GJC acts to inhibit presystemic metabolism of nifedipine (both hepatic and extrahepatic), and that both GJC and OJC slow the rate of gastric emptying in rats. As expected, OJC and OJRS did not significantly increase nifedipine bioavailability compared to (tap) water. Surprisingly, GJRS had no

significant effect on nifedipine bioavailability. Nonetheless, the suitability of the rat for in vivo investigation of the interaction of grapefruit juice with nifedipine was demonstrated.

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Chapter 10

GRAPEFRUIT JUICE DOES NOT INFLUENCE THE EXTENT OF IN VITRO NIFEDIPINE PROTEIN BINDING IN HUMAN PLASMA◆

Introduction

Grapefruit juice has been shown to increase the bioavailability of several drugs, including cyclosporine, midazolam, triazolam, terfenadine and the 1,4–dihydropyridine class of calcium channel blockers.[1-5] To date, the mechanism by which grapefruit juice exerts its effects on specific drugs has not been unequivocally established. The most probable and accepted theory is that grapefruit juice contains an active ingredient(s) specific to the juice — generally believed to be a bioflavonoid (e.g., naringin, quercetin) — that inhibits the activity of the cytochrome P450 isozymes responsible for the presystemic metabolism of various compounds.[1] However, in vivo studies investigating the interaction of specific drugs (known to interact with grapefruit juice) with certain bioflavonoids give either mixed results or show no evidence of an interaction.[1,6-8] Several explanations have been proposed for these findings[1] but none, to date, have been confirmed.

The bioavailability of nifedipine, a 1,4–dihydropyridine calcium channel blocker used in the treatment of essential hypertension and angina, is increased by grapefruit juice in human beings.[1,9-11] This compound undergoes intermediate extraction (E = 0.32 to 0.6);[12-14] hence, if it is assumed that the liver is the principle clearance organ, then absolute bioavailability (F) of the compound is influenced by hepatic intrinsic clearance (CL_i), hepatic blood flow (Q_h) and plasma protein binding (fraction unbound, f_u):[15]

'well–stirred model' $$F = \frac{Q_h}{Q_h + f_u CL_i} \qquad (10.1)$$

'parallel–tube model' $$F = e^{-f_u CL_i / Q_h} \qquad (10.2)$$

◆ A version of this chapter has been submitted for publication: J.S. Grundy, R.T. Foster. *Biopharm. Drug Dispos.* (1996).

Grapefruit juice, however, should not significantly affect Q_h as it contains very little protein, and orange juice, which contains similar basic nutrients, does not affect nifedipine bioavailability.[1] As stated above, decreased hepatic (or possibly extrahepatic?) intrinsic clearance is the most likely explanation for the effects of grapefruit juice, but alteration of plasma protein binding (i.e., the extent or affinity of nifedipine binding to plasma proteins) is another possibility that has not yet been investigated. Interestingly, Kupferschmidt et al.[16] state that an effect of grapefruit juice on plasma protein binding of midazolam cannot be excluded as a possible explanation for the increased bioavailability observed with that compound.

The in vitro protein binding of nifedipine has been previously investigated[17-20] and the results found are relatively consistent (nifedipine is 91 to 99% protein bound); even when, for example, the concentrations evaluated (1200 ng mL^{-1}) exceed the therapeutic range (< 400 ng mL^{-1}).[18] Rosenkranz et al.,[17] however, reported that nifedipine binding in pooled serum was concentration–dependent (i.e., saturable) at very high concentrations, decreasing from 98% at 200 ng mL^{-1} to 92% at 20 μg mL^{-1}. Hence, as saturation of protein binding is approached it may be possible to obtain a general estimate of nifedipine affinity to plasma or serum proteins by graphical means (e.g., Rosenthal or modified Scatchard plot)[21] using a suitable range of nifedipine concentrations. A Rosenthal plot is useful in situations where the protein concentration is not known, and is based on the equation:

$$\frac{C_B}{C_F} = nK_a P_T - K_a C_B \qquad (10.3)$$

where C_B is the bound drug concentration, C_F is the free drug concentration, n is the number of binding sites, K_a is the association constant and P_T is the total protein concentration. Plotting C_B/C_F versus C_B typically yields a straight line (assuming one class of equivalent and independent protein binding sites) whose negative slope is the association constant K_a. The inverse of K_a is the equilibrium dissociation constant (K_d) which represents the concentration of unbound drug at which exactly one-half of the binding sites are occupied, and is analogous to the Michaelis constant (K_m) described for enzymatic metabolism. A potential limitation of this approach for studying nifedipine protein binding in particular, is the low water solubility of the compound (about 10 μg mL^{-1},[22-24] or less in phosphate buffer[25,26]); hence, if the K_d exceeds this value then saturable protein binding will not be possible. However, Otto et al.[18]

report a value of 2.12×10^5 L mol^{-1} as an estimate of K_a for nifedipine in human serum, from which an estimated K_d of 1.63 μg mL^{-1} can be calculated.

In this report, the protein binding of nifedipine in human plasma at physiological pH and temperature was measured with and without the presence of grapefruit juice concentrate (GJC, 2.5%, v/v). A suitable range of total nifedipine plasma concentrations were used to give unbound nifedipine concentrations below and above the assumed K_d, so that the binding affinity of nifedipine in each case could be adequately estimated.

Materials and Methods

Materials and Chemicals

Blank human plasma, collected less than one week prior to use, was obtained from the local Red Cross. Unsweetened grapefruit juice pure frozen concentrate (Bel Air®, Lucerne Foods Ltd., Vancouver, BC, Canada) was purchased from a local supermarket. Disposable ultrafiltration units (Centrifree® Micropartition System, maximum and minimum sample volumes: 1.0 mL and 0.15 mL, respectively) were obtained from Amicon Canada Ltd. (Oakville, ON, Canada) and contained a YMT membrane capable of retaining 99.9% of serum or plasma protein with a molecular weight cutoff of 30,000 daltons. Nifedipine was purchased from Sigma Chemical Company (St. Louis, MO, USA). Nisoldipine was provided as a gift from Bayer Canada Inc. (Etobicoke, ON, Canada). All other chemicals and solvents were either reagent or HPLC grade and obtained from commercial suppliers.

Sample Preparation

All procedures were performed under sodium lamps to prevent photodegradation of nifedipine.[27] Blank human plasma was thawed at room temperature immediately prior to use, and to 9.75 mL aliquots was added either 0.25 mL of phosphate (Sorensen)[28] buffer (1/15 M, pH 7.4) or 0.25 mL of grapefruit juice concentrate. Appropriate stock solutions of nifedipine in methanol were prepared so that nifedipine could be spiked into the plasma–buffer or plasma–grapefruit juice solutions to give a range of nifedipine concentrations (2, 10, 20, 30, 50, 75 and 150 μg mL^{-1}, 10 mL of each), while keeping the final methanol concentration < 1.0% (v/v).

Ultrafiltration

Protein binding studies were conducted at 37°C in a temperature–controlled room using ultrafiltration. Immediately prior to ultrafiltration, the pH of each nifedipine spiked solution (10 mL) was measured (Model 15 pH meter, Fisher Scientific, Nepean, ON, Canada) and adjusted to pH 7.4 ± 0.1 using a small volume (50 to 200 μL) of 1 N NaOH, if necessary. The ultrafiltration of 1 mL aliquots of each sample placed in the reservoir of each ultrafiltration unit was accomplished using a Dynac II centrifuge (Becton-Dickinson, Parsippany, NJ, USA) at 1850 g for a period of time required to obtain 0.3 to 0.7 mL of ultrafiltrate (about 45 minutes). This was done after the ultrafiltration units containing the samples were allowed to incubate at 37°C for 30 minutes. The reservoir of each unit was capped to prevent sample evaporation and pH changes during the ultrafiltration procedure. Ultrafiltrate was collected in a removable cup attached to the base of each ultrafiltration unit and all samples were visually inspected for a yellow appearance to determine if protein leakage (> 20%) had occurred. In addition, protein leakage < 0.5% was tested in a small number of samples chosen at random, i.e., to an aliquot of each sample chosen was added an equal volume of acetonitrile.[18] If turbidity or a precipitate formed then the sample was discarded — note: no evidence of protein leakage was found in any of the samples taken and, hence, none were discarded.

Determination of Adsorption of Nifedipine to the Ultrafiltration Unit or Membrane

Preliminary studies were initially conducted to determine the extent of non–specific binding of nifedipine to the ultrafiltration unit or membrane. Nifedipine spiked protein–free solutions [about 2 μg mL^{-1} of nifedipine in phosphate (Sorensen) buffer (1/15 M, pH 7.4)], 1 mL samples, were placed into the reservoir of the ultrafiltration units and left undisturbed for 1.25 hours at 37°C, or were ultrafiltered by the procedure described above. Nifedipine concentrations remaining in the protein–free buffer or ultrafiltrate solutions were compared with the initial protein–free buffer concentrations of nifedipine (see equation 10.5). Six replicates were performed in each case.

Evaporative Sample Loss During Ultrafiltration

The weight of each ultrafiltration unit was measured before loading with sample, and before and after centrifugation when loaded with sample. The percent evaporative loss (%*E*) was calculated using the equation:

$$\%E = \frac{(W_{BC} - W_{AC})}{(W_{BC} - W_{BL})} \bullet 100\% \qquad (10.4)$$

where W_{BL} is the weight of the filter unit before loading with sample, and W_{BC}, W_{AC} are the weights of the loaded filter unit before and after centrifugation, respectively. Ultrafiltrate concentrations were subsequently corrected for evaporative loss.

Chromatography

Nifedipine concentrations in the plasma–buffer, plasma–grapefruit juice, protein-free solutions and ultrafiltrate samples were determined by means of an established HPLC method (lower limit of quantitation 5 ng mL^{-1})[29] using 0.1 to 0.3 mL samples diluted to 1.0 mL with HPLC–grade water. All sample analyses were conducted under sodium lamps to prevent photodegradation of nifedipine.[27] HPLC instrumentation included: a Model 600E solvent delivery system, a Model 717 autosampler; a Model 486 tunable UV–VIS absorbance detector (set at 350 nm); and an NEC 486–33 MHz computer running Millennium 2010 chromatography manager software Version 1.1 (Waters Ltd., Mississauga, ON, Canada).

Determination of Protein Bound Fraction

The percent binding of nifedipine to plasma protein, %*B*, was determined using the expression:

$$\%B = \frac{C_T - C_{UF}}{C_T} \bullet 100\% \qquad (10.5)$$

where C_T was the total (bound and unbound) nifedipine concentration in each sample before ultrafiltration, and C_{UF} was the ultrafiltrate nifedipine concentration.

Results and Discussion

Loss of nifedipine due to non–specific binding to the ultrafiltration unit or membrane averaged less than 5% (Table 10-1). Hence, the degree of error in the calculations of %*B* resulting from non–specific binding was minimal (< 1%). These results are in contrast to the work of Zhirkov et al.[30] who questioned the usefulness of ultrafiltration in drug–protein

binding studies for the drugs nifedipine, nadolol, prazosin and verapamil. This group notes that severe non-specific adsorption can occur with the compounds tested using ultrafiltration units containing one of several different types of membranes, including YMT membranes. For assessment of nifedipine adsorption, they used initial nifedipine concentrations of 10 and 30 ng mL^{-1} in buffer or primary ultrafiltrate of serum, which are concentrations within the therapeutic range of the compound. Nifedipine concentrations in the filtrates of these solutions (filtered through YMT membranes) are non-detectable (< 2 ng mL^{-1}). Thus, these findings[30] and the results reported here could suggest that nifedipine adsorption to ultrafiltration units containing YMT membranes is saturable and is significant only for levels of nifedipine within or less than the therapeutic range. Interestingly however, Otto et al.[18] reports that non-specific adsorption of nifedipine in ultrafiltration units with YMT membranes is less than 8%, using phosphate buffered protein-free solutions containing nifedipine in the range 100 to 1200 ng mL^{-1}; hence, the problems noted by Zhirkov et al.[30] may be limited to a specific type(s) of ultrafiltration unit.

Table 10-1. Adsorption of nifedipine to the ultrafiltration unit or membrane [commas separate results from multiple analyses (n = 6)].

Initial Nifedipine Concentration (μg mL^{-1}) [Theoretical]	Initial Nifedipine Concentration (μg mL^{-1}) [Measured]	Bound to ultrafiltration unit or membrane (%)
Binding to ultrafiltration unit (1.25 hour incubation)		
2	1.6	-1.1, -0.7, 2.0, 13.1, 5.9, 2.5 **(3.6 ± 5.3)**
Binding to ultrafiltration membrane		
2	1.8	3.7, -5.2, 12.1, 3.7, 4.9, 7.1 **(4.4 ± 5.7)**

Evaporative loss of samples (%E) during centrifugation can potentially result in an underestimation of protein binding. Although adjustments to the ultrafiltrate concentrations observed were made based upon %E calculations using equation 10.4, the corrections made were very small since the mean %E found was negligible (0.6 ± 0.1%) in this study.

The mean ± s values of %B, with and without the presence of GJC, were found to be relatively constant (94.9 ± 1.3% and 94.3 ± 1.8%, respectively) and independent of nifedipine

concentration up to (about) 150 μg mL^{-1} (Table 10-2). Hence, binding of nifedipine to plasma proteins was essentially unsaturable. This finding is similar to that reported with two other closely related compounds, isradipine and darodipine, both of which are highly plasma protein bound (up to 97%) but in serum and isolated solutions of human serum albumin (HSA) the binding is unsaturable.[31] However, the findings reported here are in contrast to an earlier report[17] which described saturable serum protein binding of nifedipine when the nifedipine concentration is increased to 20 μg mL^{-1}. However, nifedipine protein binding in that particular study (measured by an ultracentrifugal method) only changed from 98% to 92% over the range 0.2 to 20 μg mL^{-1} and the small differences found might be explained as experimental error. The estimated K_d value for the nifedipine–protein complex (1.63 μg mL^{-1}), as stated previously in the introduction to this chapter, was calculated using a K_a value from another study[18] and is based on the assumption of one binding site per protein molecule. However, based on the results reported here, this assumption is probably incorrect and the estimated K_d value is most likely an underestimate of the actual value. Unfortunately, further increases in the nifedipine concentration beyond the range used in the current study would probably result in precipitation of excess drug due to free fractions exceeding the water solubility of the compound (about 10 μg mL^{-1}),[22-24] giving misleading and unpredictable results.

There are at least two reasons that protein binding effects have not been seriously considered as a likely mechanism of action for grapefruit juice: (1) because nifedipine, while highly plasma protein bound (mainly to albumin, but also to α_1-acid glycoprotein and possibly lipoproteins),[18] is efficiently extracted (E = 0.32 to 0.6)[12,11] and hence blood clearance of the compound appears to be independent of plasma protein binding; and (2) it is well established from in vitro studies that bioflavonoids inhibit hepatic cytochrome P-450 3A4,[32,33] the isozyme mainly responsible for nifedipine oxidation. Nevertheless, protein binding effects by a constituent(s) of grapefruit juice have not yet been ruled out.

Table 10-2. Plasma protein binding of nifedipine. Human plasma samples spiked with different concentrations of nifedipine, containing either phosphate buffer (2.5 %, v/v, pH 7.4, 1/15 M) or grapefruit juice concentrate (2.5%, v/v), were analyzed in duplicate (individual results separated by a comma).

Initial Nifedipine Concentration (μg mL^{-1}) [Theoretical]	Initial Nifedipine Concentration (μg mL^{-1}) [Measured]	Bound to Plasma Protein (%)
	Plasma–Buffer Solutions	
2	1.9	93.8, 96.5
10	9.7	96.3, 94.7
20	19.4	95.8, 95.5
30	27.7	95.7, 95.7
50	48.2	95.7, 95.6
75	72.2	94.8, 94.7
150	140.1	92.4, 92.0
		(94.9 ± 1.3)
	Plasma–Grapefruit Juice Solutions	
2	1.8	95.4, 95.4
10	9.4	95.1, 95.7
20	19.4	95.0, 95.1
30	25.4	95.1, 95.0
50	47.0	95.1, 94.8
75	71.0	93.8, 93.6
150	139.7	90.6, 90.1
		(94.3 ± 1.8)

Protein binding of ligands can be considered 'restrictive' (e.g., warfarin) or 'permissive' (e.g., nifedipine, propranolol).[34] If grapefruit juice could affect the 'available' free fraction of nifedipine (i.e., 32% to 60% of the total plasma concentration) or alter the affinity of binding (i.e., induce a change from permissive to restrictive nifedipine protein binding) then this might account for its observed effects on nifedipine bioavailability. Brodie et al.[35] were the first to propose the 'free–drug' hypothesis which states that only the free fraction of drug determined in vitro is available for transport into tissues in vivo. However, Pardridge et al.[36,37] later proposed the 'free–intermediate' model (Figure 10-1) to explain why the transport of some drugs (e.g., propranolol, lidocaine) into tissues (e.g., liver, brain) exceeded the free drug fraction. In this model, protein bound ligand can enter tissue via a free–intermediate mechanism by which ligand dissociation from the plasma binding protein and ligand diffusion through the biological membrane are fast relative to the capillary transit time. The three primary determinants of this model are: (1) the capillary transit time, e.g., about 10

seconds for liver; (b) the rate of unidirectional dissociation of ligand from the plasma binding protein; and (c) the rate of ligand diffusion through the biological membrane lining the plasma compartment, e.g., hepatocyte cell membrane.[37] Alternatively, another explanation for the transport of bound ligands is the operation of receptor–mediated mechanisms for the uptake of plasma proteins.[15,37] Although binding sites for albumin in brain capillaries and liver sinusoids have been found, this mechanism remains controversial.[31] Nonetheless, assuming that the free–intermediate model applies to nifedipine plasma protein binding, the presence of another substrate (e.g., a constituent of grapefruit juice) could potentially affect the dissociation of nifedipine from its binding sites on plasma proteins.

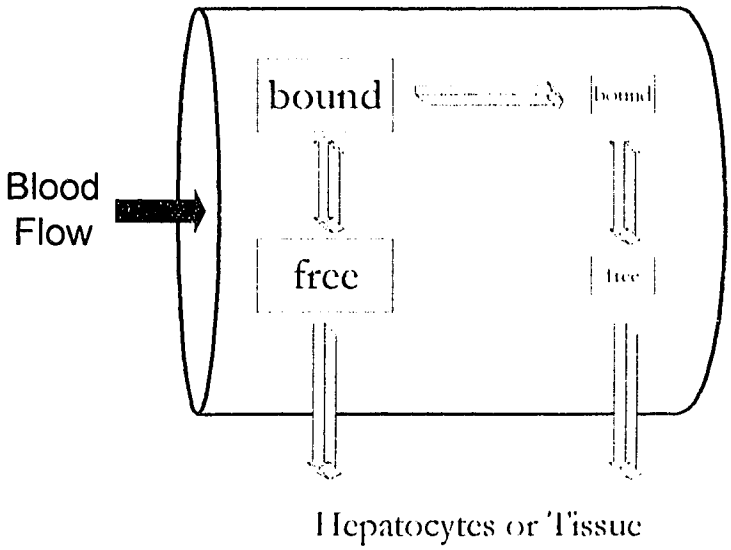

Figure 10-1. The free–intermediate model.[34,36,37] Unbound ligand in the capillary blood stream is continuously and rapidly (relative to organ transit time) exchanged with tissue, with a corresponding decrease in the free ligand concentration in the blood and subsequent displacement of bound ligand in the capillary blood stream.

Allosteric effects may occur in some interactions between HSA and its ligands, triggered by binding forces such as hydrogen bonds, Van der Waals and electrostatic interactions, and hydrophobic bonds.[38] Thus, the stability of the bound form in the microcirculation may be modified in some instances, and in vitro measurements of binding may not always reflect in vivo binding.[39] The association constant of drug–protein binding cannot help to predict the effects of the drug–protein interaction on drug transfer into tissues, however, the presence of another ligand could induce an increase in the binding affinity of the drug–protein complex and further increase the ability of the protein to retain the drug.[34] An example of this is the

nature of the warfarin–albumin complex for which the K_a is increased by small amounts of free fatty acids, resulting in reduced warfarin transfer into the brain and salivary glands.[34,40] Other examples of this phenomenon include: warfarin–benzodiazepine and tenoxicam–diazepam interactions which tend to increase each others protein binding, respectively.[38,41] Therefore, equilibrium measurements of free drug in vitro may underestimate the exchangeable fraction of drug in vivo.

Unfortunately, the results of this study preclude an assessment of the effects of GJC on the binding affinity of nifedipine to plasma proteins. The lack of an effect by GJC on the extent of nifedipine binding to plasma proteins may suggest that affinity is also unlikely to have been altered, but the latter mechanism cannot be ruled out entirely.

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Chapter 11

GENERAL DISCUSSION AND CONCLUSIONS

A series of in vitro and in vivo experiments were done with the nifedipine gastrointestinal therapeutic system (GITS) and a nifedipine dosing solution. In the course of these studies, several factors that can potentially affect nifedipine bioavailability in human beings or rats were investigated (i.e., photostability, chronopharmacokinetics, drug dissolution characteristics, hepatic and extrahepatic presystemic metabolism, grapefruit juice effects and alterations in nifedipine plasma protein binding). Evaluation of these factors is important in providing a better understanding of the mechanisms involved in delivering nifedipine to the systemic circulation and to optimize clinical outcome(s).

Prior to conducting the principal experiments a rapid, simple, sensitive and selective reversed–phase high–performance liquid chromatographic (HPLC) assay was developed to quantitate nifedipine in aqueous and biological samples.[1] This procedure involved extraction of nifedipine from plasma under alkaline conditions (pH 12), separation via reversed–phase HPLC and ultraviolet detection (350 nm). The peak corresponding to nifedipine did not overlap ($R > 1.5$) with corresponding peaks of nifedipine photodegradation products or known metabolites. The method was validated over a range of 5 to 250 ng mL^{-1} using weighted least–squares linear regression analysis. Accuracy and precision were approximately 10% or less over the entire concentration range except for the lowest concentration used which, nonetheless, was acceptable and approached 15%. The minimum quantifiable concentration of nifedipine was determined to be 5 ng mL^{-1}. The minimum detectable concentration was in the order of 1 ng mL^{-1}. Analysis of plasma samples collected from healthy volunteers demonstrated that this assay was applicable to clinical pharmacokinetic studies.

The principal studies that were conducted in this report, included: (1) a determination of the photostability of commercially available nifedipine formulations; (2) a determination of the single–dose pharmacokinetics and bioequivalency of different nifedipine GITS tablets

administered to healthy human volunteers; (3) an investigation of nifedipine GITS dissolution characteristics including development of a novel dissolution test to improve in vitro–in vivo correlations; (4) a determination of nifedipine bioavailability in rats [via intravenous (iv) and extravascular routes of administration] to establish the site(s) of presystemic nifedipine metabolism; (5) an evaluation of the rat as a suitable in vivo model for investigating the effect of grapefruit juice on nifedipine bioavailability; and (6) a determination of the effects of grapefruit juice on nifedipine protein binding in human plasma. Brief overviews of these studies and the results found are provided next.

It was hypothesized that significant decomposition of nifedipine in commercial nifedipine formulations might occur during prolonged sunlight exposure. Nifedipine undergoes photodegradation to dehydronifedipine (DNIF) upon exposure to ultraviolet light and to the nitroso–analog of dehydronifedipine (NDNIF) when exposed to sunlight.[2] Nifedipine photodegradation products do not contribute to clinical activity,[3] thus the content of nifedipine must remain uniform between equipotent formulations. Large differences in light stability between assumed bioequivalent nifedipine products could potentially result in the therapeutic failure of unstable preparations. Consequently, if large photostability differences exist between nifedipine preparations then product substitution might not be warranted. The light stability of 10 intact immediate–release (IR), prolonged–action (PA) or controlled–release (CR) oral nifedipine formulations, obtained from several European and North American manufacturers, was studied using direct continuous artificial sunlight exposure extending over a 12–week period. The content of both nifedipine and NDNIF for each product was measured using HPLC. In addition, the rate of nifedipine photodegradation was measured for both a methanolic solution of nifedipine and pure nifedipine powder to validate the effectiveness of the artificial sunlight source used in this study. After 12 weeks of artificial sunlight exposure less than 2% of NDNIF (w/w initial nifedipine content) was found in any of the formulations. Conversely, photodegradation of the methanolic nifedipine solution and pure nifedipine powder samples exceeded 10% (w/w initial nifedipine content) after about 5 to 10 minutes (mean $t_{1/2}$= 31 minutes) and 24 hours (mean $t_{1/2}$= 7.7 days) of artificial sunlight exposure, respectively. Therefore, these results showed that all the formulations evaluated provided adequate protection against nifedipine photodegradation during prolonged artificial sunlight exposure and also validated the test system. It was concluded that if bioinequivalence, therapeutic failures or pharmacodynamic differences between the

tested nifedipine formulations were observed then photoinstability would be unlikely to be a major contributory factor.

Significant daily variability occurs in the bioavailability and peak plasma concentrations of nifedipine given perorally as an IR dosage form, which is attributed to increased absorption or reduced presystemic metabolism in the morning.[4,6] The nifedipine GITS is an oral osmotic delivery device that exhibits CR properties within the gastrointestinal (GI) tract, and is designed to provide zero–order nifedipine release for nearly a full day.[7] Thus, chronopharmacokinetic properties of nifedipine might also be observed following single–dose administration of a GITS formulation by comparing plasma concentrations found in the evening with those of the following morning (e.g., 12 hour versus 24 hour postdosing levels, respectively). Hence, time–dependency and the variability in nifedipine plasma concentrations and kinetics within and between subjects were assessed in a bioequivalency study conducted with 4 commercially available nifedipine GITS formulations (2 lots of both 30 mg and 60 mg strengths obtained from the same manufacturer) given in single–dose fashion to 12 healthy subjects. As expected, mean C_{max} and AUC(0–t) values of the 60 mg doses were approximately double those of the 30 mg doses suggesting a linear dose versus concentration relationship; however, typical standards of bioequivalence were not met because of large inter– and intrasubject variability that is only partially explained by the relatively small number of study participants. This latter finding may have important ramifications for others attempting to develop a bioequivalent product. Interestingly, mean plasma nifedipine concentrations increased up to 24 hours post–dosing with each of the 4 lots tested. In the individual kinetic profiles, t_{max} was measured as 24 hours (corresponding to about 8 AM) over half the time (56%), consistent with previously reported chronopharmacokinetic observations for nifedipine. Alternatively, these results could suggest that the location of the GITS tablet in the GI tract influenced nifedipine bioavailability.

Given the results of the previous study, it was also necessary to assess the dissolution characteristics of the nifedipine GITS as a potential source of the variability and apparent chronopharmacokinetic properties observed. The nifedipine GITS incorporates a push–pull osmotic pump to release — in zero–order fashion — a finely–divided suspension of nifedipine which must then undergo dissolution in the GI tract before the drug can be absorbed.[7] Classical, differential (ALZA) and flow–through type dissolution methods adequately characterize the in vitro nifedipine suspension release rate from the nifedipine

GITS;[8,9] however, these methods fail to measure the in vitro dissolution rate of the suspended particles — a potentially significant shortcoming considering that nifedipine is poorly water–soluble (≤ 10 μg mL^{-1}).[10] Therefore, an in vitro two–phase dissolution system was developed. This system measured the rate of nifedipine 'transfer' from an aqueous phase (simulated intestinal fluid, USP, without pancreatin, containing a nifedipine GITS tablet) into an organic phase (n–octanol), a process dependent on release of the drug suspension from the tablet, dissolution of the particles in the aqueous phase and partitioning of the drug in the organic phase. For the nifedipine GITS strengths tested (30 mg and 60 mg) the two–phase method indicated that about 90% of the drug was transferred within 30 hours. In contrast, results from single–phase dissolution methods show that about 90% of the drug suspension is released within 24 hours.[8,9] Hence, the two–phase dissolution method gave results which appeared to be in better agreement with published in vivo studies of the nifedipine GITS[7,11-14] with regard to both the rate and duration of nifedipine absorption from the GI tract. However, the results also indicated that the large inter– and intrasubject variability and apparent chronopharmacokinetic properties, observed in vivo in the clinical study described previously and seen in some other reports,[13,16] could not be accounted for by the release and dissolution properties of the drug from the GITS dosage form. This study, nonetheless, emphasized the importance of differentiating between nifedipine suspension release and nifedipine dissolution for this type of formulation; but, the two–phase dissolution method may also be useful for other pharmaceutical formulations and poorly water–soluble drugs.

The nifedipine GITS produces relatively linear fractional absorption–time plots between 6 and 24 hours and about 70% of the 'available' dose is absorbed within 24 hours.[11] However, conventional single–phase in vitro dissolution tests with the nifedipine GITS demonstrate pseudo zero–order release of nifedipine suspension between about 2 to 20 hours and ≥ 90% of the labeled dose is released within 24 hours.[8,9] Using the two–phase dissolution system described above, mean in vitro data obtained from 2 strengths of the nifedipine GITS were compared with mean in vivo data from a human bioequivalency study[17] in anticipation of improved in vitro–in vivo correlations. For the in vivo data, deconvolution was performed using both the Wagner–Nelson method (model–dependent, WN) and DeMonS which is a new method of numerical deconvolution (model–independent, DS).[18,19] Calculated zero–order rates of drug absorption [0.95(WN)/1.03(DS) mg h^{-1} and 1.88(WN)/1.94(DS) mg h^{-1}; 30 mg and 60 mg strengths, respectively] compared reasonably well with the calculated zero–

order rates of drug transfer (0.96 and 2.02 mg h^{-1}; 30 mg and 60 mg strengths, respectively). These values, however, are considerably less than zero–order drug release rates obtained using single–phase dissolution methods or reported as the manufacturers design specifications (1.7 and 3.4 mg h^{-1}; 30 mg and 60 mg strengths, respectively).[8,20] Hence, improved in vitro–in vivo correlations were demonstrated with the two–phase dissolution test, yielding 1:1 (Level A) correlations ($r^2 > 0.99$ in all cases).

The peroral (po) bioavailability of nifedipine is reported to range from about 45% to 58% in the rat,[21,22] which compares favorably to human beings ($40\% \leq F \geq 68\%$).[21,23-25] The metabolism of nifedipine is similar in both rats and humans (involving oxidation of the dihydropyridine ring) and the liver is believed to be mainly responsible for the systemic clearance of the drug and the observed first–pass effect after po dosing.[21,26,27] Hence, a study was conducted to determine if intestinal metabolism also contributed to the first–pass elimination of nifedipine in the rat. The systemic availability's of a nifedipine dose given by po, intracolonic (ic) and intraperitoneal (ip) routes of administration were compared to an iv dose (in each case a nifedipine dose of 6 mg kg^{-1} was used) in adult male Sprague-Dawley rats (249 to 311 g, n = 6 or 7 per group). The geometric mean of systemic nifedipine plasma clearance after iv dosing was 10.3 mL min^{-1} kg^{-1}. The nifedipine blood–to–plasma ratio was found to be about 0.59. Therefore, the systemic blood clearance of nifedipine was about 17.5 mL min^{-1} kg^{-1}; which, compared to the hepatic blood flow in rats (55 to 80 mL min^{-1} kg^{-1})[28,29] demonstrated poor nifedipine extraction by the liver ($0.22 \leq E_h \leq 0.32$). The mean absolute bioavailabilities of the po, ip and ic doses found were 61%, 90% and 100%, respectively. Assuming complete absorption of extravascular nifedipine doses, then these results indicate that, in addition to hepatic extraction, substantial first–pass elimination of nifedipine occurs within the wall of the small intestine — but not the colon — of the rat.

The bioavailability of nifedipine in rats appears to increase and its absorption is significantly delayed by grapefruit juice concentrate (GJC).[30] Hence, the effect(s) of GJC could be to delay stomach emptying and inhibit nifedipine metabolism in the small intestinal wall and liver or, alternatively, to impede nifedipine absorption until the drug reaches the large intestine where gut wall presystemic metabolism is a less important factor. The mechanism(s) of action of GJC might be partially resolved by comparing its effect on the pharmacokinetics of nifedipine with orange juice concentrate (OJC) which has a similar consistency but lacks inhibitory effects on nifedipine presystemic metabolism, and also by

giving regular strength solutions of the two juices both of which should not significantly affect stomach emptying. Thus, a study was conducted to compare the po bioavailability of nifedipine (6 mg kg^{-1}) in male Sprague–Dawley rats coadministered GJC, OJC, grapefruit juice regular strength (GJRS), orange juice regular strength (OJRS) or (tap) water. Nifedipine plasma concentration–time profiles in the GJRS, OJRS and (tap) water groups displayed a single–peak. Both GJC and OJC groups gave double–peak profiles (indicating delayed gastric emptying); however, the majority of the nifedipine dose in both cases was absorbed during the time interval of the second peak, i.e., several hours postdosing. GJC significantly increased nifedipine bioavailability [relative bioavailability = 2.02, compared with (tap) water]. Absolute nifedipine bioavailability in the GJC group was 1.20 (relative to iv data[31]), indicating that GJC may affect both extrahepatic and hepatic first–pass metabolism, although a reduction in systemic nifedipine clearance cannot be ruled out. There appeared to be a slight increase in bioavailability after GJRS dosing but this was not statistically significant. OJC did not increase nifedipine bioavailability, further suggesting that the delay in nifedipine absorption by GJC or OJC results from delayed gastric emptying. Despite an apparent lack of affect by GJRS, the GJC results demonstrate that the rat may be a suitable animal model for further investigating the grapefruit juice–nifedipine interaction.

Interestingly, the mechanism by which grapefruit juice increases nifedipine bioavailability in human beings has not been unequivocally established to date.[32] Assuming that the liver is the principle organ responsible for the poor–to–intermediate extraction of nifedipine, bioavailability of the compound is therefore influenced by hepatic intrinsic clearance, hepatic blood flow and plasma protein binding. Because grapefruit juice does not significantly alter hepatic blood flow[32] then the most likely explanation for its effect(s) is the presence of an inhibitory substance(s) contained in the juice that temporarily decreases intrinsic clearance, such as a bioflavonoid (e.g., naringin, quercetin). However, conflicting results are reported from in vivo studies where individual bioflavonoids are coadministered with grapefruit juice.[32-35] On the other hand, nifedipine is highly plasma protein bound (91% to 99%)[36-39] but is non–restrictively cleared as its hepatic extraction ratio (32% to 60%) exceeds the free drug fraction (1% to 8%). Thus, a constituent(s) of grapefruit juice could change the free 'available' fraction of nifedipine by altering its extent or affinity of binding to plasma proteins (i.e., temporarily altering the nature of binding from non–restrictive to restrictive) during first–pass through the liver, based on the 'free–intermediate' hypothesis.[40-42] Hence, a simple

in vitro human plasma protein binding study using an ultrafiltration method was undertaken. The results of the study showed that grapefruit juice concentrate (GJC, 2.5% v/v) did not significantly affect the extent of nifedipine plasma protein binding (mean > 94%, with and without GJC) throughout the concentration range evaluated (2 to 150 $\mu g\ mL^{-1}$). Unfortunately, the affinity of nifedipine binding could not be estimated using graphical methods (i.e., Rosenthal or modified Scatchard plot) because protein binding was found to be unsaturable. In conclusion, GJC did not affect the extent of nifedipine binding to plasma proteins; suggesting, perhaps, that binding affinity was also unaffected but this cannot be completely ruled out.

In summary, the findings reported here illustrate some of the factors which are important in determining nifedipine bioavailability. Photoinstability of commercial oral nifedipine preparations and potential changes in nifedipine human plasma protein binding by grapefruit juice were not observed, whereas other factors [chronopharmacokinetics, the location of nifedipine absorption (e.g., gut wall metabolism), drug dissolution, and grapefruit juice] appeared to significantly influence the bioavailability (rate or extent) of nifedipine in human beings or rats. The development of future oral nifedipine dosage forms (or formulations of similar compounds) should be done in the expectation of improving oral bioavailability and therapeutic response, and reducing the inter– and intrasubject variability in drug delivery to the systemic circulation and the incidence and severity of adverse effects. The in vitro and in vivo studies conducted in this report provide a somewhat better understanding of several factors which should be taken into consideration during the design, development and clinical use of nifedipine products. However, these findings also demonstrate the need for more clinical studies to be conducted with existing nifedipine formulations such as the nifedipine GITS, especially since the data that was obtained from in vitro studies and rats cannot be necessarily extrapolated to the human clinical setting. Finally, given recent concerns that have been raised regarding the clinical use of IR nifedipine formulations and subsequent uncertainty about the use of longer–acting formulations, more attention needs to be focused on the mechanisms and magnitude of factors involved in determining nifedipine bioavailability in human beings as related to clinical outcome(s).

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