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UNIVERSITY OF ALBERTA

Parenteral Nutrition and Drug Interaction

BY

June Ke (C)

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

PHARMACEUTICAL SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

SPRING 1990

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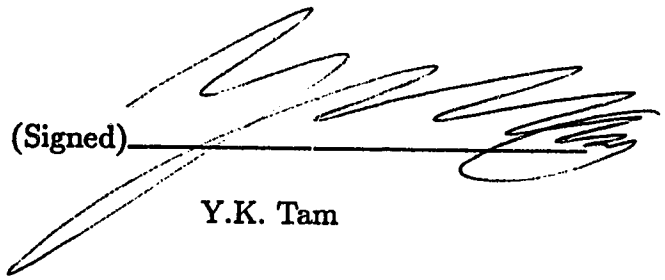
(Signed) 

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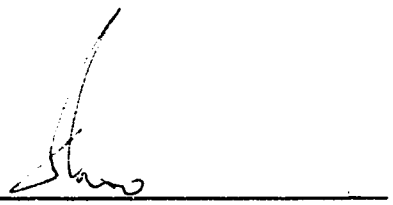
(Signed) 

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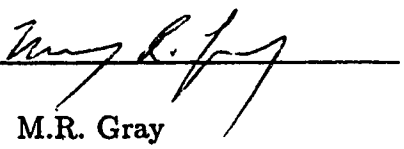
(Signed) 

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(Signed) Sohair Tawfik

S.R. Tawfik

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H.A. Semple

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(Signed) Barbara Marriage M.Sc. R.D.

B. Marriage

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NAME OF AUTHOR: **June Ke**

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YEAR THIS DEGREE GRANTED: **1990**

(Signed) June Ke

Permanent Address:
Faculty of Pharmacy and
Pharmaceutical Sciences
University of Alberta
Edmonton, Alberta
Canada T6G 2N8

Date: April 24, 1990

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Yun K. Tam

Ronald T. Coutts

Murray R. Gray

Fakhreddin Jamali

David Lalka

Fredric J. Nugent

Date: March 29, 1990

To My Teacher Weiqin Liu

ABSTRACT

The primary objectives of this project are to study the acute and short-term effects of parenteral nutrition (PN), a specific route of nutrient delivery, on metabolism, protein binding and renal excretion of drugs. Lidocaine and ampicillin were chosen as model compounds. Before this project was conducted, an HPLC assay was developed to evaluate lidocaine metabolism in detail.

The acute effects of PN on lidocaine and ampicillin pharmacokinetics were studied in healthy human volunteers. There was no acute effect of PN on the pharmacokinetics of ampicillin, and, renal functions, including glomerular filtration rate (GFR), remain unchanged in healthy human volunteers. Similarly, there was no acute effect of PN on pharmacokinetics of lidocaine in healthy human subjects.

The effects of acute (co-infusion) and short-term (7 days *in vivo* infusion) PN treatments on hepatic lidocaine elimination were studied using an isolated rat liver perfusion technique. No acute effect of PN on lidocaine elimination was observed. However, compared to livers isolated from chow-fed animals, hepatic clearance and hepatic extraction ratio for lidocaine were reduced by 29.8% and 31.1%, respectively, in rats after 7 days of PN ($p < 0.05$). Moreover, mass balance at steady state showed that recovery was higher for lidocaine (46.5% (PN) vs. 25.6% (chow-fed), $p < 0.05$) and lower for the aryl methyl hydroxylation product of lidocaine, MeOH-LIDO, (0.21% (PN) vs. 2.75% (chow-fed), $p < 0.05$) in the

PN group compared to controls. These data combined with the results from the human studies show that PN and drug interaction is highly dependent upon the period of PN exposure; and PN-induced alteration of lidocaine elimination is more likely due to a secondary effect on hepatic function of parenteral nutrients rather than a direct competition between drug and nutrient for hepatic enzyme sites.

The effects of PN and nutritional status on lidocaine pharmacokinetics were conducted in a pilot study in pigs. The preliminary results indicate that PN and nutritional states significantly affect lidocaine disposition; PN lowers lidocaine binding to serum proteins, however the extent of PN and drug interaction is unlikely to be dependent upon the subject's nutritional status.

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AAG	α-1-acid glycoprotein
ANOVA	Analysis of variance
AUC	Area under the serum concentration-time curve
C_{in}	Inlet concentration
C_{max}	Maximum concentration
C_{out}	Outlet concentration
CL_{Cr}	Creatinine clearance
CL_H	Hepatic clearance
CL_R	Renal clearance
CL_T	Total body clearance
cm	centimeter(s)
E	Extraction ratio
F_u	Fraction of drug excreted unchanged in the urine
f_u	Fraction of unbound drug in serum
g	gram
GC/MS	Gas Chromatography-Mass Spectrometry
GFR	Glomerular filtration rate
GX	N-glycyl-2,6-xylidine
h	hour(s)
HPLC	High-performance liquid chromatography
ICG	Indocyanine green

I.D.	Internal diameter
I.S.	Internal standard
iv	Intravenous
kcal	kilocalorie(s)
kg	kilogram(s)
kJ	kilojoule(s)
L	Liter(s)
LIDO	Lidocaine
MEGX	N-(N-ethylglycyl)-2,6-xylidine
MeOH-LIDO	N-(N,N-diethylglycyl)-2-hydroxymethyl-6-methylaniline
MeOH-MEGX	N-(N-ethylglycyl)-2-hydroxymethyl-6-methylaniline
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mmol	millimolar
n	number of observations
nm	nanometer(s)
4-OH-GX	4-hydroxy-N-glycyl-2,6-xylidine
3 or 4-OH-LIDO	3 or 4-hydroxy-lidocaine
3 or 4-OH-MEGX	3 or 4-hydroxy-N-(N-ethylglycyl)-2,6-xylidine
4-OH-XYL	4-hydroxy-2,6-xylidine
PN	Parenteral Nutrition

Q	Hepatic blood flow rate or buffer perfusion rate
r^2	Correlation
RNI	Recommended nutrient intakes
RPF	Renal plasma flow
SD	Standard deviation
$T_{\frac{1}{2}}$	Half-life
T_{max}	Time to reach maximum concentration
t_{ss}	Time to reach steady state during continuous drug infusion
UV	Ultraviolet
v/v	volume to volume ratio of mixtures
V_{dss}	Steady state volume of distribution
V_{ss}	Velocity of drug elimination
2,6-XYL	2,6-xylidine, 2,6-dimethylaniline
ΣX_u	Cumulative urinary excretion of drug
μl	microliter(s)
μg/ml	micrograms per milliliter
°C	degree celcius

CHAPTER 1

INTRODUCTION

1.1 Parenteral Nutrition (PN) —— An Overview

Parenteral Nutrition (PN) is defined as the administration of nutrients by the intravascular route. Common PN solutions contain amino acids, carbohydrates, lipids and other essential components such as vitamins, minerals and other trace elements. As a general guide, PN therapy is indicated when a patient is unable to tolerate adequate enteral nutrition for prolonged periods. Criteria for placing a patient on PN include inadequate food intake for 7 to 15 days, loss of 7% to 10% of pre-illness body weight, and abnormalities in certain anthropromorphic measures (*Corcoran 1986*).

After many years of investigation in parenteral feeding (*Barker 1904, Dudrick and Rhoads, 1971, Cuthbertson 1980, Levenson et al 1984*), the ability to supply complete nutritional support intravenously was finally realized in 1967 (*Dudrick et al. 1968, Wilmore and Dudrick, 1968*). Since then, the use of PN has become widespread and has proved to be one of the major advances in medical care in this century (*Klein and Nealon, 1988*). According to 1984 statistics, $432 million was spent on PN solutions in the United States alone (*Piech 1985*).

The success of PN therapy is due to its widespread application in surgical and medical disorders. Surgical recovery has been improved with the incorporation of PN therapy (*Mullen et al. 1980, 1982*). Several medical disorders which

have been resistant to other therapy are treated with PN therapy (*McSweeney 1983*). Patients suffering from a variety of gastrointestinal diseases, pancreatitis, major burns and cancer are the prime candidates for PN therapy. These patients are in negative nitrogen balance because of rapid protein breakdown (catabolism) in meeting the daily energy requirements. A 7-day fast depresses liver protein by 40%, gastrointestinal protein by 28%, and kidney protein by 20% (*Addis et al. 1936*). Catabolic states vary from the less extreme condition of protein or calorie deficiency to the more extreme cases of classic malnutrition and starvation. Good nutrition is necessary for normal growth and development as well as cell function and regeneration. Without adequate nutrient intake, synthesis of protein, including drug metabolizing enzymes, is slow, healing is delayed and body resistance to disease is decreased. PN therapy is designed to prevent and reverse these adverse effects of inadequate enteral nutrient intake.

However, since 1971 it has been realized that the use of PN, may also cause catheter-related and metabolic complications (*Peden et al. 1971*). With increasing experience and subsequent improvement in insertion techniques, catheter design, patient monitoring, and formula composition, the rate of complications associated with the use of PN has been dramatically reduced. Abnormalities associated with the liver and biliary tract, however, still occur often and in some instances may result in significant morbidity (*Bower, 1983, Roy and Belli, 1985, Baker and Rosenberg 1987, Klein and Nealon, 1988*).

1.2 Parenteral Nutrition and Pharmacokinetics

Numerous studies have shown that nutrient and nutritional states can substantially alter drug disposition in animals and humans (*Hathcock and Coon, 1978, Roe, 1988, Anderson 1988, Yang and Yoo, 1988*). However, the pharmacokinetic behavior of drugs in parenterally nourished patients has received little attention. This may be partly because PN therapy is often associated with different disease states, variable nutritional status and/or multiple pharmacotherapy administered during PN, complicating PN-drug interaction.

1.2.1 PN and Nutritional Status

The elimination of most drugs from the body involves the processes of both metabolism and excretion. The hepatic metabolic enzymes, which are proteins, play an important role for the biotransformation and subsequent elimination of drugs not easily cleared through the kidney. Clearly, proteins are required for the synthesis of these enzymes and therefore any nutritional state which reduces the availability of amino acids could be expected to reduce the amounts of drug metabolizing enzymes. This can occur in the patients who require PN therapy. Because the enteral calorie intake is low, protein will be catabolized and used as a source of energy, reducing the availability of amino acids for enzyme synthesis.

It is conceivable that PN may have conflicting effects on the hepatic elimination of drugs. First, therapeutic use of PN is designed to maintain normal nutritional status and to supply extra or special nutrients to correct deficits or

heal injuries (*McSweeney 1983*). This rehabilitation, in theory, should enhance drug elimination from the body on a chronic basis by improving the nutritional status of the patient because a good nutritional status is necessary for maintaining normal function of the hepatic enzyme system. In humans, the clearance of antipyrine has been used as an index to assess the microsomal oxidative capacity of the liver. Studies have shown that a reduction in the metabolism of antipyrine in protein-calorie malnourished children can be reversed within a month of starting on a high-protein, high-calorie diet (*Homeida et al. 1979, Narang et al. 1977*). This observation suggested that drug metabolism is impaired in malnourished children and that impairment can be rehabilitated with an improved diet.

On the other hand, in humans, prolonged infusion of PN may cause hepatic dysfunction (*Bowyer et al. 1988*), the frequency of which was related to the duration of PN infusion (*Merritt, 1980*). Serum transaminases, bilirubin, and alkaline phosphatase levels become elevated, representing hepatocyte injury and intrahepatic cholestasis (*Grant et al. 1977, Stein et al. 1980*). Theoretically, these PN-associated abnormalities in the liver should induce a decrease in hepatic drug elimination.

Several studies in animals and humans suggested that PN infusion can significantly influence hepatic drug elimination. For example, in humans, antipyrine clearance, which is reduced in protein-calorie malnourished patients, could return to normal after a month of PN therapy (Tranvouex et al. 1985). In animals, healthy rats had 24 % and 57 % lower content and activity, respectively, of hepatic cytochrome P_{450} and 71 % lower hepatic pentobarbital clearance after 7 days

of PN compared with animals fed with standard laboratory rat chow *ad libitum* (*Knodell et al. 1980 and 1984*). There is an apparent discrepancy between animal and human studies. Differences in the duration of PN and nutritional status or species differences in hepatic drug clearance could conceivably account for some of the discrepancy. However, both an increase and a decrease in antipyrine clearance have been reported in two separate human studies. Antipyrine clearance was decreased by 34% in post-operative patients receiving an amino acid-dextrose solution for 7 days (*Burgess et al. 1987*) but increased by 87% in previously malnourished subjects undergoing nutritional repletion with 8 days of PN therapy (*Pantuck et al. 1985*). The major difference between the two studies is the nutritional status of the subjects used. Therefore, these results suggest that underlying nutritional status is an important determinant for the study of PN and drug interaction. The effects of PN on hepatic drug elimination could be qualitatively and quantitatively different in different nutritional status.

Moreover, the ability to clear drug from the body is nutritional status dependent. With improvement of the nutritional status of a patient during a course of PN therapy, the ability to clear drug from the body would be enhanced accordingly. Thus, it is obvious that PN therapy poses some very fundamental and practical clinical questions about the safety and efficacy of drug therapy. Adjustment of dosage regimens may be required in patients with variable nutritional states during a course of PN, particularly for drugs that have narrow therapeutic indices. Unfortunately, the effects of PN on drug elimination in different nutritional status have not been investigated.

1.2.2 PN and Protein Binding

Many nonpolar weakly acid/weakly basic drugs are present in the blood in the form of a complex with plasma proteins such as albumin and α-1-acid glycoprotein, to facilitate their movement through the circulation. The protein bound drug is a large complex which cannot easily cross cell membranes and therefore has a restricted distribution. The protein bound drug is also assumed to be pharmacologically inactive. In contrast, the unbound drug crosses cell membranes and interacts with receptors to produce pharmacologic effects. Moreover, only unbound drug is assumed to be cleared by eliminating organs, including the liver and kidney. Clearly, drug binding rests at the very interface between pharmacokinetics and pharmacodynamics.

Previously, it had been assumed that the extraction of low clearance drugs was sensitive to changes in the protein binding, whereas the extraction of high clearance drugs was not (*Pang and Rowland, 1977*). Experimental findings of alteration in diazepam extraction with alteration in protein binding (*Rowland et al. 1983, 1984*) prompted re-examination of these assumptions. The experimental results suggested that hepatic extraction of high clearance drugs (and hence bioavailability) is also sensitive to protein binding changes (*Rowland et al. 1984, Byrne et al. 1985*).

In addition to drugs, plasma proteins bind and facilitate the transport of a number of physiologic substances through the plasma. Among these are bilirubin (*Odell, 1973*), bile acids (*Gree et al 1971*), nonpolar amino acid such as tryptophan (*Cunningham et al. 1975*) and free fatty acid (FFA) (*Spector, 1975*). High

concentrations of circulating nutrients in the blood stream during PN may alter drug plasma protein binding by either competing with the drug for common protein binding sites or producing conformational changes in the structure of the drug binding sites. This competition theoretically alters the pharmacokinetics and pharmacologic effectiveness of the drugs because of an increase in the fraction of unbound drug. It is obvious that drugs which are extensively bound to the plasma proteins are affected more. For example, both concentration and turnover of FFA are increased by PN supplemented with lipid (*Skutches et al. 1980, Tulikoura and Huikuri, 1982*). The drug displacing action of elevated FFA is well known and occurs with a number of highly bound drugs such as salicylic and valproic acids and phenytoin (*Zimmerman et al. 1981*).

Decreased drug protein binding could also result indirectly from PN. Progressive hepatobiliary dysfunction is a common side effect of PN. With advancing liver damage, basal hepatic functions, including albumin synthesis and export, diminish, and albumin concentrations in intravascular and interstitial spaces fall (*Lanza-Jacoby et al. 1982*). This trend toward reduced drug binding capacity may be further exacerbated by the elevated bilirubin and bile acids that accompany PN-induced liver injury (*Grant et al. 1977, Riely et al. 1979*). Unfortunately, this important aspect of PN-drug interaction has received little attention.

1.2.3 PN and Drug Metabolism

Many drugs are cleared from the body by metabolism and the chemical reactions concerned in the drug metabolism are classified as Phase I and Phase

II reactions. The liver is by far the most important organ in drug metabolism even though intestinal tissue, lung, and kidney also contain appreciable amounts of metabolizing enzymes.

Phase I (or called functionalization) reactions usually convert the parent drug to a more polar metabolite by oxidation, reduction, or hydrolysis. Oxidation processes are by far the most common and important Phase I reactions and are catalyzed by a cytochrome P_{450}-dependent enzyme system generally known as mixed function oxidase (MFO). The resulting metabolite may be more active than the parent molecule (which may then be designated as a prodrug), less active, or inactive. The purpose of Phase I reactions is to introduce a polar functional group into the drug molecule.

Phase II reactions, which are also called synthetic or conjugation reactions, involve coupling between the drug or its metabolite and an endogenous substrate, such as glucuronic acid, sulfate, or an amino acid to form water-soluble conjugated products. Phase II reactions are catalyzed by many conjugative enzymes and need a source of energy for their accomplishment. Conjugated metabolites are readily excreted in the urine and are generally devoid of pharmacological activity and toxicity.

PN may affect Phase I and/or Phase II metabolism of drugs by altering the composition and activity of drug metabolizing enzymes. With drugs that are metabolized by different and/or competing parallel pathways, a change in composition of cytochrome P_{450} isoenzymes may alter the relative importance of these

pathways because the same nutrient component may induce and/or inhibit different isoenzymes differently. For example, a study has shown that the food-induced increase in plasma propranolol concentration is accompanied by a reduction of area under drug concentration-time curve (AUC) of conjugated propranolol (*Liedholm and Melander, 1986*). As there seems to be no consistent reduction of the hydroxylated or dealkylated metabolites of propranolol (*Walle et al. 1981*), it seems possible that one or more specific nutrients may selectively inhibit the primary conjugation of propranolol. Therefore, it is extremely important to evaluate individual metabolic pathways while considering a nutrient effect on drug metabolism.

Some experiments have shown that hepatic drug clearance changed with the use of PN in human (*Anderson et al. 1988*). Both an increase (*Tranvouex et al. 1985, Pantuck et al. 1984 and 1985*) and a decrease (*Burgess et al. 1987*) in antipyrine clearance have been reported. However, the concentrations of antipyrine metabolites were not measured in these studies, therefore, how PN affects drug metabolism is unclear. Knodell *et al.* (*1979, 1980 and 1984*) studied the metabolism and disposition of meperidine and pentobarbital in healthy rats and found that PN significantly changed pharmacokinetic and metabolic parameters. Decreased clearances of meperidine and pentobarbital and decreased maximal velocities (V_{max}) for pentobarbital hydroxylation and meperidine demethylation were found in the livers of parenterally fed animals. Unfortunately, these studies did not systematically investigate the effects of PN on the same substrate in different and/or competing parallel metabolic pathways. Therefore, the mechanisms by which PN may affect various hepatic drug metabolic pathways remain to be

established. Moreover, the way PN influences drug elimination by conjugation is unclear.

1.2.4 PN and Hepatic Blood Flow

Besides hepatic metabolizing enzymes, hepatic blood flow may also be an important factor for hepatic drug elimination, especially for drugs with a high extraction ratio. This is because, for a highly extracted drug, the hepatic extraction ratio is a monotone decreasing function of hepatic blood flow. For example, acute exercise may produce large increases in cardiac output, but redistribution of blood leads to a significant reduction in liver blood flow. Not surprisingly, the steady state plasma concentration of lidocaine, a drug with a high hepatic extraction, was increased by strenuous exercise (*Sweeney 1981*). On the other hand, as expected for a drug with a low hepatic extraction, the clearance of antipyrine was unaffected by exercise (*Swartz et al.1974*).

Some studies found that in humans, a protein-rich meal increased splanchnic blood flow (of which hepatic blood flow is a part) by an average of 35% in the first hour and hepatic blood flow by an average of 91% 2 hours after eating, but found no change in hepatic blood flow after a carbohydrate-rich meal (*Brandt et al. 1955, Orrego et al. 1965*). These observations suggest that intake of certain enteral nutrients does influence hepatic blood flow rate. However, according to preliminary results in our laboratory, electromagnetically measured hepatic blood flow was only increased by 15% when anesthetized pigs received PN, and there was no change in the hepatic blood flow when the animals received normal saline (unpublished

data). Recently, Fabri *et al.* (*1987*) reported that after 7 days of PN treatment there was no statistically significant change in the clearance of indocyanine green (ICG), an indicator used to measure hepatic blood flow, in patients who were not on PN prior to the experiments and had normal liver function. Based on these results, it is unlikely that PN infusion could cause a substantial alteration in hepatic blood flow. Therefore, hepatic blood flow may not be an important contributor to PN induced alteration in hepatic drug elimination.

1.2.5 PN and Renal Drug Clearance

Renal excretion is a major route of elimination for many drugs. In general, drugs which are water soluble, have low molecular weight ($\leq$300), or are not significantly metabolized by the liver will be eliminated by renal excretion. The processes by which a drug is excreted via the kidney may include any combination of three processes, i.e. glomerular filtration, active tubular secretion and tubular reabsorption.

Amino acid infusion has been shown to double creatinine clearance and increase renal plasma flow (*Graf et al. 1983, Meyer et al. 1983*). This observation implies that renal drug clearance may be altered by PN therapy. In theory, an increase in renal plasma flow during PN may elevate the clearance of a number of drugs excreted mainly by the kidney. This potential influence of PN on renal drug elimination may be involved in different processes of renal drug excretion. It has been reported that in humans, decreased protein intake can reduce renal plasma flow, creatinine clearance, and the clearance of some substances excreted

principally through the kidney (*Kitt et al. 1989*). However, there was no available information in the literature with respect to the effect of PN on renal drug elimination.

1.2.6 Acute PN-Drug Interaction

It has been noticed that while the concomitant intake of food can enhance the bioavailability of certain drugs by reducing their presystemic metabolism; repeated intake of protein-rich and/or charcoal-broiled food can reduce the plasma level of many drugs by the enzyme-inducing effect of protein/benz(a)pyrene (*Melander and McLean, 1983, Anderson et al. 1979 and 1985, Kappas et al. 1976 and 1978, Fagan et al. 1987*). These observations suggest that the food effect on drug disposition is dependent upon the duration of the food intake; concomitant and repeated food intake may cause contrary effects on drug disposition via different mechanisms. Accordingly, acute and chronic PN infusion may also have different effects on drug disposition. This necessitates both acute and chronic studies concerning the PN-drug interaction.

The acute effects of food on the presystemic metabolism of drugs have been extensively studied (*Welling, 1977, Roe, 1979, Melander and McLean, 1983, Melander et al. 1988.*). Some of the presystemically cleared drugs have a higher bioavailability when taken together with food. While concomitant food intake may increase the bioavailability of propranolol by 50-100 % when the drug is given in the conventional rapid-release formulation, there is no such effect when it is given in a slow-release formulation (*Byrne et al. 1984, Liedholm and Melander, 1986*).

These findings indicate that, while this food effect can be quite pronounced, it is also quite transient.

It was believed that transient changes in hepatic blood flow in response to a meal could account for nearly all of the increase in the AUC of propranolol (*McLean et al. 1978 and 1980, Olanoff et al. 1986*), but it is becoming apparent that mechanisms involving changes in hepatic blood flow, protein binding, and intestinal and hepatic metabolism may all contribute to the food effects (*Svensson et al. 1983, Corbett et al. 1986, Modi et al. 1988*). Recently a new, plausible, mechanism was proposed in which hormonal regulations may play an important role in metabolic enzyme activities in the presence of enteral nutrients (*Semple et al. 1990*).

It is conceivable that PN infusion may cause an instant elevation of the concentration of circulating nutrients in the blood stream. These high concentrations of nutrients in the blood may acutely compete with drugs for the metabolic enzyme sites in the liver because the major enzyme systems that metabolize drugs are also responsible for the metabolism of a variety of other exogeous and endogenous compounds, including macronutrients. These nutrients may also acutely compete with drugs for the plasma protein binding sites because some drugs and nutrients may share the same binding sites. As a result of the competition, lipophilic drugs could be displaced from both the hepatic metabolizing enzyme sites and the protein binding sites, which in turn would acutely alter drug elimination from the body during PN infusion. In contrast to studies of the acute effects of food, there are no published reports dealing with the acute effects of PN on drug elimination.

1.2.7 Nutrient Components and Drug Disposition

Studies to identify dietary components that can influence drug metabolism have been conducted more recently. The metabolic clearance of antipyrine was increased in patients following consumption of a high protein diet for two weeks (*Conney et al. 1977*). The consumption of a high carbohydrate diet for the same period of time did not significantly alter antipyrine clearance from its baseline values determined when patients were on their usual diet. Fagan et al. (*1987*) have also observed in human volunteers that a high-protein diet (40% of total calories) caused a higher metabolic clearance of theophylline and propranolol than a low-protein diet (10% of total calories). These results indicate that dietary protein can accelerate the metabolism of these representative substrates of the mixed function oxidase system in humans.

The individual effects of protein, fat and carbohydrate on the metabolism of some aromatic or chlorinated hydrocarbons have been separately assessed by feeding rats with various test diets (*Nakajima et al. 1982*). The results have shown that the rate of microsomal metabolism of these chemicals increased linearly with increasing carbohydrate intake independently of protein or fat intake. This finding is not at all in conflict with the earlier findings that a high-protein diet increases and a low-protein diet decreases, the metabolism of some foreign compounds, and it seems that it is not protein but carbohydrates in the diet that actually determines the hepatic metabolism (*Sato and Nakajima, 1987*).

The mechanisms of protein and, in particular, carbohydrate effects on the mixed function oxidase in humans have not been established. Increased dietary

protein augments hepatic microsomal cytochrome P450 content, liver weight and mitotic indices in animals (*Argyris 1971, Campbell and Hayes 1974*); these effects are similar in many respects to the inducing effects of phenobarbitals. Certain amino acids such as tryptophan and oxidised sulphur amino acids may increase liver protein synthesis and induce the mixed function oxidase system in animals and in liver cell cultures (*Arcos et al. 1980, Evarts and Mostafa 1981, Paine 1976, Sidransky 1986, Wheeler et al. 1985*).

Dietary protein not only affects the mixed function oxidase system in humans, but also alters kidney function. Decreased protein intake can reduce renal plasma flow, creatinine clearance, and the clearance of a number of substances excreted principally by the kidney in human (*Kitt et at 1989*).

In comparison to a fat-free diet, feeding a corn oil diet to rats caused an increase in the microsomal metabolism of a variety of substrates, including hexobarbital, aminopyrine and ethylmorphine (*Wade, 1986*). Many studies have suggested that a 10% corn oil diet increases the activities of drug-metabolizing enzymes, whereas others have reported that 3% dietary corn oil is sufficient for such increases (*Norred and Wade, 1979, Rowe and Wills, 1976*). However, isocaloric exchanges of saturated fat, unsaturated fat and carbohydrate do not appear to influence the metabolism of antipyrine and theophylline by the mixed function oxidase system in humans (*Anderson 1979*).

Studies to identify the specific nutrient component in PN regimens which may alter drug disposition are limited. Antipyrine metabolism was increased by

24% in healthy adults after one day of intravenous infusion of amino acids (*Pantuck et al. 1984*) and antipyrine metabolism remained the same with a change of dextrose concentration in PN solution from 13% to 25% (*Pantuck et al. 1985*). These observations suggest that amino acids in PN solution may be a determinant for altering drug disposition. Burgess *et al* (*1987*) found that antipyrine clearance was decreased by 34% in post-operative patients receiving an amino acid-dextrose solution for 7 days; but the reduction in antipyrine clearance could be prevented by the inclusion of lipid calories within the PN regimen. Thus, it is possible that specific nutrient components in PN solution may significantly affect drug disposition.

In summary, according to the above discussion, it is highly probable that, with PN therapy, the metabolism and excretion patterns of drugs will be significantly different from those seen in the absence of PN therapy. The result could vary from one in which the concentration of drug in the blood is reduced to a sub-therapeutic level, to one in which the blood drug concentration is so high that it is toxic. To minimize this possibility, it is essential to have a thorough understanding of PN and drug interaction. This is the objective of the present proposed research. Furthermore, to understand the influence of PN on drug elimination, it is necessary to systematically assess various possible mechanisms and factors which may affect PN and drug interaction, such as different hepatic pathways of drug metabolism, hepatic and renal blood flow, protein binding and the time of exposure to PN therapy.

1.3 Lidocaine

Lidocaine, N,N-diethylglycine-2,6-xylidide (Fig. 1.1), is the most frequently used drug for initial parenteral therapy of acute ventricular arrhythmias (*Benowitz and Meister 1978*). It is also widely used as a local anesthetic and significant systemic absorption often occurs (*Covino 1972*).

1.3.1 Pharmacokinetics

1.3.1.1 Absorption

Lidocaine is well absorbed after an oral administration but it is subjected to extensive hepatic first pass metabolism (*Boyes et al. 1971*). Only one-third of the drug reaches the general circulation after oral administration in human (*Boyes et al. 1971*). Consequently, the concentration of the drug attained in plasma is lower after an oral dose as compared to an identical intravenous dose. The lower plasma lidocaine concentration may have caused therapeutic failure in some patients (*Parkinson et al. 1970*). After a large oral dose (> 500 mg), many patients experienced nausea, vomiting and abdominal discomfort which is thought to be associated with high plasma concentrations of toxic metabolites produced by first pass metabolism (*Boyes et al. 1971*). Thus, this route of administration is not preferred.

1.3.1.2 Distribution

Lidocaine is extensively distributed in tissues (*Benowitz et al. 1974, Ahmad and Medzihradsky 1971*). Approximately 50 to 70% of the drug is bound to plasma

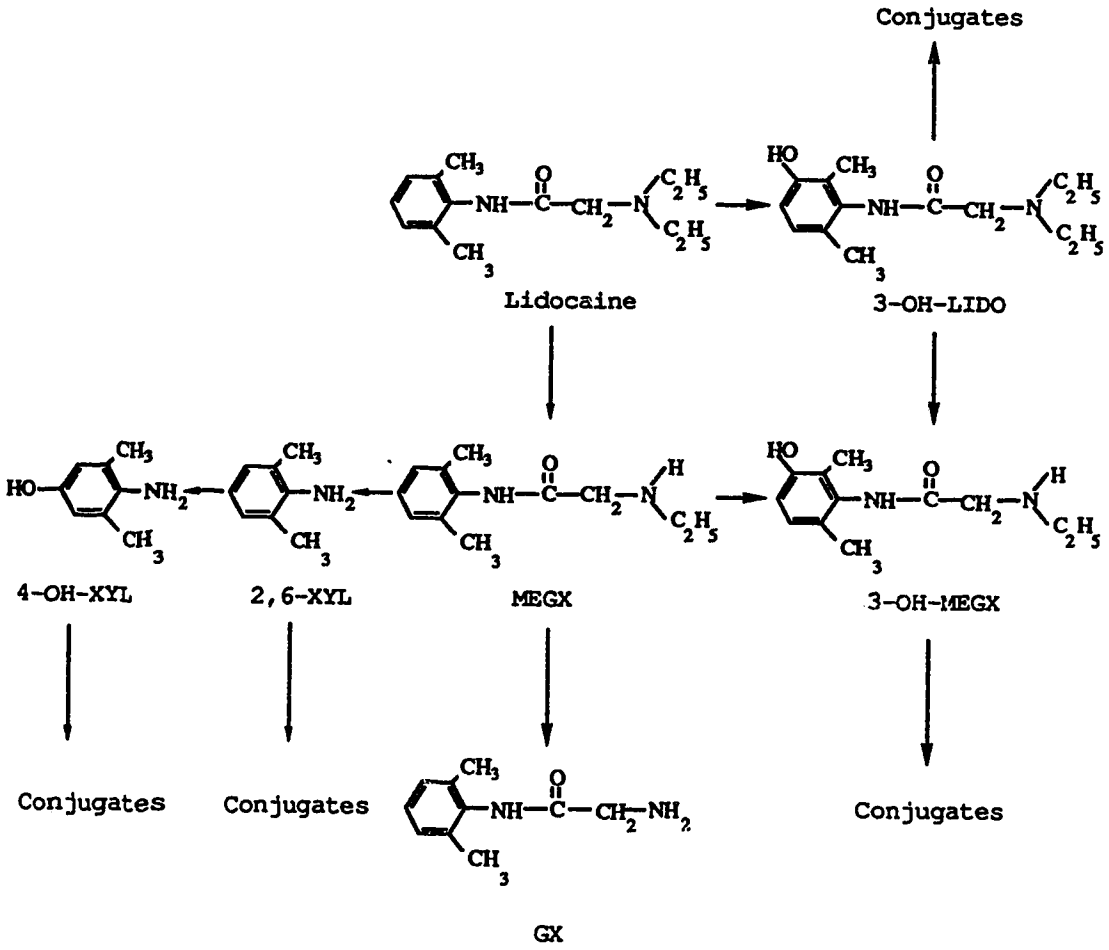


Figure 1.1 Schematics of lidocaine metabolism in humans and animals.

protein. Lidocaine has been shown to specifically bind to α-1-acid glycoprotein (AAG) with high affinity and low capacity characteristics (*Routledge et al. 1980*). In addition to binding to AAG, approximately 30 to 40% of lidocaine that is bound at therapeutic concentrations is associated with albumin (*McNamara et al 1981*).

After an intravenous bolus injection in normal subjects, the plasma lidocaine profile can be described by either a two or three compartment open model (*Tucker and Boas 1971*). Pharmacokinetic parameters (clearance, half-life and volume of distribution) calculated from either model showed no significant difference (*D'Argenio and Schumitzky 1979*). From the practical point of view, a two-compartment open model adequately describes lidocaine's plasma profile (*Singh and Kocot 1976, Boyes et al. 1971, Thomson et al. 1973 and Nation et al. 1977*).

1.3.1.3 Metabolism

Based on the structure of the drug, three sites are susceptible to metabolic attack: the tertiary amino group, the amide linkage and the aromatic ring. The tertiary amino group is susceptible to N-oxidation and oxidative N-deethylation. The amide linkage is susceptible to hydrolysis by microsomal amidases. The aromatic ring and the aryl methyl groups are also susceptible to hydroxylation.

The metabolic fate of lidocaine has been extensively studied in various species (*Keenaghan and Boyes 1972, Strong et al. 1973, Nelson et al. 1977, Mihaly et al. 1978, Coutts et al. 1987*). Three new *in vitro* metabolites have been recently reported. The products of N-oxidation (*Patterson et al. 1986*) and hydroxylation of the aryl methyl group (*Fujita et al. 1985, Kawai et al. 1986*) have

been identified in rat liver microsomes. The third *in vitro* new metabolite identified in the rabbit-liver homogenate was 2-amino-3-methyl-benzoic acid, a product of oxidation of aryl methyl group and amide hydrolysis of lidocaine (*Kammerer and Schmitz, 1986*). More recently, a new *in vivo* metabolite, 3-hydroxyl-N-glycyl-2,6-xylidine which is a product of *m*-hydroxylation and N-deethylation of lidocaine, has also been isolated and identified in rat urine by Coutts *et al* (*1987*). However, the Phase II metabolism of lidocaine is not well characterized.

Lidocaine is almost completely metabolized in the liver (*Keenaghan and Boyes 1972*). In human less than 5 % of intact drug was recovered in urine after an oral or parenteral dose. The major metabolic pathways for lidocaine involve N-deethylation and aromatic ring hydroxylation. Ring hydroxylation is viewed as a high-affinity, low-capacity system and N-deethylation as a low-affinity, high-capacity system in lidocaine metabolism (*Bahr et al. 1977*). The deethylation processes are similar in humans and animals, whereas, the hydroxylation processes are species dependent (*Keenaghan and Boyes 1972*). For example, in humans, *p*-hydroxylation dominates, whereas *m*-hydroxylation is more important in rats (*Keenaghan and Boyes 1972*). The proposed metabolic pathways in humans and animals are shown in Fig. 1.1. However, there was no HPLC assay available to simultaneously separate and quantitate lidocaine and its all known and/or possible metabolites, especially the *p*-hydroxylated metabolites of lidocaine.

N-deethylation metabolites, N-(N-ethylglycyl)-2,6-xylidine (MEGX) and N-glycyl-2,6-xylidine (GX), were commonly detectable in the blood of patients after

receiving lidocaine (*Strong et al. 1973*). They share some of the pharmacological properties of lidocaine, and may be responsible for central nervous system toxicity (*Halkin et al. 1975*). Narang et al. (*1978*) studied the anti-arrhythmic and toxic effects of MEGX and GX. They have found that MEGX has around 83%, and GX around 10% of the antiarrhythmic activity of lidocaine.

1.3.1.4 Excretion

Although biliary secretion has been reported in rats, there is no evidence of biliary secretion in humans (*Gillis 1989*). In humans approximately 80 % of an intravenous dose was recovered in urine, of which about 70 % was 4-hydroxy-2,6-xylidine (4-OH-XYL), a compound without any demonstrated pharmacologic effect, which was found mainly in conjugated forms. Because about 20% of a dose remains unaccounted for in human urine, it is possible that hepatic metabolism of lidocaine involves as yet unidentified metabolic pathway(s) in humans. Another possibility is that some of the known metabolites were lost during the storage and/or analysis procedures due to the instability of those metabolites.

1.3.2 Rationale for Choosing Lidocaine as A Model Drug

Lidocaine is a highly extracted drug and its clearance is dependent upon hepatic blood flow (*Nation et al. 1977*). Therefore, a change in hepatic blood flow potentially induced by PN could have a significant impact on lidocaine clearance.

Lidocaine is highly extracted and almost completely metabolized by the liver (*Pang and Rowland 1977, Keenaghan and Boyes, 1972*), therefore it is suitable

for use in "one-pass" isolated perfused rat liver studies. Lidocaine is also extensively used in drug metabolism studies (*Pang and Rowland 1977*), and recently, assessment of lidocaine metabolism as a quantitative liver function test has been suggested (*Oellerich et al. 1989*). Its major primary elimination pathways are N-deethylation and aromatic ring hydroxylation. In addition, primary metabolites of lidocaine either undergo further N-deethylation, ring hydroxylation and amide hydrolysis or conjugate with sulfate or glucuronic acid. Thus, the measurement of various metabolites of lidocaine allow the evaluation of the effects of PN on various drug metabolic pathways.

Like many basic and lipophilic drugs, lidocaine is specifically bound to AAG (*Routledge et al. 1980*) and has been used as a model drug to study protein binding changes during different disease states. Lidocaine is about 70 % bound to plasma proteins (*Routledge et al. 1980*) and has a similar chemical structure to certain aromatic amino acids, such as L-tryptophan and L-phenylalanine in PN solution. Therefore, measurement of alteration of plasma lidocaine protein binding during PN infusion may reflect the potential competition for binding sites from certain aromatic amino acids.

Choosing lidocaine as a model drug in the study of PN and drug interaction may allow the elucidation of the effects of PN on hepatic blood flow, various drug metabolic pathways in the liver, protein binding and volume of distribution of drugs.

1.4 Ampicillin

Ampicillin, (6R)-6-[α-D-phenylglycylamino]-penicillanic acid (Fig. 1.2) is the prototypical agent of β-lactam antibiotics and frequently used in the treatment of respiratory-tract infections, urinary-tract infections and enteric infections (*Reynolds, 1989*).

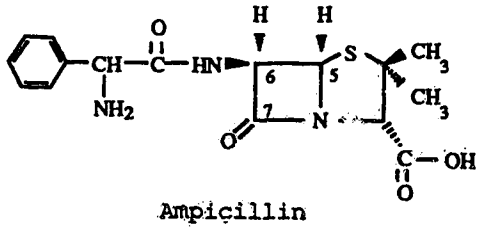
1.4.1 Pharmacokinetics

1.4.1.1 Absorption

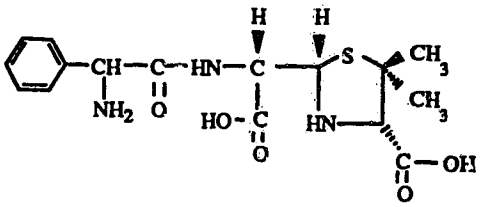
Ampicillin is stable in acid and is well absorbed from the gastrointestinal tract after oral administration (*Mandell and Sande, 1985*). Food can interfere with the absorption of ampicillin so doses should be taken 30 minutes to an hour before meals. Peak concentrations in plasma are obtained in about 1 to 2 hours and following a dose of 500 mg by mouth are reported to range from 2 to 6 μg/ml (*Barza and Weinstein 1976*).

1.4.1.2 Distribution

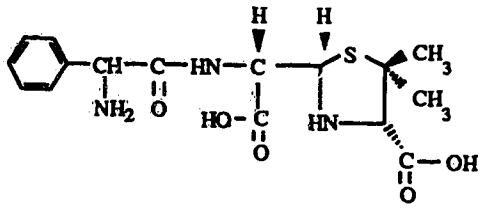
Ampicillin is widely distributed and therapeutic concentrations can be achieved in ascitic, pleural, and joint fluids. This drug diffuses across placenta into fetal circulation and the drug can be detected in the milk of nursing mothers (*Takyi 1970*). About 20 % of the drug is bound to plasma proteins and the plasma half-life is about 1 to 2 hours, but this may be increased in neonates and the elderly (*Triggs et al. 1980, Sabra et al. 1973*).



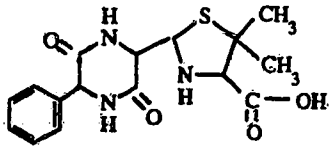
Ampicillin



5R, 6R - penicilloic acid



5S, 6R - penicilloic acid



piperazine - 2,5 - dione

Figure 1.2 Chemical structures of ampicillin and its metabolites.

1.4.1.3 Metabolism

In studies involving healthy subjects, about 21% of a dose of ampicillin (250 or 500 mg) has been reported to be metabolized. Ampicillin is metabolized to 5R, 6R-penicilloic acid and 5S, 6R-penicilloic acid in man (*Bird et al. 1983*), but piperazine-2,5-dione has also been detected in human urine following administration of ampicillin by mouth (*Haginaka and Wakai 1986*). The chemical structures of ampicillin and its metabolites are depicted in Fig. 1.2..

1.4.1.4 Excretion

Renal elimination of ampicillin occurs partly by glomerular filtration and partly by tubular secretion. In renal failure patients, there is decreased renal tubular secretion as well as decreased glomerular filtration of ampicillin and ampicillin plasma half-lives of 7 to 20 hours have been reported (*Hori et al. 1983*). About 20 to 40 % of an orally administered dose is excreted unchanged in the urine in the first 6 hours after a dose; urinary concentrations range from 0.25 to 1 mg/ml following a dose of 500 mg. Following parenteral administration about 60 to 80% is excreted in the urine within 6 hours (*Reynolds 1989*). Ampicillin appears in the bile, undergoes enterohepatic circulation, and is excreted in appreciable quantities in the feces (*Mandell and Sande, 1985*).

1.4.2 Rationale for Choosing Ampicillin as A Model Drug

In order to examine the potential effects of PN on renal drug elimination, ampicillin is chosen as another model drug in this project. More than 80% of

this drug is removed by the kidney in the intact form. Its renal clearance is substantially higher than glomerular filtration rate which indicates that renal active secretion does occur. Since creatinine clearance is to be monitored throughout the study, measurement of ampicillin clearance during PN will give more information with respect to the effects of PN on renal drug excretion by various processes via the kidney.

1.5 Rationale for Choosing the *in vitro* and *in vivo* Animal Models

1.5.1 *In vitro* Isolated Rat Liver Perfusion Model

Isolated rat liver perfusion experiments offer a convenient means of studying the liver's contribution to drug metabolism. Since both organ and cellular integrity are maintained, these studies more adequately represent the *in vivo* situation than do studies with microsomes or isolated cell preparations. The advantage of using rat liver perfusion, in particular the single pass system, is that it allows researchers to single out parameters which may influence hepatic drug clearance and study them individually. In addition, the use of a single pass system allows drug metabolism to be investigated at steady state. The ability to measure the rate of drug metabolism at a constant drug input rate is an unique feature of this system compared with other preparations (i.e., cell preparations or microsomal preparations). Although hydroxylation of lidocaine is probably different in rats compared to humans (*m*-hydroxylation in rat vs. *p*-hydroxylation in human), the deethylation pathways are similar (*Keenaghan and Boyes 1972*).

1.5.2 *In vivo* Pig Model

The pig has been used frequently as an *in vivo* model in PN research (*Buckley et al. 1985*) because its nutritional requirements are very similar to humans (*McCraken 1980 and Dodds 1982*). In addition, there are many similarities in cardiovascular system, gastrointestinal tract, renal physiology, and liver blood flow ($\sim$ 25 ml/min.kg) between the pig and humans (*Egger et al. 1974 and Earl et al. 1964*). Furthermore, according to our laboratory's preliminary pig study, the metabolic profile of lidocaine in the pig is closest to that of humans in that *p*-hydroxylation is an important metabolic pathway.

1.6 Hypotheses

This project aims at testing the following hypotheses:

1. PN increases drug renal clearance secondary to an elevation in glomerular filtration rate (GFR) and renal plasma flow (RPF).
2. PN and drug interaction depends upon the duration of PN administration.
3. PN inhibits different metabolic pathways in the liver differently.
4. PN lowers binding of drugs to serum and/or tissue proteins.
5. The extent of PN and drug interaction depends upon the subject's nutritional state.

1.7 Objectives

The objectives of this project are to use two model drugs, lidocaine and ampicillin to study the detailed mechanisms of PN and drug interactions. The specific objectives of this project are:

1. to develop sensitive, specific and convenient HPLC assays capable of measuring lidocaine and its aromatic ring and aryl methyl hydroxylated metabolites in biological samples.
2. to study lidocaine metabolism in detail.
3. to evaluate acute effects of macronutrients in PN on ampicillin pharmacokinetics, specifically on renal clearance in human.
4. to delineate acute effects of macronutrients in PN on lidocaine pharmacokinetics, specifically on hepatic metabolism in human.
5. to study both the acute and the short-term effects of PN on lidocaine hepatic elimination using an isolated rat liver perfusion model.
6. to initiate a pilot study in pigs:

   a. to study the effects of PN and nutritional state on the disposition of lidocaine.

   b. to study the effects of PN and nutritional state on the serum protein binding of lidocaine.

1.8 References

Addis T, Poo LJ, Lew W., "Quantities of protein lost by various organs and tissues of body during fast.", *J Biol Chem*, 115, pp.111-116 (1936).

Ahmad K, Medzihradsky F., "Lidocaine in blood and tissues after single dose and steady state infusion.", *Res Commun Chem Pathol Pharmacol*, 2, pp.813-828 (1971).

Anderson KE., "Nutrition and oxidative drug metabolism in man: relative influences of dietary lipids, carbohydrate,and protein.", *Clin Pharmacol Ther*, 26, pp.493-501 (1979).

Anderson KE, Pantuck EJ, Conney AH, Kappas A., "Nutrient regulation of chemical metabolism in humans.", *Fed Proc*, 44, pp.130-133 (1985).

Anderson KE., "Influence of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet.*, 14, pp.325-346 (1988).

Arcos JC, Myers SC, Neuburger BJ, Argus MF.. "Comparative effects of indole and aminoacetonitrile derivatives on dimethylnitrosamine-demethylase and aryl hydrocarbon hydroxylase activities.", *Cancer Lett*, 9, pp.161-167 (1980).

Argyris TS., "Additive effects of phenobabital and high protein diet on liver growth in immature male rats ", *Dev Biol*, 25, pp.293-309 (1971).

von Bahr C, Hedlund I, Karlen B, Backtrom D, Grasdalen H., "Evidence for two catalytically different binding sites of liver microsomal cytochrome P450 : Importance for species and sex differences in oxidation pattern of lidocaine.", *Acta Pharmacol Toxicol*, 41, pp.39-48 (1977).

Baker AL, Rosenberg IH., "Hepatic complications of total parenteral nutrition.", *Am J Med*, 82, pp.489-497 (1987).

Baker AE., "Use of subcutaneous injections of saline and carbohydrate.", *Am Med*, 9, pp.234 (1904).

Barza M, Weinstein L., "Pharmacokinetics of the penicillins in man.", *Clin Pharmacokinet*, 1, pp.297-308 (1976).

Benowitz N, Forsyth RP, Melmon KL, Rowland M., "Lidocaine disposition kinetics in monkey and man I. Prediction by a perfusion model.", *Clin Pharmacol Ther*, 16, pp.87-98 (1974).

Benowitz NL, Meister W., "Clinical pharmacokinetics of lidocaine.", *Clin Pharmacokinet*, 3, pp.177 -201 (1978).

Bird AE, Cutmore EA, Jennings KR, Marshall AC., "Structure re-assignment of a metabolite of ampicillin and amoxycillin and epimerization of their penicilloic acids.", *J Pharm Pharmacol*, 35, pp.138-143 (1983).

Bower RH., "Hepatic complications of parenteral nutrition.", *Semin Liver Dis*, 3, pp.216-224 (1983).

Bowyer BA, Miles JM, Haymond MW., "L-carnitine therapy in home parenteral nutrition patients with abnormal liver tests and low plasma carnitine concentrations.", *Gastroenterology*, 94, pp.434-438 (1988).

Boyes RN, Scott DB, Jebson PJ, Grodman MV, Julian DG., "Pharmacokinetics of lidocaine in man.", *Clin Pharmacol Ther*, 12, pp.105-116 (1971).

Brandt JL, Castleman L, Ruskin HD, Greenwald J, Kelly JHJr., "The effect of oral protein and glucose feeding on splanchnic blood flow and oxygen utilization in normal and cirrhotic subjects.", *J Clin Invest*, 34, pp.1017-1025 (1955).

Buckley DC, Mirtallo JM, Kudsk KA., "Total parenteral nutrition in swine.", In: Tumbleson ME, ed. **Swine in biomedical research.**, (New York and London: Plenum Press) pp.1147-1151 (1985).

Burgess P, Hall RI, Bateman DN, Johnston IDA., "The effect of total parenteral nutrition on hepatic drug oxidation.", *JPEN*, 11, pp.540-543 (1987).

Byrne AJ, McNeil J, Harrison PM, Louis W, Tonkin AM, McLean AJ., "Stable oral availability of sustained release propranolol when coadministered with hydralazine or food: evidence implicating substrate delivery rate as determinant of presystemic drug elimination.", *Br J Clin Pharmacol*, 17 Suppl.1, pp.45-50 (1984).

Byrne AJ, Morgan DJ, Harrison PM, McLean AJ., "Variation in hepatic extraction ratio with unbound drug fraction discriminates between models of hepatic drug elimination.", *J Pharm Sci*, 74, pp.205-207 (1985).

Campbell TC, Hayes TR., "Role of nutrition in the drug-metabolizing enzyme system.", *Pharmacol Rev*, 26, pp.171-197 (1974).

Conney AH, Pantuck EJ, Kuntzman R, Kappas A, Anderson KE, Alvares AP, "Nutrition and chemical biotransformations in man", *Clin Pharmacol Ther*, 22, pp.707-720 (1977).

Corbett H, Cahill CM, Heinzow B, Harrison PM, Byrne AJ, McLean AJ., "Interaction between

oral hydralazine and propranolol. II. Assessment of altered splanchnic blood flow as the determinant of altered presystemic extraction.", *J Pharmacol Exper Ther*, 239, pp.517-521 (1986).

Corcoran GB, "Pharmacokinetic considerations during total parenteral nutrition", In: Lebenthal E, ed. **Total Parenteral Nutrition**, (New York: Raven Press) pp.125-140 (1986).

Coutts RT, Torok-Both GA, Tam YK, Chu LV, Pasutto FM., "Acetylation and pentafluorobenzoylation of lidocaine metabolites in aqueous solution and identification of derivatives by combined gas chromatography/mass spectrometry", *Biomed Environ Mass Spectrom*, 14, pp.173-182 (1987).

Covino BG., "Local anesthesia", *N Engl J Med*, 286, pp.1035-1042 (1972).

Cunningham VJ, Hay L, Stoner HB., "The binding of l-tryptophan to serum albumins in the presence of nonesterified fatty acids.", *Biochem J*, 146, pp.653-658 (1975).

Cuthbertson D., "Historical background to parenteral nutrition", *Acta Chir Scand Suppl*, 489, pp.1-11 (1980).

D'Argenio DZ, Schumitzky A., "A program package for simulation and parenteral estimation in pharmacokinetic systems.", *Comput Programs Biomed*, 9, pp.115-134 (1979).

Dodds WJ., "The pig model for biomedical research.", *Fed Proc*, 41, pp.247-256 (1982).

Dudrick SJ, Wilmore DW, Vars HM, Rhoads JE., "Long-term total parenteral nutrition with growth, development, and positive nitrogen balance.", *Surgery*, 64, pp.134-142 (1968).

Dudrick SJ, Rhoads JE., "New horizons for intravenous feeding.", *JAMA*, 215, pp.939-949 (1971).

Earl FL, Tegeris AS, Whitmore GE, Morison R, Fitzhugh OG., "The use of swine in drug toxicity studies.", *Ann NY Acad Sci*, 111, pp.671-688 (1964).

Egger G, Kutz K, Strebel H, Bircher J, Weber M, Scholl E, Preisig R., "Bile formation in the intact pig.", *Am J Vet Res*, 35, pp.1203-1208 (1974).

Evarts RP, Mostafa MH., "Effects of indole and tryptophan on cytochrome P-450, dimethylnitrosamine demethylase, and arylhydrocarbon hydroxylase activities.", *Biochem Pharmacol*, 30, pp.517-522 (1981).

Fabri PJ, Deutsch S, Gower W. Mirtallo JM, Staubus A, Love JE., "Parenteral nutrition: Short term effects on hepatic clearance of sodium taurocholate and indocyanine green.", *Clin Biochem*, 20, pp.57-60 (1987).

Fagan TC, Walle T, Oexmann MJ, Walle UK, Bai SA, Gaffney TE., "Increased clearance of propranolol and theophylline by high-protein compared with high-carbohydrate diet.", *Clin Pharmacol Ther*, 41, pp.402-406 (1987).

Fujita S, tatsuno J, Kawai R, Kitagawa H, Suzuki T, Kitani K., "Age associated alteration of lidocaine metabolism is position selective.", *Biochem Biophys Res Commun*, 126, pp.117-122 (1985).

Gillis MC, Bisson R, Blais D., "Compendium of pharmaceuticals and specialties.", (Canadian Pharmaceutical Association) 29th, pp.547 (1989).

Graf H, Stummvoll HK, Luger A, Prager R., "Effect of amino acid infusion on glomerular filtration rate.", *N Engl J Med*, 308, pp.159-160 (1983).

Grant JP, Cox CE, Kleinman LM, Maher MM, Pittman MA, Tangrea JA, Brown JH, Cross E, Beazley RM, Jones RS., "Serum hepatic enzyme and bilirubin elevations during parenteral nutrition.", *Surg Gynecol Obstet*, 145, pp.573-580 (1977).

Gree HO, Mortiz J, Lack L., "Binding of sodium taurocholate by bovine serum albumin.", *Biochem Biophys Acta*, 231, pp.550-552 (1971).

Haginaka J, Wakai J., "A sensitive high performance liquid chromatographic assay of a new metabolite of ampicillin in man.", *J Pharm Pharmacol*, 38, pp.225-226 (1986).

Halkin H, Meffin P, Melmon KL, Rowland M., "Influence of congestive heart failure on blood levels of lidocaine and its active monodeethylated metabolites.", *Clin Pharmacol Ther*, 17, pp.669-676 (1975).

Hathcock JN and Coon J., **Nutrition and drug interrelations.**, In: Hathcock JN and Coon J, ed. (New York: Academic Press, Inc.) pp.1-835 (1978).

Homeida M, Karrar ZA, Roberts JC., "Drug metabolism in malnourished children: a study with antipyrine.", *Arch Dis Child*, 54, pp.299-302 (1979).

Hori R, Okumura K, Kamiya A, Nihira H, Nakano H., "Ampicillin and cephalexin in renal insufficiency.", *Clin Pharmacol Ther*, 34, pp.792-798 (1983).

Kammerer RC and Schmitz DA., "Lidocaine metabolism by rabbit-liver homogenate and detection of a new metabolite.", *Xenobiotica*, 16, pp.681-690 (1986).

Kappas A, Anderson KE, Conney AH, Alvares AP., "Influence of dietary protein and carbohydrate on antipyrine and theophylline metabolism in man.", *Clin Pharmacol Ther*, 20, pp.643-653

(1976).

Kappas A, Alvares AP, Anderson KE, Pantuck EJ, Pantuck CB, Chang P, Conney AH., "Effect of charcoal-broiled beef on antipyrine and theophylline metabolism.", *Clin Pharmacol Ther*, 23, pp.445-450 (1978).

Kawai R, Fujita S, Suzuki T., "A new lidocaine metabolite, ω-Diethylamino-2-hydroxymethyl-6-methylacetanide.", *Drug Metab Dispos*, 14, pp.277-279 (1986).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man.", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Kitt TM, Park GD, Spector R, Tsalikian E., "Reduced renal clearance of oxypurinol during a 400 calorie protein-free diet.", *J Clin Pharmacol*, 29, pp.65-71 (1989).

Klein S, Nealon W., "Hepatobiliary abnormalities associated with total parenteral nutrition", *Semin Liver Dis*, 8, pp.237-246 (1988).

Knodell RG, Spector MH, Keller FX, Brooks DA, Kyner WT., "Effects of enteral and parenteral alimentation on intestinal metabolism of pentobarbital in the rat.", *Clin Res*, 27, pp.268A (1979).

Knodell RG, Spector MH, Brooks DA, Keller FX, Kyner WT., "Alterations in pentobarbital pharmacokinetics in response to parenteral and enteral alimentation in rat.", *Gastroenterology*, 79, pp.1211-1216 (1980).

Knodell RG, Steele NM, Cerra FB, Cross JB, Solomon TE., "Effects of parenteral and enteral hyperalimentation on hepatic drug metabolism in the rat", *J Pharmacol Exper Ther*, 229, pp.589-597 (1984).

Lanza-Jacoby S, Sitren HS, Stevenson NR, Rosato FE., "Changes in circadian rhythmicity of liver and serum parameters in rats fed a total parenteral nutrition solution by continuous and discontinuous intravenous or intragastric infusion.", *JPEN*, 6, pp.496-502 (1982).

Levenson SM, Hopkins BS, Waldron M., "Early history of parenteral nutrition.", *Fed Proc*, 43, pp.1391-1406 (1984).

Liedholm H, Melander A., "Concomitant food intake can increase the bioavailability of propranolol by transient inhibition of its presystemic primary conjugation.", *Clin Pharmacol Ther*, 40, pp.31-36 (1986).

Mandell GL, Sande MA., "Antimicrobial agents.", In: Gilman AG, Goodman LS, Rall TW, Murad

F., ed. **Goodman and Gilman's the pharmacological basis of therapeutics, 7th.**, (New York: Macmillan Publishing Company.) pp.1115-1169 (1985).

Merritt RJ., "Cholestasis associated with total parenteral nutrition.", *J Pediatr Gastrointerol Nutr*, 5, pp.9-22 (1986).

McCraken KJ, Eddie SM, Stevenson WG., "Energy and protein nutrition of early-weaned pigs. 1. Effect of energy intake and energy: Protein on growth, efficiency and nitrogen utilization of pigs between 8-32d.", *Br J Nutr*, 43, pp.289-304 (1980).

McLean AJ, McNamara P, du Souich P, Gibaldi M, Lalka D., "Food, splanchnic blood flow and bioavailability of drugs subject to first-pass metabolism.", *Clin Pharmacol Ther*, 24, pp.5-10 (1978).

McLean AJ, Skews H, Bobik A, Dudley FJ., "Interaction between oral propranolol and hydralazine-mechanism and therapeutic implications.", *Clin Pharmacol Ther*, 30, pp.31-34 (1980).

McNamara PJ, Slaughter RL, Pieper JA, Wyman MG, Lalka D., "Factors influencing serum protein binding of lidocaine in humans.", *Anesth and Analg*, 60, pp.395-400 (1981).

McSweeney GW., "Parenteral nutrition", In: Katcher BS, Young LY, Koda K, Koda-Kimble MA., ed. **Applied therapeutics, the clinical use of drugs, 3rd.**, (Spokane: Applied Therapeutics, Inc.) pp.629-650 (1983).

Melander A, McLean A., "Influence of food intake on presystemic clearance of drugs.", *Clin Pharmacokinet*, 8, pp.286-296 (1983).

Melander A. Lalka D, McLean A., "Influence of food on the presystemic metabolism of drugs.", *Pharmacol Ther*, 38, pp.253-267 (1988).

Meyer TW, Ichikawa I, Zatz R, Brenner BM., "The renal hemodynamic response to amino acid infusion in the rat.", *Trans Assoc Am Physicians*, 96, pp.76-83 (1983).

Mihaly GW, Moore RG, Thomas J, Triggs EJ, Thomas D, Shanks CA., "The pharmacokinetics and metabolism of the anilide local anaesthetics in neonates.", *Eur J Clin Pharmacol*, 13, pp.143-152 (1978).

Modi MW, Hassett JM, Lalka D., "Influence of posture on hepatic perfusion and the presystemic biotransformation of propranolol: Simulation of the food effect.", *Clin Pharmacol Ther*, 44, pp.268-274 (1988).

Mullen TL, Buzby GP, Matthews DC, Smale BF, Rosato EF., "Reduction of operative morbidity

and mortality by combined preoperative and postoperative nutritional support.", *Ann Surg*, 192, pp.604-613 (1980).

Mullen JM, Brenner U, Dienst C, Pichlmaier H., "Preoperative parenteral feeding in patients with gastrointestinal carcinoma.", *Lancet*, 1, pp.68-71 (1982).

Nakajima T, Koyama Y, Sato A., "Dietary modification of metabolism and toxicity of chemical substances — with special reference to carbohydrate. ", *Biochem Pharmacol*, 31, pp.1005-1011 (1982).

Narang RK, Mehta S, Mathur VS., "Pharmacokinetic study of antipyrine in malnourished children.", *Am J Clin Nutr*, 30, pp.1979-1982 (1977).

Narang PK, Crouthamel WG, Carliner NH, Fisher ML, "Lidocaine and its active metabolites.", *Clin Pharmacol Ther*, 24, pp.654-662 (1978).

Nation R, Triggs EJ, Selig M., "Lignocaine kinetics in cardiac patients and aged subjects.", *Br J Clin Pharmacol*, 4, pp.439-448 (1977).

Nelson SD, Garland WA, Breck GD, Trager WF., "Quantification of lidocaine and several metabolites utilizing chemical-ionization mass spectrometry and stable isotope labelling.", *J Pharm Sci*, 66, pp.1180-1189 (1977).

Norred WP, Wade AE., "Effect of dietary lipid ingestion on the induction of drug-metabolizing enzymes by phenobarbital.", *Biochem Pharmacol*, 22, pp.432-436 (1973).

Odell GB., "Influence of binding to human plasma albumin.", *Ann NY Acad Sci*, 226, pp.225-237 (1973).

Oellerich M, Burdelski M, Ringe B, Lamesch P, Gubernatis G, Bunzendahl H, Pichlmayr R, Hermann H., "Lignocaine metabolite formation as a measure of pre-transplant liver function.", *Lancet*, 3, pp.640-642 (1989).

Olanoff LS, Walle T, Cowart TD, Walle K, Oexmann MJ, Conradi EC., "Food effects on propranolol systemic and oral clearance: support for a blood flow hypothesis.", *Clin Pharmacol Ther*, 40, pp.408-414 (1986).

Orrego H, Mena I, Baraona E, Palma R., "Modifications in hepatic blood flow and portal pressure produced by different diets.", *Am J Dig Dis*, 10, pp.239-248 (1965).

Paine AJ., "Effect of amino acids and inducers on the activity of the microsomal mono-oxygenase system in rat liver cell culture.", *Chem Biol Interact*, 13, pp.307-315 (1976).

Pang KS, Rowland M., "Hepatic clearance of drug. II. Experimental evidence for acceptance of the "well-stirred" model over the "parallel-tube" model using lidocaine in the perfused rat liver in situ preparation.", *J Pharmacokinet Biopharm*, 5, pp.655-699 (1977).

Pantuck EJ, Pantuck CB, Weissman C, Askanazi J, Conney AH., "Effects of parenteral nutritional regimens on oxidative drug metabolism.", *Anesthesiology*, 60, pp.534-536 (1984).

Pantuck EJ, Pantuck CB, Weissman C, Gil KM, Askanazi J., "Stimulation of oxidative drug metabolism by parenteral refeeding of nutritionally depleted patients.", *Gastroenterology*, 89, pp.241-245 (1985).

Parkison PI, Margolin L, Dickson DSP., "Oral lignocaine: Its absorption and effectiveness in ventricular arrhythmia control.", *Br Med J*, 2, pp.29-30 (1970).

Patterson LH, Hall G, Nijjar BS, Khatra PK, Cowan DA., "In-vitro metabolism of lignocaine to its N-oxide.", *J Pharm Pharmacol*, 38, pp.326 (1986).

Peden VH, Witzleben CL, Skelton MA., "Total parenteral nutrition.", *J Pediatr*, 78, pp.180-181 (1971).

Piech CT., "Parenteral/enteral nutrition a $1.2 billion market in 1984.", *Am Pharm*, NS25 (5), pp.11-12 (1985).

Reynolds JEF., **Martindale The extra pharmacopoeia, 29th.**, pp.116-119 (1989).

Riely CA, Fine PL, Boyer JL., "Progressively rising serum bile acids. A common effect of parenteral nutrition.", *Gastroenterology*, 77, pp.A34 (1979).

Roe DA., "Interactions between drugs and nutrients.", *Med Clin Nor Amer*, 63, pp.985-1007 (1979).

Roe DA., "A comparison of drug-nutrient and nutrient-nutrient interaction.", In: Bodwell CE, John W, Erdman JR, ed. **Nutrient interactions.**, (New York: Marcel Dekker) pp.365-377 (1988).

Routledge PA, Barchowsky A, Bjornsson TD, Kitchell BB, Shand DG., "Lidocaine plasma protein binding.", *Clin Pharmacol Ther*, 27, pp.347-351 (1980).

Rowe L, Wills ED., "The effect of dietary lipids and vitamin E on lipid peroxide formation, cytochrome P_{450} and oxidative demethylation in the endoplasmic reticulum.", *Biochem Pharmacol*, 25, pp.175-179 (1976).

Rowland M, Leitch D, Fleming G, Smith B., "Protein binding and hepatic extraction of diazepam

across the rat liver.", *J Pharm Pharmacol*, 35, pp.383-384 (1983).

Rowland M, Leitch D, Fleming G, Smith B., "Protein binding and hepatic clearance: discrimination between models of hepatic clearance with diazepam, a drug of high intrinsic clearance, in the isolated perfused rat liver preparation.", *J Pharmacokinet Biopharm*, 12, pp.129-147 (1984).

Roy CC, Belli DC., "Hepatobiliary complications associated with TPN: An enigma.", *J Am Coll Nutr*, 4, pp.651-660 (1985).

Sabra A, Metrau F, Feitosa N, Santi JM, Trabulsi LR., "Serum levels of anhydrous ampicillin administered orally in normal infants.", *Curr Ther Res*, 15, pp.866-871 (1973).

Sato A, Nakajima T., "Pharmacokinetics of organic solvent vapors in relation to their toxicity.", *Scand J Work Environ Health*, 13, pp.81-93 (1987).

Semple HA, Tam YK, Coutts RT., "A computer simulation of the food effect: transient changes in hepatic blood flow and michaelis-menten parameters as mediators of hepatic first pass metabolism and bioavailability of propranolol.", *Biopharm Drug Dis*, In press, (1990).

Sidransky H., "Effects of tryptophan on protein synthesis by liver.", In: Scarpelli DG, Migaki G., ed. **Nutritional diseases: research directions in comparative pathobiology**, (New York: Alan R. Liss, Inc.,) pp.81-90 (1986).

Singh JB, Kocot BZ., "A controlled trial of intramuscular infarction.", *Am Heart J*, 91, pp.430-436 (1976).

Skutches CL, Myers RN, Paul P, Smink RD, Reichard GA., "Plasma free fatty acid turnover and oxidation during fat-free and intra-lipid TPN.", *JPEN*, 4, pp.572-574 (1980).

Spector AA., "Fatty acid binding to plasma albumin.", *J Lipid Res*, 16, pp.165-179 (1975).

Stein TP, Buzby GP, Gertner MH, Hargrove WC, Leskiw MJ, Mullen JL., "Effect of parenteral nutrition on protein synthesis and fat metabolism in man.", *Am J Physiol*, 239, pp.G280-287 (1980).

Strong JM, Parker M, Atkinson AJ., "Identification of glycinexylidide in patients treated with intravenous lidocaine.", *Clin Pharmacol Ther*, 14, pp.67-72 (1973).

Svensson CK, Edwards DJ, Mauriello PM, Barde SH, Foster AC, Lanc RA, Middleton EJr, Lalka D., "Effect of food on hepatic blood flow; implications in the "food effect" phenomenon", *Clin Pharmacol Ther*, 34, pp.316-323 (1983).

Swartz RD, Sidell FR, Cucinell SA., "Effects of physical stress on the disposition of drugs elimi-

nated by the liver in man.", *J Pharmacol Exper Ther*, 188, pp.1-7 (1974).

Sweeney GD., "Drugs—some basic concepts.", *Med Sic Sports Exercise*, 13, pp.247-251 (1981).

Takyi BE., "Excretion of drugs in human milk.", *J Hosp Pharm*, 28, pp.317-326 (1970).

Thomson PD, Melmon KL, Richardson JA., "Lidocaine pharmacokinetics in advanced heart failure, liver disease and renal failure in humans. ", *Ann Intern Med*, 78, pp.499-508 (1973).

Tranvouex JL, Lerebours E, Chretien P, Fortunet HF, Colin R., "Hepatic antipyrine metabolism in malnourished patients:influence of the type of malnutrition and course after nutritional rehabilitation.", *Am J Clin Nutr*, 41, pp.1257-1264 (1985).

Triggs EJ, Johnson JM, Learoyd B., "Absorption and disposition of ampicillin in the elderly.", *Eur J Clin Pharmacol*, 18, pp.195-198 (1980).

Tucker GT, Boas RA., "Pharmacokinetic aspects of intravenous regional anesthesia.", *Anesthesology*, 34, pp.538-549 (1971).

Tulikoura I, Huikuri K., "Morphological fatty changes and function of the liver, serum free fatty acids, and triglycerides during parenteral nutrition.", *Scand J gastroenterol*, 17, pp.177-185 (1982).

Wade AE., "Effects of dietary fat on drug metabolism.", *J Environ Path Toxic Oncol*, 6, pp.161-189 (1986).

Walle T, Fagan TC, Walle K, Oexmann MJ, Conradi EC, Gaffney TE., "Food-induced increase in propranolol bioavailability—relationship to protein and effects on metabolites.", *Clin Pharmacol Ther*, 30, pp.790-795 (1981).

Welling PG., "Influence of food and diet on gastrointestinal drug absorption: a review.", *J Pharmacokinet Biopharm*, 5, pp.291-334 (1977).

Wheeler EL, Schwass DE, Crawford L, Berry DL., "Modulation of Benzo[a]pyrene metabolism by dietary sulfur amino acids.", Finley JW, Schwass DE., **Xenobiotic metabolism: nutritional effects.**, (ACS symposium series, American Chemical Society, Washington, DC.) (1985).

Wilmore DW, Dudrick SJ., "Growth and development of an infant receiving all nutrients exclusively by vein.", *JAMA*, , pp.860-864 (1968).

Yang CS, Yoo TSH., "Dietary effects on drug metabolism by the mixed-function oxidase system", *Pharmacol Ther.*, 38, pp.53-72 (1988).

Zimmerman CL, Patel IH, Levy RH, Edwards D, Nelson SD, Hutchinson M., "Protein binding of valproic acid in the presence of elevated free fatty acids in patient and normal human serum.", *Epilepsia*, 22, pp.11-17 (1981).

CHAPTER 2

A NEW HPLC ASSAY FOR LIDOCAINE AND ITS METABOLITES: APPLICATION TO STUDY 4-HYDROXYLATION[1]

2.1 Introduction

The *in vivo* metabolism of lidocaine has been studied extensively in various species (*Keenaghan and Boyes, 1972, Strong et al. 1973, Nelson et al. 1977, Mihaly et al. 1978, Coutts et al. 1987, Thomson et al. 1987*). Lidocaine is rapidly metabolized mainly to phenolic compounds which are excreted in urine as conjugates in all species, but the relative amounts of these phenolic metabolites vary with the species. In humans, for example, the most abundant urinary metabolite of lidocaine is 4-hydroxy-2,6-xylidine (4-OH-XYL), while in rats, 3-hydroxy-lidocaine (3-OH-LIDO) and 3-hydroxy-N-(N-ethylglycyl)-2,6-xylidine (3-OH-MEGX) are more prominent. It has also been claimed that 4-hydroxy-lidocaine (4-OH-LIDO) is a significant metabolite (up to 5%) of lidocaine in rats (*Thomas and Meffin 1972*), and may be an intermediate in the metabolism of lidocaine to 4-OH-XYL in man (*Mihaly et al. 1978*). Other investigators (*Nelson et al 1977*) have speculated that 4-hydroxylated metabolites of lidocaine may be excreted in low concentrations in human urine.

Preliminary results in our laboratory suggest that the major urinary metabolite of lidocaine in human, 4-OH-XYL, is formed from a number of probable precursors such as 4-OH-LIDO, 4-hydroxy-N-(N-ethylglycyl)-2,6-xylidine (4-OH-MEGX)

[1] A version of this chapter has been published in *J Chromatogr Biomed Appl* 423: 199-206, (1987)

and 4-hydroxy-N-glycyl-2,6-xylidine (4-OH-GX) in addition to 2,6-xylidine (2,6-XYL). This observation has revived an interest in studying lidocaine metabolism in detail because *p*-hydroxylation is the most dominant process for the elimination of lidocaine and its metabolites in human. To date, no one has isolated and quantified all of these 4-hydroxylated precursors of 4-OH-XYL in human.

In order to study lidocaine metabolism in human and animals, and to elucidate mechanisms by which PN may influence lidocaine metabolism, it is imperative to have an assay which is capable of measuring lidocaine and all its known and/or possible metabolites. A search in the literature showed that most assays provide measurements of lidocaine and its two active N-deethylated metabolites N-(N-ethylglycyl)-2,6-xylidine (MEGX) and N-glycyl-2,6-xylidine (GX) (*Hignite et al. 1978, Nation et al. 1979, Hill et al. 1980, Ahmad and Medzihradsky 1971, Benowitz and Rowland 1978, Benowitz et al. 1974, Holt et al. 1979, Luzzi et al. 1984*). Tam and coworkers (*1984*) have separated five and quantitated four of the lidocaine metabolites (MEGX, GX, 3-OH-LIDO and 3-OH-MEGX) from perfused rat liver samples. Kawai *et al.* (*1985*) reported a similar separation of the same metabolites. There is no HPLC assay available to separate and quantitate lidocaine and its known and/or possible metabolites, especially the *p*-hydroxylated precursors of 4-OH-XYL, in human. In this study we decided to develop a simple high-performance liquid chromatographic (HPLC) method which could be used to measure lidocaine and nine of its known and/or possible metabolites in human. In addition we have initiated a pilot study in pigs specifically to investigate the *p*-hydroxylation pathway of lidocaine.

2.2 Experimental

2.2.1 Reagents and Chemicals

The hydrochloride salts of lidocaine and its metabolites, MEGX, GX, 3-OH-LIDO, 3-OH-MEGX, 2,6-XYL and 4-OH-XYL, were kindly supplied by Astra Pharmaceuticals (Mississauga, Canada). 4-Hydroxylated products of lidocaine (4-OH-LIDO, 4-OH-MEGX and 4-OH-GX) were synthesized in our laboratory from N-chloroacetyl-4-hydroxy-2,6-xylidine and structures were confirmed by combined gas chromatography-mass spectrometry (GC-MS) (*Coutts et al. 1987*). Procaine hydrochloride, purchased from K & K Labs. (Plainview, NY, grade), was used as the internal standard. Acetonitrile (HPLC grade), ethyl acetate (BDH analytical reagent), acetic anhydride, phosphoric acid and triethylamine (TEA) were purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.). The other chemicals used were reagent grade.

2.2.2 Human Studies

Patients with suspected myocardial infarction were recruited into the lidocaine trial conducted at the Foothills Hospital. The sub-protocol published by Rademaker *et al.* (*1986*) for lidocaine infusion and blood sampling was used with slight modifications. Briefly, the patient was given two 100-mg lidocaine·HCL loading doses 30 minutes apart. Infusion (3 mg/min) began immediately after the first loading dose for a period up to 48 hours. Additional blood samples were withdrawn from the patient up to 10 hours post-infusion. Total urine was collected

from the beginning of lidocaine infusion until 72 hour post-infusion. Since most of the plasma samples collected in this trial were analyzed prior to the development of this method, the post-infusion profile of only one patient is reported to demonstrate the applicability of this assay. Urine samples from another patient were also analyzed.

2.2.3 Pig Studies

Following an overnight fast, a Yucatan Miniature pig received a bolus injection of 4-OH-LIDO and 4-OH-MEGX (4 mg/kg) intravenously on separate occasions 10 days apart. The urine samples were collected and recorded for 48 hrs. A 20 ml aliquot of each urine samples were stored at -20°C.

2.2.4 Sample Treatment

2.2.4.1 Plasma

All labware used was made of glass. The caps of tubes were either made of or lined with Teflon. A stock solution containing the hydrochloride salts of lidocaine and its metabolites (except 4-OH-XYL) was prepared by dissolving these compounds in blank human plasma. This stock solution was stable for at least a week. The stock solution of 4-OH-XYL was also prepared in the blank human plasma and had to be prepared daily because it was unstable. Adequate concentrations of lidocaine and its nine metabolites were obtained by spiking appropriate quantities of the above two stock solutions and diluting to the proper volume with blank human plasma. The concentration range of the standards studied was between 0.2

and 5.0 μg/ml for all the components except 4-OH-XYL (highest concentration was 20 μg/ml). To 0.5 ml of plasma standard or patient plasma was added 0.5 ml of 6 N hydrochloric acid. To hydrolyze conjugates, the mixture, in a tightly capped glass tube, was incubated at 100°C for 45 min. After cooling to room temperature, 0.5 ml of 6 N sodium hydroxide, 1 ml of 0.15 M sodium phosphate buffer (pH 6.8) and 0.1 ml of internal standard (20 μg/ml in water) were added. The pH of the resultant mixture was approximately 8.5.

2.2.4.2 Urine

The preparation of urine standards was similar to that of the plasma standards except the concentration range of 4-OH-XYL was extended to 500 μg/ml. To 0.1 ml of urine standards or patient or pig urine was added 0.10 ml of 6 N hydrochloric acid. The rest of the acid hydrolysis procedures were the same as described for the plasma samples except 0.1 ml of 6 N sodium hydroxide was used to neutralize the acid after incubation.

2.2.5 Aqueous Acetylation

The method of Coutts *et al.* (*1987*) was used. Approximately 1.5 g of potassium bicarbonate were added to the acid-hydrolyzed plasma or urine samples. To the mixture were added 300 μl of acetic anhydride and when effervescence stopped, the solution was vortexed (IKA-VIBRAX-VXR, Terrochem, setting at 1000) with 6 ml of ethyl acetate for 10 min. After centrifugation (1000 g) for 10 min, 5 ml of the organic layer were removed and dried under a gentle stream

of nitrogen. The residue was reconstituted with 300 μl of the mobile phase, and 50-200 μl were injected into the HPLC system. Because it lacks free amino or phenolic groups, lidocaine was not acetylated during the derivatization procedure.

2.2.6 Chromatography

The HPLC system (Waters Assoc.) consisted of two M-45 pumps, and M-480 variable-wavelength UV detector (set at 200 nm), an automatic sampler (WISP, model 721) and data processing station (Model 840). Separation of lidocaine and its derivatized metabolites was achieved using a 5μm C_{18} reversed-phase Nova-Pak cartridge (11.5 cm $\times$ 8 mm I.D.). The aqueous mobile phase ($\sim$ pH 3) consisted of 12% (v/v) acetonitrile, 0.1% (v/v) phosphoric acid and 0.15% (v/v) triethylamine, and was pumped at a flow rate of 2 ml/min.

2.3 Results

2.3.1 Human

Figs. 2.1 and 2.2 are the HPLC traces of human plasma and urine, respectively. The blank samples showed the absence of endogenous interference. Lidocaine and the acetylated derivatives of the metabolites were well separated to allow accurate quantification. Calibration curves prepared for the standards were linear (r^2 >0.99) in the concentration ranges studied (0.2 - 500 μg/ml for 4-OH-XYL and 0.2 - 5 μg/ml for the rest). Each calibration curve consisted of at least five points and each point was determined in triplicate. The coefficients

of variation (mean ± %S.D.) were 4.68 ± 3.88% for plasma and 3.10 ± 3.43% for urine. The ranges were 0.2 - 13.5 and 0.13 - 17.4%, respectively.

4-OH-XYL was the major metabolite present in plasma (Fig. 2.1). Its concentration was higher than that of lidocaine. The level of MEGX and GX was approximately an order of magnitude lower than that of 4-OH-XYL. The *m*-hydroxylated metabolites of lidocaine and MEGX and *p*-hydroxylated metabolites of lidocaine, MEGX and GX were absent in plasma. 2,6-XYL, the reported precursor of 4-OH-XYL (*Keenaghan and Boyes 1972*), was not detectable. The post-infusion profiles of lidocaine, MEGX, GX and 4-OH-XYL are shown in Fig. 2.3. The time courses of lidocaine, MEGX and 4-OH-XYL were parallel.

Although the decline of lidocaine concentration was apparently non-linear, it cannot be established at this time whether the elimination of lidocaine after prolonged infusion is non-linear because samples were not collected at frequent enough intervals. More studies are required.

Similar to the plasma profiles, 4-OH-XYL was the most abundant metabolite in the urine (Table 2.1). It accounted for 80% of the administered dose. The rest of the metabolites consisted of approximately 5% of the dose. In this particular patient, both the *m*- and *p*-hydroxylated metabolites of lidocaine and its N-deethylated metabolites were present.

In addition to the *p*-hydroxylated precursors of 4-OH-XYL, the levels of the other metabolites were similar to those reported in the literature (*Keenaghan and Boyes 1972*). It is interesting to note that 2,6-XYL was not detected in plasma and urine.

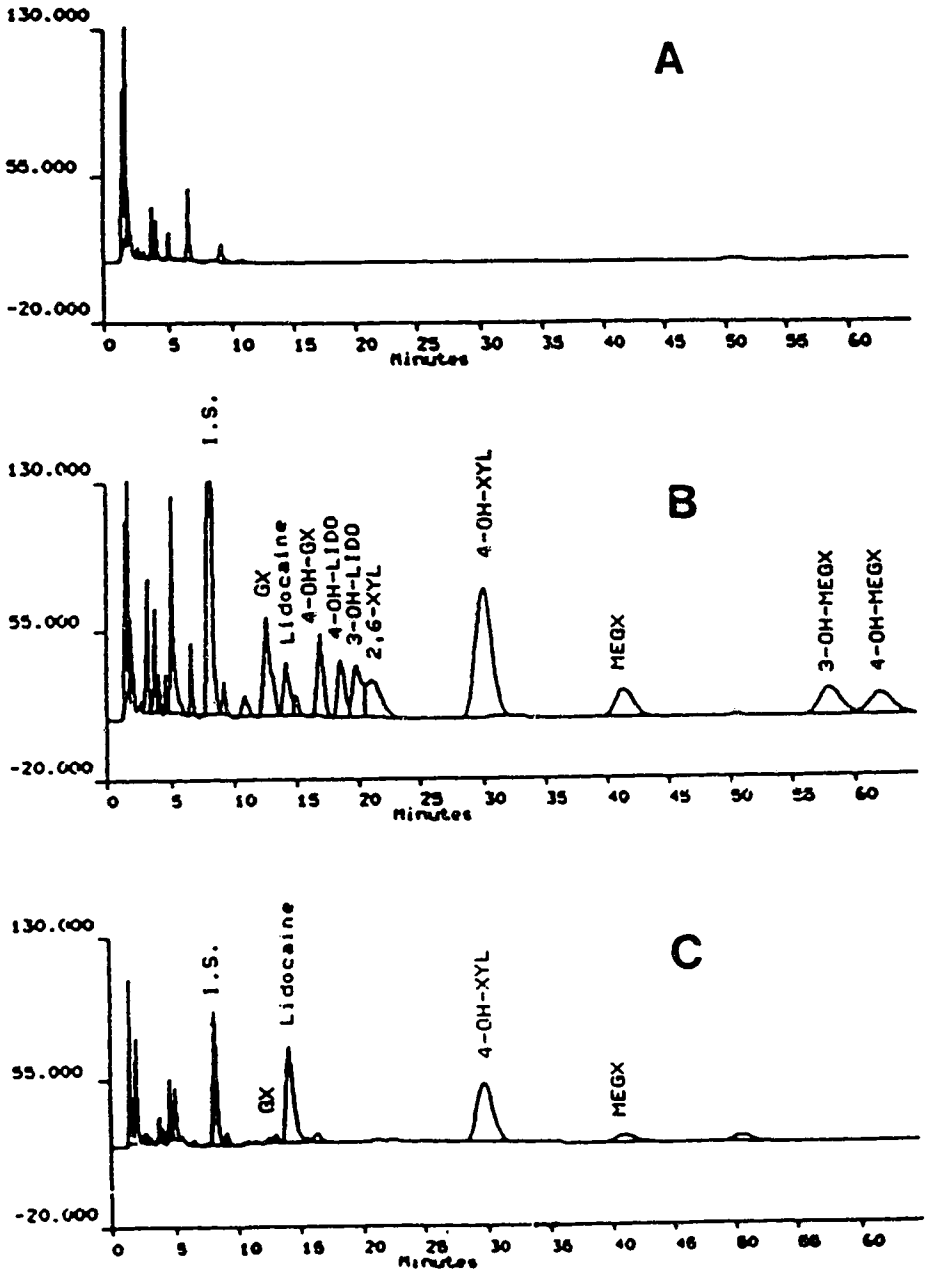


Figure 2.1 Chromatograms obtained from blank (A), spiked (B) and patient plasma (C) samples. The concentration of each spiked component was 5 μg/ml, except 4-OH-XYL (20 μg/ml). The signals were measured in mV.

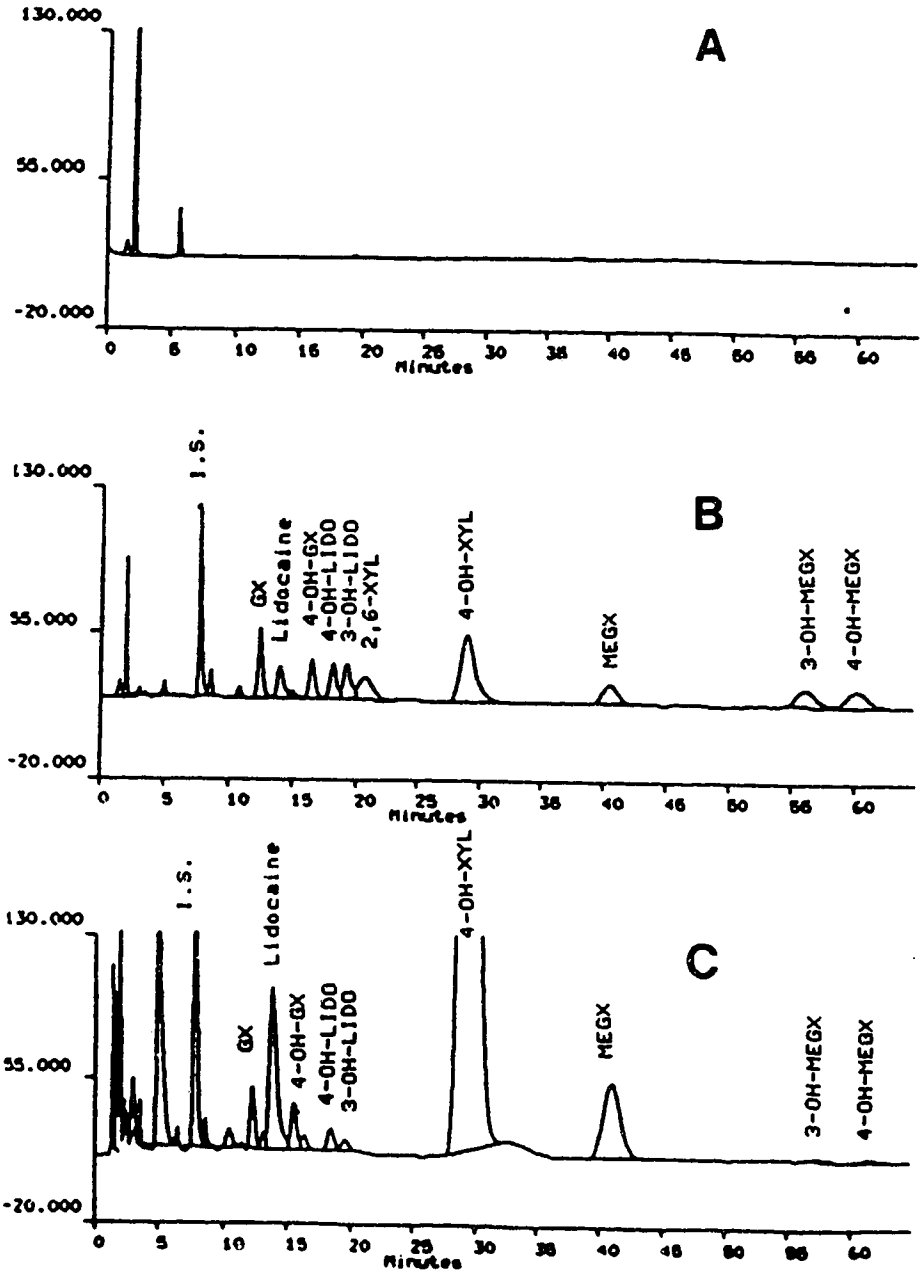


Figure 2.2 Chromatograms obtained from blank (A), spiked (B) and patient urine (C) samples. The concentration of each spiked component was 3 μg/ml, except 4-OH-XYL (12 μg/ml). The signals were measured in mV.

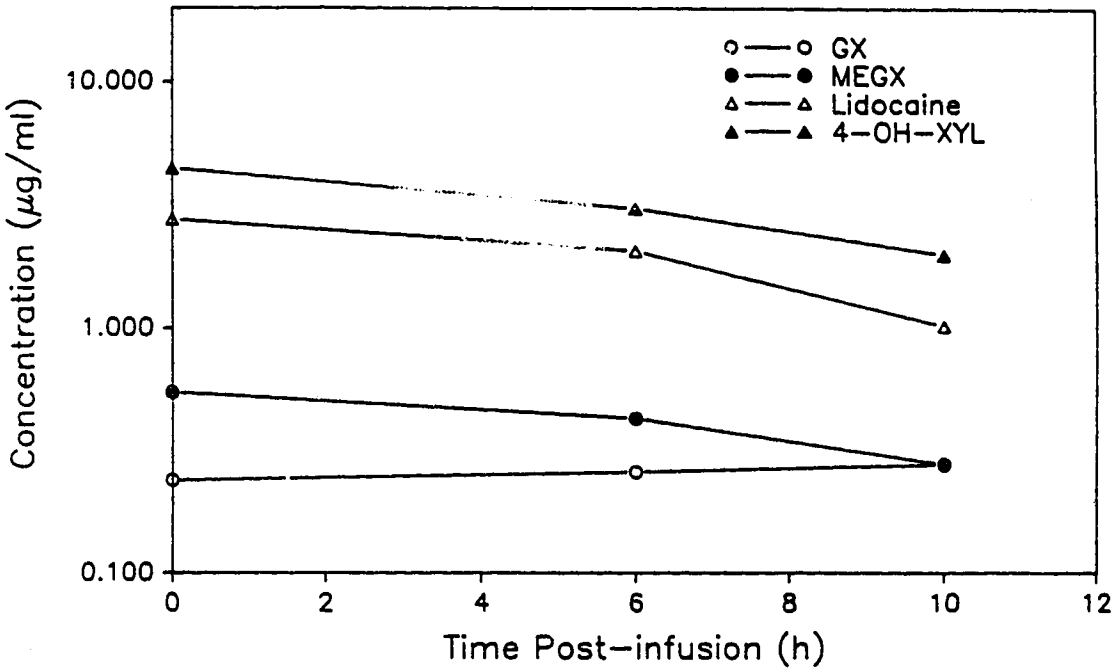


Figure 2.3 A representative patient plasma profiles of lidocaine, MEGX, GX and 4-OH-XYL during the post-infusion phase.

Table 2.1 Urinary recovery of lidocaine and its metabolites after a course of lidocaine infusion in a patient with suspected myocardial infarction.

Compound	Recovery (%)
Lidocaine	2.1
MEGX	1.7
GX	0.55
4-OH-LIDO	0.28
4-OH-MEGX	0.06
4-OH-GX	0.24
4-OH-XYL	80.1
3-OH-LIDO	0.13
3-OH-MEGX	0.04
Total	85.2

2.3.2 Pig

The total recoveries of 4-OH-LIDO and 4-OH-MEGX and their metabolites are quantitated as molar concentration of the free base and shown in Table 2.2. About 17 % and less than 1 % of 4-OH-XYL was formed from a separate intravenous bolus of 4-OH-MEGX and 4-OH-LIDO, respectively.

2.4 Discussion

In order to characterize the metabolism of lidocaine in detail, it became clear in our study that an improved analytical procedure capable of isolating, separating

Table 2.2 Urinary recoveries of 4-OH-LIDO and 4-OH-MEGX and their metabolites after an intravenous dose of 4-OH-LIDO and 4-OH-MEGX (4 mg/kg) on separate occasions in a Yucatan Miniature pig.

Compound	Recovery (% dose)	
	4-OH-LIDO	4-OH-MEGX
4-OH-LIDO	60.80	-.-
4-OH-MEGX	3.95	68.66
4-OH-GX	1.57	0.88
4-OH-XYL	0.72	16.64
Total	67.04	86.18

and quantifying known and possible *in vivo* metabolites of lidocaine was required. In the present study, a new HPLC assay has been developed.

Phenolic metabolites of lidocaine, MEGX and GX are amphoteric compounds and difficult to extract efficiently from aqueous solutions. Using the aqueous acetylation method which was developed by Coutts *et al.* (*1987*), it was found that the efficiency of acetylation and extraction was complete. This conclusion was drawn when the HPLC trace of a second ethyl acetate extract was found to be devoid of any metabolites of lidocaine and derivatives of these metabolites. The derivatives formed were stable and no significant degradation of these derivatives was observed after 10 days of storage at -20°C.

The acetylation procedure also enhanced the separation of lidocaine and

its metabolites. An essential feature of the envisaged method was that it would distinguish structural isomers. For example, underivatized *m*- and *p*-hydroxylated lidocaine and MEGX isomers failed to be separated by us using the same HPLC condition and by Nelson *et al* (*1977*) using the direct insertion probe and chemical-ionization mass spectrometry, whereas separation was complete after acetylation (see Figs. 2.1 and 2.2).

A number of β-blockers, such as propranolol, nadolol, pindolol, acebutolol and metoprolol, and calcium channel blockers, such as verapamil and diltiazem, which might be given to the patients along with lidocaine, have been found not to interfere with the assay.

The separation of 2,6-XYL and 3-OH-LIDO was less than optimal, but the accuracy of measuring these compounds was not adversely affected. It was determined that the level of 2,6-XYL was very low in our patient population and, in many cases, it was undetectable. Furthermore, 3-OH-LIDO was not always detected in our subjects either. In addition the acetate derivatives of 3-OH-MEGX and 4-OH-MEGX had long retention times, which made HPLC separation time-consuming (run time > 60 minutes). The third major disadvantage of this assay was that the acetylation products had low detector sensitivities. The detection limit of this assay is 0.1 μg/ml for lidocaine and its metabolites when 0.5 ml of plasma or 0.1 ml of urine was used.

Lidocaine metabolism has been a major subject of research in the past (*Keenaghan and Boyes 1972, Nelson et al. 1977, Mihaly et al. 1978*). The previous

identified major lidocaine metabolic pathway in human was that first, lidocaine was N-deethylated to form MEGX, which could either further N-deethylated to GX or amide-hydrolized to 2,6-XYL. Then, 2,6-XYL was *p*-hydroxylated to 4-OH-XYL which was a major urinary metabolite and excreted in urine mainly as conjugates. No one has investigated the process by which lidocaine and its two N-deethylated metabolites, MEGX and GX were first converted in man to their 4-hydroxylated counterparts, and one, two or all three of these 4-hydroxylated metabolites were subsequently enzymatically (amidase) hydrolyzed to 4-OH-XYL (Fig. 2.4), even though this possibility was implied by the detection of 4-OH-LIDO in infants (*Mihaly et al. 1978*).

In the present study, using this newly developed HPLC assay all of three *p*-hydroxylated precursors of 4-OH-XYL have been isolated and identified in human (Table 2.1). Traces of 4-OH-LIDO, 4-OH-MEGX and 4-OH-GX (< 0.6 % of an intravenous dose) were recovered in the urine of patients with or without myocardial infarction. This observation suggests that in addition to N-deethylation, *p*-hydroxylation could also be an important primary pathway in human. Therefore, lidocaine metabolism was more complicated than previously reported.

We have further investigated the possibility that in the pig model, 4-OH-XYL was also formed from 4-OH-LIDO and 4-OH-MEGX in addition to 2,6-XYL. Preliminary experimental data in the pig indicated that 4-OH-XYL was formed from both of 4-OH-LIDO and 4-OH-MEGX in the different ratios. Compared with 4-OH-MEGX, even less 4-OH-LIDO (< 1%) was converted to 4-OH-XYL.

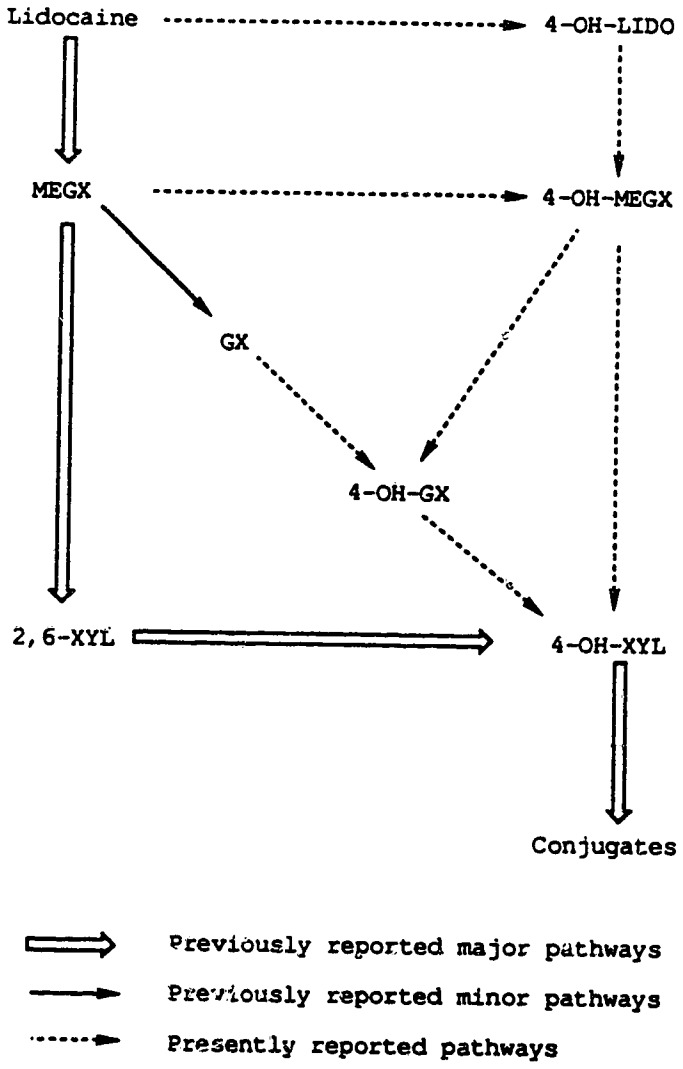


Figure 2.4 The previously reported and presently proposed pathways of lidocaine metabolism in humans.

This observation is consistent with the previous results obtained in the rabbit liver microsomal study in which MEGX was found to be rapidly hydrolyzed to 2,6-XYL, while lidocaine or GX could not be hydrolyzed in this way (*Hollunzer, 1960*). It is possible that both MEGX and its 4-hydroxylated product may be more susceptible to the amidase hydrolysis; whereas lidocaine and 4-OH-LIDO are not. More studies are required to confirm this deduction.

In conclusion, we have demonstrated that this newly developed HPLC assay can be used to study lidocaine pharmacokinetics in humans and animals. Traces of 4-OH-LIDO, 4-OH-MEGX and 4-OH-GX were isolated and quantitated in human urine, confirming that lidocaine, MEGX and GX were converted to their 4-hydroxylated counterparts. Two of these precursors of 4-OH-XYL, 4-OH-LIDO and 4-OH-MEGX, do metabolize to 4-OH-XYL in the pig. These results suggested that lidocaine metabolism in human was more complicated than previously identified and that *p*-hydroxylation could also be an important primary pathway in human.

2.5 References

Ahmad K, Medzihradsky F., "Lidocaine in blood and tissues after single dose and steady state infusion."; *Res Commun Chem Pathol Pharmacol*, 2, pp.813-828 (1971).

Benowitz N, Rowland M., "Determination of lidocaine in blood and tissues.", *Anesthesiology*, 39, pp.639-641 (1973).

Benowitz N, Forsyth RP, Melmon KL, Rowland M., "Lidocaine disposition kinetics in monkey and man I. Prediction by a perfusion model.", *Clin Pharmacol Ther*, 16, pp.87-98 (1974).

Coutts RT, Torok-Both GA, Tam YK, Chu LV, Pasutto FM., "Acetylation and pentafluorobenzoylation of lidocaine metabolites in aqueous solution and identification of derivatives by combined gas chromatography/mass spectrometry", *Biomed Environ Mass Spectrom*, 14, pp.173-182 (1987).

Hignite CE, Tschanz C, Steiner J, Huffman DH, Azarnoff DL., "Quantitation of lidocaine and its deethylated metabolites in plasma and urine by gas chromatography-mass fragmentography.", *J Chromatogr*, 161, pp.243-249 (1978).

Hill J, Roussin A, Lelorier J, Gailie G., "High-pressure liquid chromatographic determination of lidocaine and its active deethylated metabolites.", *J Pharm Sci*, 69, pp.1341-1343 (1980).

Hollunger G., "Solubilisation and purification of an amide-hydrolysing microsomal enzyme.", *Acta Pharmacol Toxicol*, 17, pp.374-383 (1960).

Holt DW, Loizou M, Wyse RKH., "Gas-liquid chromatographic measurement of lignocaine in small samples of canine myocardium after enzymatic digestion.", *J Clin Pathol*, 32, pp.225-228 (1979).

Kawai R, Fujita S, Suzuki T., "Simultaneous quantitation of lidocaine and its four metabolites by high-performance liquid chromatography:application to studies on in vitro and in vivo metabolism of lidocaine in rats.", *J Pharm Sci*, 74, pp.1219-1224 (1985).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man.", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Lazzi FA, Wenger TL, Klinger J, Barchowsky A, Strauss H., "Simultaneuos determination of lidocaine and its metabolites in plasma and myocardium.", *J Chromatog Biomed Appl*, 311, pp.291-299 (1984).

Mihaly GW, Moore RG, Thomas J, Triggs EJ, Thomas D, Shanks CA., "The pharmacokinetics and metabolism of the anilide local anesthetics in neonates.", *Eur J Clin Pharmacol*, 13, pp.143-152 (1978).

Nation RL, Peng GW, Chiou WL., "High-performance liquid chromatographic method for the simultaneous determination of lidocaine and its N-dealkylated metabolites in plasma.", *J Chromatog Biomed Appl*, 162, pp.466-473 (1979).

Nelson SD, Garland WA, Breck GD, Trager WF., "Quantification of lidocaine and several metabolites utilizing chemical-ionization mass spectrometry and stable isotope labelling.", *J Pharm Sci*, 66, pp.1180-1189 (1977).

Rademaker AW, Kellen J, Tam YK, Wyse DG., "Character of adverse effects of prophylactic lidocaine in the coronary care unit.", *Clin Pharmacol Ther*, 40, pp.71-80 (1986).

Strong JM, Parker M, Atkinson AJ., "Identification of glycinexylidide in patients treated with intravenous lidocaine.", *Clin Pharmacol Ther*, 14, pp.67-72 (1973).

Tam YK, Yau ML, Sim HA, Tawfik S., "Simultaneous quantitation of lidocaine and five its metabolites in biological fluids using HPLC. ", *Clin Invest Med*, 7, pp.A7 (1984).

Thomas J. Meffin P., "Aromatic hydroxylation of lidocaine and mepivacaine in rats and humans.", *J Med Chem*, 15, pp.1046-1049 (1972).

Thomson A, Elliott H, Kelman A, Meredith P, Whiting B., "The pharmacokinetics and pharmacodynamics of lignocaine and MEGX in healthy subjects", *J Pharmacokinet Biopharm*, 15, pp.101-115 (1987).

CHAPTER 3

QUANTIFICATION OF THREE MAJOR LIDOCAINE METABOLITES AND THEIR CONJUGATES IN HUMAN URINE[2]

3.1 Introduction

Lidocaine metabolism has been a subject of interest for a number of years (*Keenaghan and Boyes 1972, Nelson et al. 1977, Pang et al. 1986, Coutts et al. 1987*). The major primary pathway of lidocaine biotransformation in human is apparently N-deethylation followed by secondary oxidation, hydrolysis and conjugations (*Keenaghan and Boyes 1972*). Recently, we found that in addition to N-deethylation, *p*-hydroxylation could also be an important primary pathway in human (*Tam et al. 1987*). Consistent with the literature results (*Keenaghan and Boyes 1972, Boyes and Keenaghan 1971*), we have also found that N-(N-ethylglycyl)-2,6-xylidine (MEGX), N-glycyl-2,6-xylidine (GX) and 4-hydroxy-2,6-xylidine (4-OH-XYL) are the major metabolites in patients with suspected myocardial infarction (*Tam et al. 1987*).

The Phase II metabolism of lidocaine is not well characterized because most studies used acid to hydrolyse the conjugates (*Boyes and Keenaghan 1971, Mihaly et al. 1978*). It is well known that this method is not discriminatory. Enzyme hydrolysis was used by Nelson *et al.* (*1977*) to study the conjugates of lidocaine metabolites. These investigators reported that in healthy human volunteers

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MEGX was not conjugated, 60 to 68% of an oral dose of lidocaine was excreted in the form of glucuronide and/or sulfate conjugates of 4-OH-XYL, and the enzymes used to hydrolyze conjugates somehow interfered with the extraction of GX.

We have also found that 4-OH-XYL, in its free form, is highly unstable in neutral and alkaline solution. More than 50% decomposition occurs within 24 hours at room temperature. It is questionable, therefore, whether the recovery of 4-OH-XYL after the traditional 24 hour incubation with enzymes is quantitative.

Based on the available information, it is apparent that the traditional enzyme hydrolysis method for quantifying conjugates of lidocaine metabolites is not of an adequate accuracy. The method reported by Eichelbaum *et al.* (*1981*) was found by us to be unsuitable for improving 4-OH-XYL stability. Therefore we decided to develop an analytical procedure which would permit quantitative measurements of these lidocaine metabolites without significant decomposition and allow study of Phase II metabolism of lidocaine.

3.2 Materials and Methods

3.2.1 Chemicals and Enzyme Solution

The HCl salts of MEGX, GX and 4-OH-XYL were kindly supplied by Astra Pharmaceuticals (Missasauga, Ontario, Canada). β-Glucuronidases (E. Coli type VII, lyophilized; and H. pomatia type H-2) and sulfatase (limpets type V, lyophilized) were purchased from Sigma (St. Louis, U.S.A.). All other chemicals were reagent grades.

Three kinds of enzyme solutions were prepared by dissolving appropriate amounts of each of the three enzymes in appropriate solutions. β-Glucuronidases type VII solution (400 Sigma Unit, U/ml) was prepared in 0.075 M pH 6.8 potassium phosphate buffer solution; β-Glucuronidase type H-1 solution (500 U/ml) and sulfatase type V solution (9 U/ml) were prepared in 0.2% sodium chloride solution.

3.2.2 Urine Samples

Aliquots of urine samples from seven patients with suspected myocardial infarction who were admitted to the lidocaine trail (*Rademaker et al. 1986*) were pooled. These patients were given two 100 mg intravenous bolus injections of lidocaine HCl 30 minute apart., followed by infusion (3 mg/min) immediately after the first bolus dose. The duration of infusion ranged from 16 to 48 hour. Individual urine samples, including a pre-dose specimen, were collected until 72 hour post-infusion. Unless otherwise specified, all of the following studies relating to hydrolysis are performed in tightly capped (teflon lined) glass tubes.

3.2.3 Enzyme and Acid Hydrolysis

3.2.3.1 Enzyme hydrolysis

To an aliquot of 0.1 ml patient or spiked urine sample containing a mixture of MEGX, GX and 4-OH-XYL (concentration range from 4 - 600 μ g/ml) was added one of the three enzyme solutions (0.1 ml) and 0.5 ml buffer solution (0.075 M pH 6.8 potassium phosphate and 0.1 M pH 5 sodium acetate for type VII and type

H-1 of β-Glucuronidase respectively, and 0.2 M pH 5 sodium acetate for sulfatase type V). Then each sample was diluted to 1.5 ml with deionized water. For each of the control samples, 0.1 ml of the buffer solution was added in place of the enzyme solution to maintain the sample volume. All samples were incubated at 37°C for a maximum of 36 hours. For quantitative measurements, these samples were purged with a gentle nitrogen stream for 1 minute prior to incubation.

3.2.3.2 Acid hydrolysis

To 0.1 ml of patient urine or spiked urine sample containing a mixture of MEGX, GX and 4-OH-XYL (concentration range from 4 - 1,000 μ g/ml) was added an equal volume of 6 N HCl. All samples were incubated at 100°C for a maximum of 2 hours. After cooling to room temperature, 0.1 ml of 6 N sodium hydroxide and 1 ml of 0.15 M sodium phosphate buffer (pH 6.8) were added.

3.2.4 Analytical Methods

The treated samples were quantified using the method of Tam *et al.* (*1987*). Briefly, to the treated samples about 1.5 g of potassium bicarbonate and 0.1 ml of internal standard solution (Procaine, 20 μg/ml) were added. The samples were aqueous acetylated under mild basic condition (pH $\sim$ 8.5) using acetic anhydride. The derivatives were extracted with ethyl acetate. Organic layer was removed and dried under a gentle stream of nitrogen. The residue was reconstituted with mobile phase for HPLC analysis. Chromatographic separation was achieved using a 5 μm C_{18} Nova-Pak cartridge (11.5 cm $\times$ 8 mm I.D.). Mobile phase, consisted

of acetonitrile 12 % (v/v), phosphoric acid 0.1 % (v/v) and triethylamine 0.15 % (v/v) in water, was pumped at 2 ml/min. The UV response of each derivative was measured at 214 nm. The concentration ranges of standard solutions prepared for the three metabolites in this study were from 10 to 1,200 μg/ml for 4-OH-XYL, 10 to 25 μg/ml for MEGX and 4 to 8 μg/ml for GX.

3.2.5 Data Analysis

All samples were prepared in triplicate and the results were recorded as mean ± SD. It was assumed that acid hydrolysis released free MEGX, GX, and 4-OH-XYL from all forms of conjugates of these three lidocaine metabolites. A two-tailed t test ($p = 0.05$) was used to evaluate differences between groups.

3.3 Results

3.3.1 4-OH-XYL

Type VII β-Glucuronidase, which contained no sulfatase, is the most efficient enzyme in hydrolyzing 4-OH-XYL glucuronide (Fig. 3.1a). The maximum yield of aglycone was achieved within 30 minutes, whereas it required 24 hours for the type H-1 enzyme, which is a mixture of β-Glucuronidase and sulfatase, to hydrolyze the conjugate. The yield was significantly lower with type H-1 than with type VII (346 ± 20 vs. 484 of ± 23 μg/ml, $p<0.05$). Sulfate-conjugate and free (unconjugated)

4-OH-XYL were not detected in the patient urine when the samples were treated with type V Sulfatase or buffer containing no enzyme (Fig. 3.1a).

The decrease of free 4-OH-XYL levels in the enzyme hydrolysis-time curve (Fig. 3.1a.) is most likely due to the instability of 4-OH-XYL. This hypothesis is supported by the continuous decomposition of authentic 4-OH-XYL when it was incubated with enzymes (Fig. 3.1b). However, the enzyme preparations apparently make little contribution to the rate of 4-OH-XYL decomposition since the concentration profiles derived from the use of enzyme preparations and buffer controls are superimposable. It is shown in Fig. 3.1b that the rate of 4-OH-XYL decomposition is pH dependent, higher at pH 6.8 than at pH 5.0.

nade to stabilize 4-OH-XYL using the method of Eichelbaum

bic acid and sodium bisulfite were added to the incubation

ounts up to 0.43 and 32 mg/ml of each agent, respectively.

to prevent decomposition. However, 4-OH-XYL was sta-

n the samples containing its conjugates were purged with nitrogen for 1 minute prior to incubation with enzymes (Fig. 3.2a & 3.2b).

Maximal hydrolysis of the conjugates was achieved within 45 minutes with the type VII enzyme plus nitrogen pretreatment. This result is not significantly different from that obtained after acid hydrolysis (1164 $\pm$ 27 vs. 1204 $\pm$ 40 μg/ml), suggesting that 4-OH-XYL is present in the patient urine only in the form of glucuronide. This observation is further substantiated by the absence of sulfate conjugate (Fig. 3.2a). It is interesting to note that the yield of 4-OH-XYL after

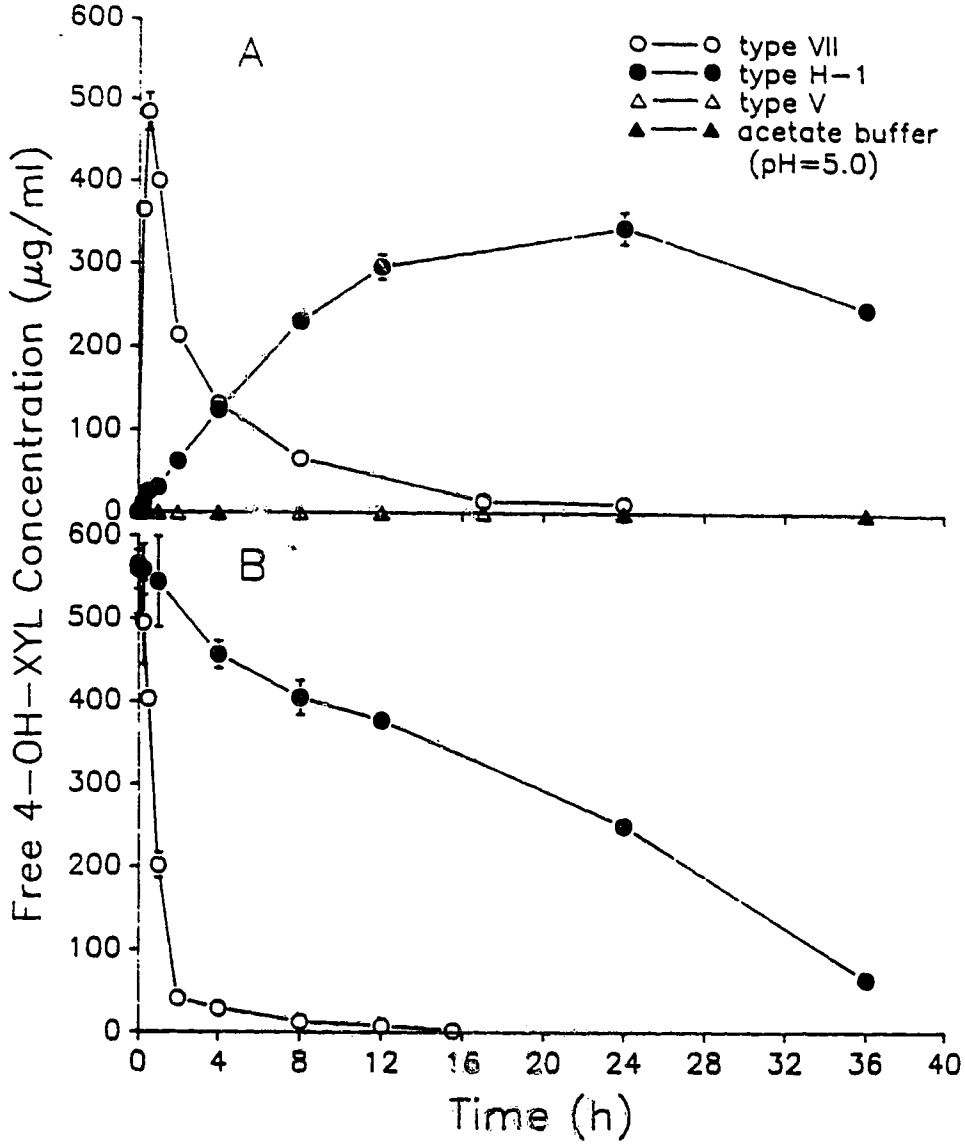

Figure 3.1 A. Profiles of 4-OH-XYL release from its conjugates in type VII (pH = 6.8) and H-1 (pH = 5.0) β-glucuronidase, type V sulfatase (pH = 5.0) and acetate buffer (pH = 5.0) preparations. B. Time courses of spiked 4-OH-XYL in the two buffered β-glucuronidase solutions studied. No samples were purged with nitrogen before incubation.

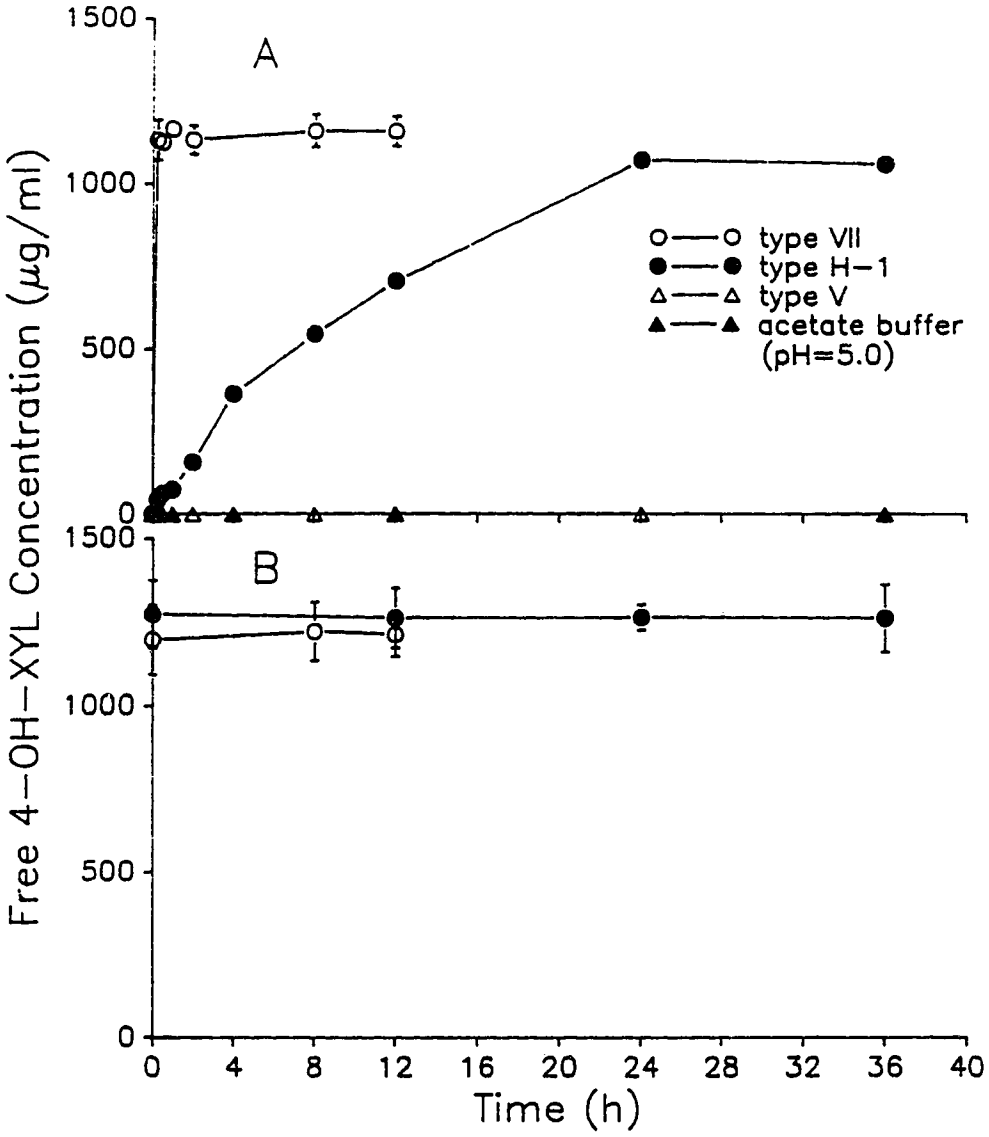

Figure 3.2 A. Profiles of 4-OH-XYL release from its conjugates in type VII (pH = 6.8) and H-1 (pH = 5.0) β-glucuronidase, type V sulfatase (pH = 5.0) and acetate buffer (pH = 5.0) preparations. B. Time courses of spiked 4-OH-XYL in the two buffered β-glucuronidase solutions studied. All of the samples were purged with nitrogen before incubation.

type H-1 treatment is slightly lower ($\sim$ 8%) than that obtained using type VII enzyme ($p < 0.05$). This could reflect some decomposition of 4-OH-XYL after prolonged reaction (36 hours or more).

3.3.2 MEGX

MEGX is present in the patient urine in its free form, and as sulfate and glucuronide conjugates (Table 3.1). The hydrolysis of the glucuronide and sulfate conjugates was relatively rapid. A maximum yield of MEGX was obtained within an hour with type VII β-Glucuronidase and within 2 hours with type V sulfatase treatment. However, it took 12 hours for the glucuronide and sulfate conjugates to hydrolyze when type H-1 enzyme was used. The recovery of free MEGX from type H-1 enzyme hydrolysis was equal to the sum of the free MEGX released from the type VII β-glucuronidase and type V sulfatase, suggesting that these enzymes could be used to quantitatively measure the conjugates of MEGX. Hydrolysis of the patient urine samples with acid provided a higher MEGX yield than that obtained by hydrolysis with type H-1 enzyme (31.3 ± 3.4 vs 21.5 ± 1.1 μg/ml, $p < 0.05$), indicating the presence of other conjugates of MEGX in the urine. MEGX is stable during acid or enzyme treatments. Purging the sample with nitrogen did not change the yield of MEGX.

3.3.3 GX

The treatment of patient urine with type H-1 and type VII β-glucuronidase showed that no significant amounts of glucuronide and sulfate conjugates of GX

Table 3.1 Mean ± SD recovery of various forms of 4-OH-XYL, MEGX and GX in pooled patient urine.

Form of Excretion	Recovery (%)		
	4-OH-XYL	MEGX	GX
Glucuronide	96.7±7.01	16.6±4.5	--
Sulfate	--	6.6 ±1.8	--
Other conjugates	--	31.9±4.4	9.4±10.0
Free	--	44.9±6.8	90.6±10.5

were excreted (Table 3.1). Although acid hydrolysis gave a slight increase in the GX response (6.04 ± 0.37 vs. 5.09 ± 0.33 μg/ml $p < 0.05$), this result suggests GX is present mostly in the free form. GX was stable to both enzyme and acid treatments, and nitrogen treatment did not change the yield.

3.4 Discussion

3.4.1 Methods for Stabilizing 4-OH-XYL

The mechanism of 4-OH-XYL decomposition has been studied (*Torok-Both 1987*). It was found that this metabolite is primarily oxidized to the corresponding *p*-quinone *via* the intermediate imine. This reaction requires oxygen and is pH dependent. The metabolite is most stable under highly acidic condition and the stability of this compound decreases rapidly as pH increases. Since the enzymes function optimally at pH 5 and 6.8, it is at these pH values that the rate of 4-OH-XYL decomposition are high (Fig. 3.1b). There are two approaches to stabilize

this metabolite. One is to use antioxidants. This method was used successfully to stabilize norantipyrine (*Eichelbaum et al. 1981*), but 4-OH-XYL could not be stabilized using this method. The reason could be due to the higher affinity of 4-OH-XYL towards the primary reactant, oxygen. The second approach is to remove oxygen entirely from the reaction mixture. Purging the aqueous sample with nitrogen seemed to provide the answer to this stability problem. Whether this simple method is applicable to other unstable metabolites remains to be tested. We have used this method to study the conjugation of the three lidocaine metabolites and the results are listed in Table 3.1.

There was no free 4-OH-XYL detected in the pooled urine samples. Extensive conjugation and/or instability of unconjugated 4-OH-XYL could contribute to this observation. It would be extremely difficult to accurately measure free 4-OH-XYL in urine, if it were present, because the decomposition of this metabolite is extremely rapid (Fig. 3.1b). This suggests that significant decomposition can occur in the bladder and/or upon storage.

It is interesting to note that previous investigators (*Boyes and Keenaghan, 1971, Keenaghan and Boyes 1972, Mihaly et al 1978*) have not identified any problems with the stability of 4-OH-XYL. This could be due to the fact that acid hydrolysis was used most of the time to release 4-OH-XYL from the conjugate, and 4-OH-XYL is more stable under acidic than basic and/or neutral conditions. We have noticed that, under acidic conditions and at concentrations higher than 20 μg/ml, the decomposition kinetics of 4-OH-XYL is apparently zero order ($\sim$

0.14 μg/min). Since the rate of reaction is slow, the impact on the recovery of 4-OH-XYL at high concentrations (> 1,000 μg/ml) is minimal. Again the nitrogen pretreatment to remove dissolved oxygen corrects the problem. Therefore, it is advisable to use nitrogen pretreatment for all samples prior to acid hydrolysis.

Based on the results of this study, the quantification method reported by Nelson *et al.* (*1977*) may not be appropriate. These investigators added deuterated 4-OH-XYL to fresh urine samples and assumed that the deuterated standard had the same stability as the conjugated metabolite. This assumption is incorrect because the decomposition profile of 4-OH-XYL is vastly different from that of the conjugate, which accounts for most if not all of the 4-OH-XYL in urine. The conjugate, according to our experience, is stable for at least six months upon storage (-20°C), whereas, the half-life of 4-OH-XYL is only about 10 days under identical conditions.

3.4.2 MEGX and GX

Although a literature report indicates that MEGX is not excreted in conjugated form (*Nelson et al. 1977*), at least three conjugates of MEGX are found in the patient urine (Table 3.1). This discrepancy could be related to a number of factors, which include difference in analytical methods, in subject populations and in the dosages used. The discrepancy cannot be attributed to the different enzymes used to hydrolyze the urine samples. The type H-2 enzyme used by Nelson *et al.* (*1977*) was similar to the type H-1 enzyme used in this study. In fact, we have confirmed that type H-2 β-glucuronidase (500 U/ml) does hydrolyze

our urine samples. The amount of free MEGX released by type H-2 enzyme was virtual identical to that produced by type H-1 enzyme. There is not enough information to evaluate potential differences in the subject populations, therefore, no conclusion can be made. The dosage of lidocaine used in this study is higher than that reported by Nelson *et al.* (*1977*), therefore, it is likely that Phase II metabolism of MEGX could be concentration related. In a lidocaine study, where healthy subjects received single intravenous doses of lidocaine (1 mg/kg), which was considerably less than the 250 mg oral dose used in the study of Nelson *et al.* (*1977*), we found MEGX glucuronide in the urine of these human volunteers. Although other MEGX conjugates could not be found, the reasons for the discrepancy between the two studies remain unclear.

The analytical method used by Nelson *et al.* (*1977*) did not permit the investigators to study GX conjugation because the type H-2 enzyme used to hydrolyze GX conjugates interfered with the extraction of the metabolite. We have circumvented the problem by acetylating free GX in aqueous solution prior to its extraction (*Tam et al. 1987*).

3.4.3 Effects of Enzyme Concentration

The concentrations of enzyme preparations used in the present study were confirmed to be adequate. When the concentration of type VII and type H-1 enzymes were increased threefold (from 400 to 1200 and 500 to 1500 U/ml, respectively), the difference in yields of 4-OH-XYL and MEGX was found to be less than 2%.

In conclusion, we have shown that the traditional methods of hydrolyzing conjugates with acid and enzyme preparations have to be used with caution when dealing with an unstable metabolite. A case in point is the quantification of 4-OH-XYL conjugates. Additional procedures such as pretreatment of aqueous solutions of conjugates with nitrogen gas may have to be incorporated in order to ensure proper quantification of unstable metabolites. Furthermore, we have revealed that MEGX forms at least three conjugates and GX exists mainly in its free form in the urine of patients receiving lidocaine.

3.5 References

Boyes RN, Keenaghan JB., "Some aspects of the metabolism and distribution of lidocaine in rats, dogs and man.", In: Scott DB, Julian DG., ed. **Lidocaine in the treatment of ventricular arrhythmias**, (Edinburgh: Livingston) pp.140-151 (1971).

Coutts RT, Torok-Both GA, Tam YK, Chu LV, Pasutto FM., "Acetylation and pentafluorobenzoylation of lidocaine metabolites in aqueous solution and identification of derivatives by combined gas chromatography/mass spectrometry", *Biomed Environ Mass Spectrom*, 14, pp.173-182 (1987).

Eichelbaum M, Sonntag B, Dengler HJ., "HPLC determination of antipyrine metabolites.", *Pharmacology*, 23, pp.192-202 (1981).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man.", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Mihaly GW, Moore RG, Thomas J, Triggs EJ, Thomas D, Shanks CA., "The pharmacokinetics and metabolism of the anilide local anaesthetist in neonates.", *Eur J Clin Pharmacol*, 13, pp.143-152 (1978).

Nelson SD, Garland WA, Breck GD, Trager WF., "Quantification of lidocaine and several metabolites utilizing chemical-ionization mass spectrometry and stable isotope labelling.", *J Pharm Sci*, 66, pp.1180-1189 (1977).

Pang KS, Terrell JA, Nelson SD, Feuer KF, Clements MJ, Endrenyl L., "An enzyme-distributed system for lidocaine metabolism in the perfused rat liver preparation.", *J Pharmacokinet Biopharm*, 14, pp.107-130 (1986).

Rademaker AW, Kellen J, Tam YK, Wyse DG., "Character of adverse effects of prophylactic lidocaine in the coronary care unit.", *Clin Pharmacol Ther*, 40, pp.71-80 (1986).

Tam YK, Tawfik SR, Ke J, Coutts RT, Gray MR, Wyse DG., "HPLC assay of lidocaine and its nine metabolites in human plasma and urine.", *J Chromatogr Biomed Appl*, 423, pp.199-206 (1987).

Torok-Both G., "Analysis of phenylzine, lidocaine and doxapram: Application to the study of their metabolism and pharmacokinetics.", **Ph.D. Thesis, University of Alberta**, pp.172-177 (1987).

CHAPTER 4

THE ACUTE EFFECTS OF PN ON RENAL AMPICILLIN CLEARANCE IN HEALTHY HUMAN VOLUNTEERS[3]

4.1 Introduction

Parenteral nutrition (PN) is a widely used medical technique whereby hypertonic solutions of glucose and protein, with or without a lipid component, are delivered intravenously to maintain normal nutrition in subjects unable to ingest adequate oral nutrients. At the same time, it has been recognized that renal physiologic function, e.g. glomerular filtration rate (GFR), significantly altered with the use of PN (*Bengoa et al. 1983, Graf et al. 1983*). However, the influence of administration of PN solution on renal drug clearance has received little attention. For example, intravenous infusion of an admixture of ampicillin and PN solution may result in > 10 fold difference in plasma ampicillin concentrations from similar doses of ampicillin (*Colding et al. 1982*). It is not clear whether the altered pharmacokinetic profile of ampicillin in patients receiving PN is the result of their physiochemical interaction in the delivery system (*Feigin et al. 1973, Colding and Andersen 1978*) or the result of altered physiologic function due to PN, for example, altered GFR (*Bengoa et al. 1983, Graf et al. 1983*), which could in turn affect ampicillin pharmacokinetics. Many antibiotics including ampicillin, are thought to be reasonably compatible with PN solutions when administered as

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a co-infusion rather than as an admixture with PN solution in the bag or bottle (*Trissel 1988*). However, there are limited data on the pharmacokinetics of ampicillin when co-infused with PN.

This study aims to determine the effects of PN solution, in particular, amino acid infusion on the pharmacokinetics of ampicillin given as a co-infusion (via a separate vein) during PN. The hypothesis to be tested is that ampicillin clearance increases during PN secondary to the amino acid induced elevation in GFR.

4.2 Methods

4.2.1 Subjects

Eight healthy, young adult non-smokers (4 males and 4 females, 20-28 years of age) were recruited for the study. The demographic data for these subjects are listed on Table 4.1. Their height and weight ranges were 155 to 179.5 cm and 46 to 82 kg respectively. Body mass index (weight (kg) / height $(m)^2$) (*Garrow and Webster 1985*) was in the generally acceptable range of 19 to 26 for all subjects.

Written informed consent was obtained from each subject. This research protocol was approved by the Ethics Review Committee for Human Experimentation of the University of Alberta. The study was performed at the Clinical Investigations Unit at the University of Alberta Hospital.

Table 4.1 Demographic data for eight healthy human volunteers.

Subject	Sex	Age(yrs)	Height(cm)	Weight(kg)	Smoking
MF	F	26	155.0	46.0	No
CE	F	20	177.5	80.4	No
GW	F	21	170.5	63.5	No
LD*	F	25	167.0	71.2	No
GT	M	25	169.2	55.4	No
ED	M	28	172.2	65.0	No
TR	M	24	177.0	68.0	No
GB	M	25	179.5	82.0	No
Mean		24	171.0	66.4	
±SD		2.6	7.8	12.0	

* Voluntarily withdrew after 1 study period.

4.2.2 Study Design and Sample Collection

Following an overnight fast, each subject received a single intravenous dose (250 mg) of ampicillin sodium (Penbrient, Ayerst Lab, Montreal, PQ.) in association with each of three nutrient regimens.

Two intravenous regimens and one enteral regimen were used. The first intravenous regimen consisted of a standard solution for peripheral PN in use at the University of Alberta Hospital; each liter of PN solution contains 100 g dextrose, 37.5 g amino acids (Travasol, Baxter-Travenol Lab., Malton, Canada), 50 mmol sodium, 50 mmol potassium, 3 mmol magnesium, 3 mmol calcium, 11

mmol phosphate, 1 ml multiple trace elements (MTE-4, NovaPharm/LyphoMed, Pharmaceutical Co., Markham, Canada) and 10 ml multiple vitamins (MV1-1000, SABEX international Ltd., Montreal, Canada). The second intravenous regimen consisted of 10% dextrose water (D10W) with all additives as the PN solution except for amino acids, calcium and phosphate. All intravenous solutions were prepared by the hospital pharmacy under aseptic conditions. No intravenous lipid emulsion was used in this study. The third nutrient regimen consisted of a meal containing similar fluid volume (1 L), caloric (490 kcal / 2058 kJ), protein (37.5 g) and sodium (66 - 77 mmol/L) content as the PN solution and prepared in the diet kitchen.

Two peripheral intravenous catheters were inserted in each subject, one in each arm prior to the experiment. One catheter was used for nutrient infusion and the other was for ampicillin infusion and blood sampling. One liter of each intravenous solution was infused over a 12 hour period. The intravenous solutions were infused in a random order within a 24 hour period. Four patients received PN as the initial infusion. The parenteral and enteral studies were performed at least 28 days apart. Each subject was allowed water as the only enteral intake and remained on bed rest during each study.

Each (250 mg) dose of ampicillin was reconstituted with sterile water immediately prior to intravenous injection over 5 minutes into a vein in an extremity not receiving the intravenous nutrient. Ampicillin was administered 2 hours after commencement of each intravenous nutrient regimen. For the measurement

of ampicillin concentration, blood samples (5 ml) were taken at 0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes, and thereafter at 1 to 2 hourly intervals up to a total of 10 hours from the time of ampicillin administration. The blood samples were collected into polypropylene tubes and stored at 4°C for one hour prior to centrifugation. After separation, serum was stored at −20°C until analysis. Each subject was asked to void prior to Ampicillin injection, and the volume of each subsequent void, including the last sample at 10 hours after injection, were recorded. A 20 ml aliquot of each urine sample was frozen immediately at −20°C, and all urine and serum samples were analyzed for ampicillin within 12 hours after completion of each study. In addition, serum and urine creatinine concentrations were measured. A sample of each void was also tested for glucosuria (Chemstrip, Boehringer Mannheim Canada, Dorval, PQ). With the enteral nutrient regimen, ampicillin was given 4 hours after the ingestion of a standardized meal. Blood and urine samples were collected over a 10 hour period and measured for ampicillin and creatinine and tested for glucosuria as described above.

4.2.3 Sample Analysis

4.2.3.1 Measurement of ampicillin

Ampicillin was measured by a modified high performance liquid chromatography (HPLC) technique as described by Vree *et al.* (*1978*). Briefly, each 100 μl aliquot of serum or urine sample, diluted 1 : 10 with water, was mixed with 0.1 ml of 10 μg/ml solution of N-ethyl-N-methyl-glycine-2,6-xylidide as internal standard (I.S.) and 0.4 ml of Perchloric acid (0.33 N). After centrifugation (13,500 g for

3 minutes), 100 μl of the clear supernatant was injected onto the HPLC system (Waters Associates, Mississauga, Ont. Canada) which consisted of a M-45 pump, a Model 441 UV detector (set at 214 nm), an automatic sampler (WISP, Model 721) and a data processing station (Model 840).

Separation was achieved using a 5 μm C_{18} reversed-phase Nova-Pak cartridge (11.5 cm $\times$ 8 mm I.D.). No interfering peaks were observed in blank serum and urine (Fig 4.1 and 4.2). The aqueous mobile phase: for serum samples consisted of acetonitrile (12% v/v), phosphoric acid (0.09% v/v) and triethylamine (0.07% v/v); for urine samples consisted of acetonitrile (11% v/v), phosphoric acid (0.06% v/v) and triethylamine (0.05% v/v), and was pumped at a flow rate of 2 ml/min. Before each series of determination two standard curves were constructed for serum and urine, respectively. The concentration ranges for the standards used in this study were 0.3 - 5 μg/ml for serum and 0.3 - 50 μg/ml for urine. The limit of detection for ampicillin was 0.5 μg/ml for both serum and urine samples. The coefficient of variation over the examined concentration ranges for serum and urine was $< 5\%$.

4.2.3.2 Measurement of creatinine

Creatinine was measured by Jaffe reaction after removal of protein and non-creatinine chromogens following adsorption of the creatinine onto a cation exchange (Dowex) resin. Creatinine was eluted from the resin with alkaline picrate prior to its determination (*Stoten 1968*). Coefficient of variation for the assay was $< 6\%$. The reference range of serum creatinine concentrations in healthy adults is 50 to 110 μmol/L.

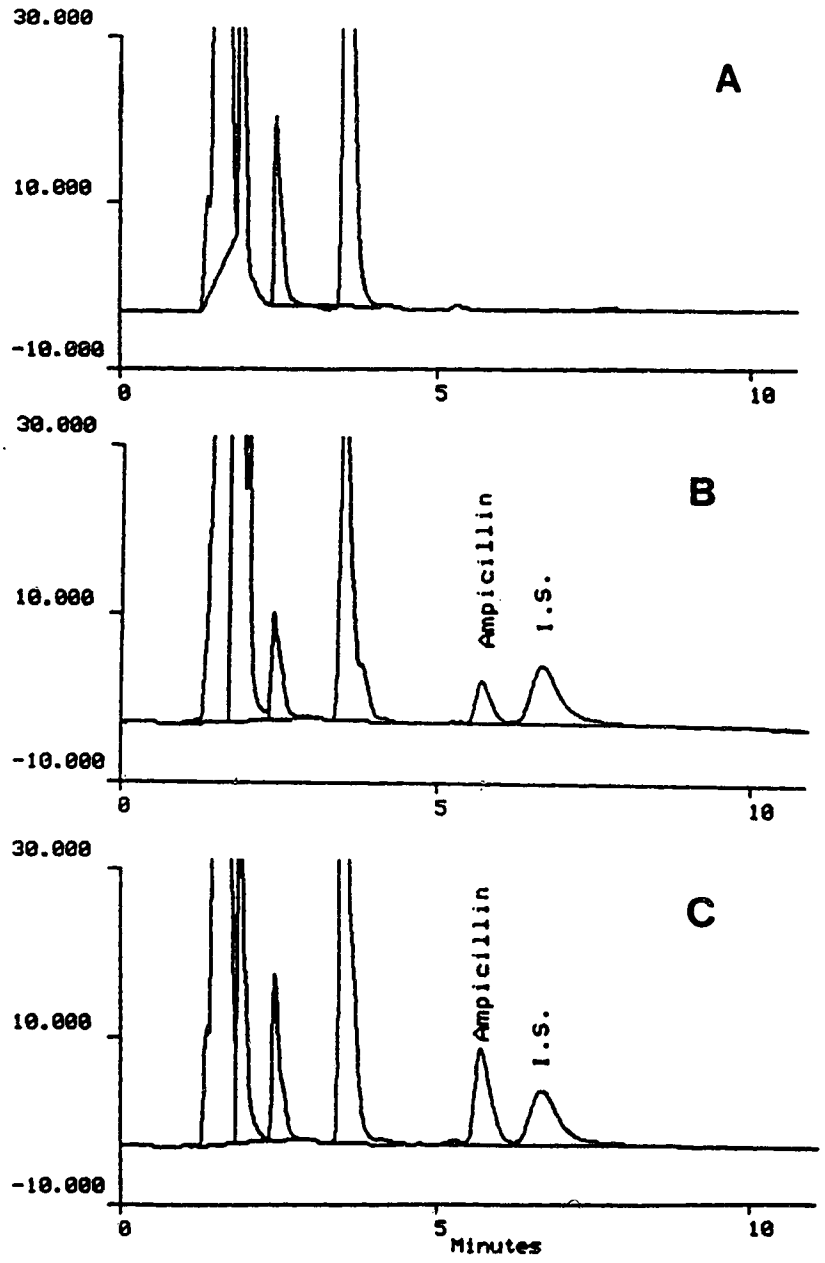

Figure 4.1 Chromatograms obtained from blank (A), spiked (B) and patient serum (C) samples. The concentration of the spiked ampicillin was 5 μg/ml and the signals were measured in mV.

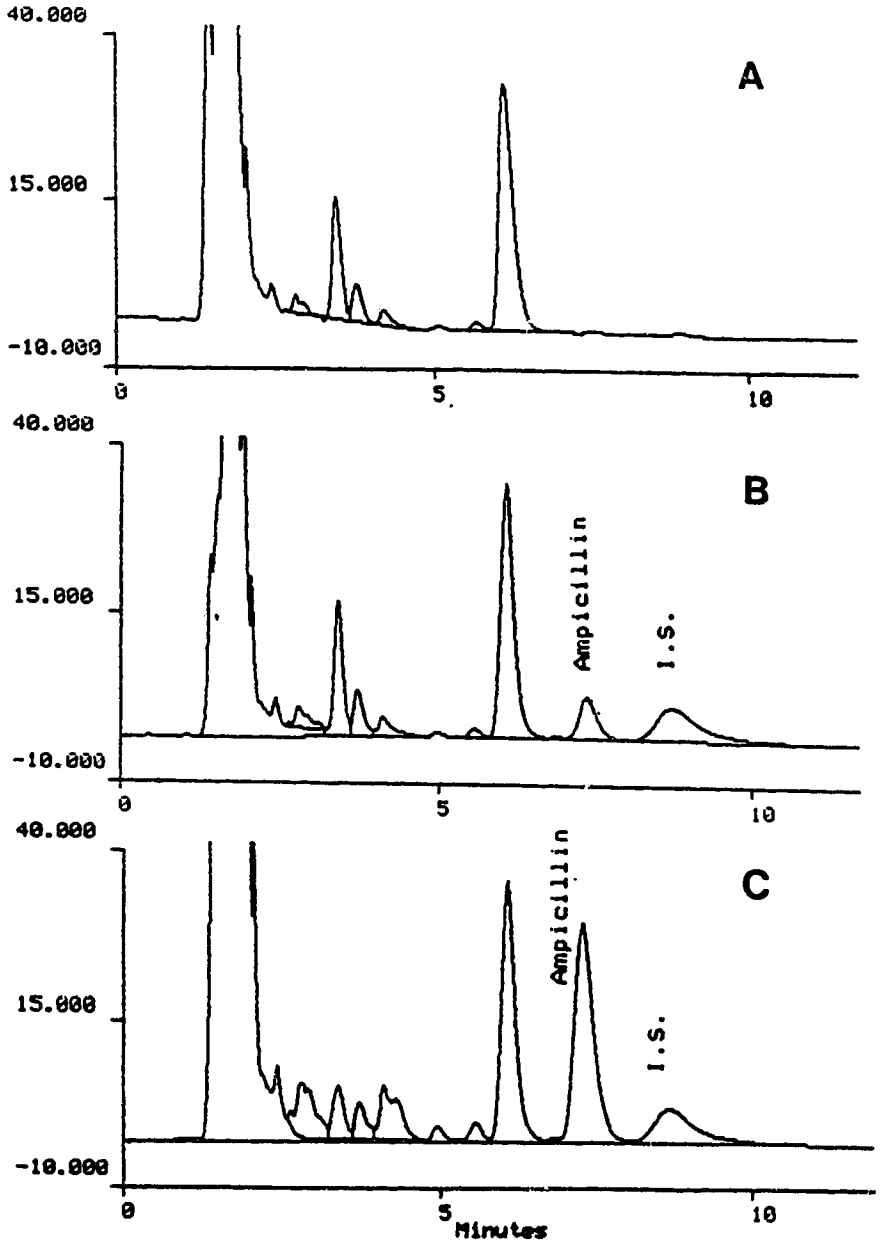

Figure 4.2 Chromatograms obtained from blank (A), spiked (B) and patient urine (C) samples. The concentration of the spiked ampicillin was 10 μg/ml and the signals were measured in mV.

4.2.4 Treatment of Data

4.2.4.1 Pharmacokinetic analyses

Serum drug concentration-time data were analyzed using model-independent pharmacokinetic methods (*Gibaldi and Perrier 1982*). The terminal elimination rate constant, β, was calculated from the best-fit line through the log-linear terminal phase of the curves. The area under the plasma drug concentration versus time curves was calculated from time zero to infinity ($AUC = AUC_{0-t} + AUC_{t-\infty}$). AUC_{0-t} was calculated using the linear trapezoidal method and $AUC_{t-\infty}$ was calculated by dividing the concentration measured at time t (last sampling point) by β. Terminal elimination half-life ($T_{\frac{1}{2}}$) was obtained from the quotient of $0.693/\beta$. Total body clearance (CL_T), renal clearance (CL_R), volume of distribution at steady state (V_{dss}) and fraction of drug excreted unchanged in the urine (F_u) were calculated with the following equations:

$$CL_T = \text{Dose} / AUC$$

$$CL_R = \sum X_u / AUC$$

$$V_{dss} = \text{Dose} / (\beta \times AUC)$$

$$F_u = \sum X_u / \text{Dose}$$

where, $\sum X_u$ is the total amount of unchanged ampicillin excreted in the urine. Pharmacokinetic data analyses were performed using JANA and NONLIN software programs (Statistical Consultaints Inc., Lexington, KY).

4.2.4.2 Creatinine clearance

Creatinine clearance (CL_{Cr}) was calculated using the standard formula UV/P (*Pitts 1974*), where CL_{Cr} is expressed in ml/min.; U = urine concentration of creatinine in μmol/L; V = urine flow in ml/min.; P = plasma or serum creatinine concentration in μmol/L.

4.2.4.3 Statistical methods

Group comparison for each measured and calculated variable employed one way analysis of variance with repeated measures (*Bolton, 1984*). A p value of 0.05 was used to judge significance. All values are presented as Mean ± SD.

4.3 Results

The results for 7 subjects are reported here; one female subject voluntarily withdrew after 1 study period. Pharmacokinetic parameters for individual subjects after the three nutrient regimens are reported on Tables 4.2 to 4.4. There were no significant differences in any of the measured or calculated variables regardless of the nutrient regimens (Table 4.5). Crossover analysis of our data showed no significant differences in ampicillin kinetics between intravenous nutrient groups regardless of the sequence of infusion. Ampicillin was undetectable in the serum beyond 6 hours after injection in all subjects (Fig. 4.3). Serum creatinine concentrations ranged from 54 to 94 μmol/L and were normal. Creatinine clearance with enteral, intravenous D10W and PN were 119 ± 21 ml/min., 125 ± 32 ml/min. and 125 ± 48 ml/min respectively, and were not significantly different. Based on our data, the statistical power for detecting a change in ampicillin clearance at one standard deviation above the mean using conventional method (*Bolton, 1984*) was 77% at an α level of 0.05.

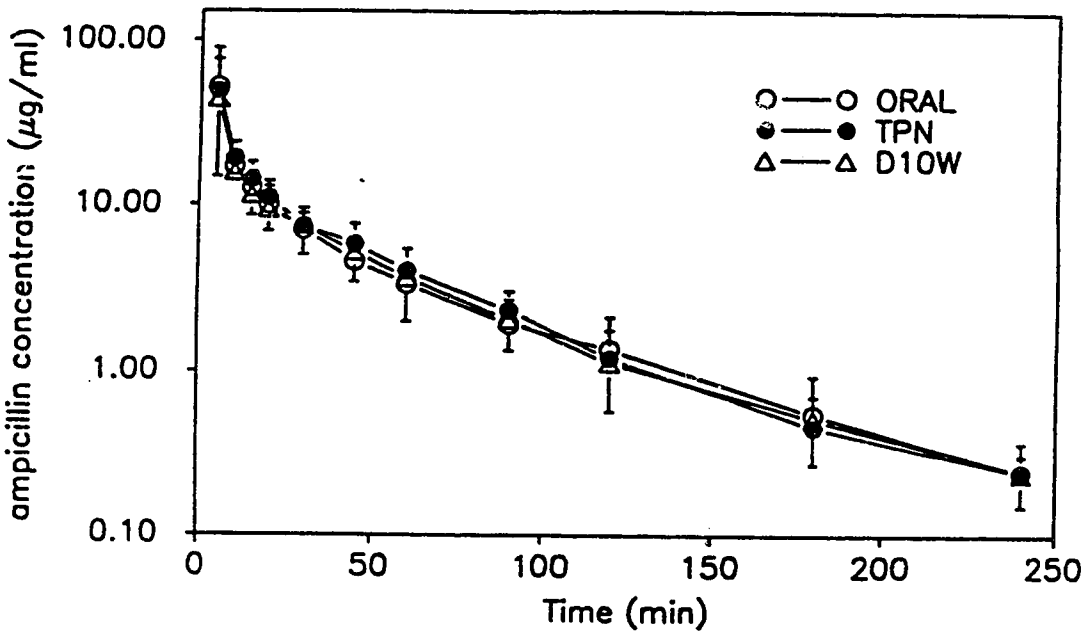

Figure 4.3 Serum ampicillin profiles after single 250 mg dose of intravenous ampicillin with three different nutrient regimens.

Table 4.2 Pharmacokinetic parameters of ampicillin in individual subjects who received single intravenous dose of ampicillin (250 mg) with enteral nutrient.

Subject	Pharmacokinetic Parameters*					
	AUC	$T_{1/2}$	V_{dss}	CL_R	CL_T	$F_u \times 100\%$
MF	965	50	0.26	1.6	5.4	29
CE	493	36	0.21	4.5	6.2	72
GW	835	43	0.21	1.8	4.5	39
GT	1137	36	0.14	3.6	4.0	89
ED	1516	57	0.11	1.8	2.5	74
TR	718	39	0.19	4.9	4.9	99
GB	583	35	0.19	4.3	5.3	81
Mean	892	42	0.19	3.2	4.7	69
±SD	352	8.4	0.05	1.4	1.2	25.7

* AUC, $\mu g \times min/ml$; $T_{1/2}$, min; V_{dss}, L/kg; CL_R, $ml/min \times kg$; CL_T, $ml/min \times kg$.

4.4 Discussion

PN is a widely used medical technique. However, the potential influences of PN on drug disposition have not been investigated properly. This study was specifically designed to evaluate the acute effects of PN on renal drug clearance. Ampicillin was chosen as a model drug in this study because renal clearance is its major elimination pathway and occurs partly by glomerular filtration and partly by tubular secretion (*Hori et al. 1983*). Therefore its use may reflect a number of potential mechanisms by which PN can induce alterations in drug renal clearance.

Table 4.3 Pharmacokinetic parameters of ampicillin in individual subjects who received single intravenous dose of ampicillin (250 mg) with D10W.

Subject	Pharmacokinetic Parameters*					
	AUC	$T_{1/2}$	V_{dss}	CL_R	CL_T	$F_u \times 100\%$
MF	1167	43	0.23	3.9	4.7	83
CE	638	33	0.17	4.9	4.8	102
GW	877	37	0.16	4.6	4.4	105
GT	543	42	0.35	4.8	8.2	58
ED	938	44	0.20	3.9	4.0	97
TR	1092	32	0.15	2.7	3.2	82
GB	612	50	0.28	5.1	5.0	102
Mean	838	40	0.22	4.3	4.9	90
±SD	246	6.5	0.07	0.8	1.6	16.8

* AUC, $\mu g \times min/ml$; $T_{1/2}$, min; V_{dss}, L/kg; CL_R, $ml/min \times kg$; CL_T, $ml/min \times kg$.

Pharmacokinetic studies on the disposition of ampicillin are extensively reported (*Foulds, 1986*). However, there are limited data on the specific role of parenteral nutrient regimen on the pharmacokinetics of ampicillin. The marked variability in plasma ampicillin concentration when it is delivered as an admixture with PN (*Colding et al. 1982*) presumably was associated with the variable loss of ampicillin in PN in vitro (*Feigin et al. 1979, Colding et al. 1978, Trissel, 1988*). We did not study patients already receiving PN to avoid potential interference with the pharmacokinetics of ampicillin from underlying disorders, different nu-

Table 4.4 Pharmacokinetic parameters of ampicillin in individual subjects who received single intravenous dose of ampicillin (250 mg) with PN.

Subject	Pharmacokinetic Parameters*					
	AUC	$T_{1/2}$	V_{dss}	CL_R	CL_T	$F_u \times 100\%$
MF	1441	40	0.16	3.1	3.8	81
CE	577	27	0.20	5.5	5.4	103
GW	684	38	0.25	4.5	5.5	81
GT	598	25	0.29	5.3	7.5	71
ED	880	43	0.17	2.7	4.2	65
TR	1089	30	0.14	2.1	3.2	64
GB	1024	58	0.14	3.3	3.0	109
Mean	899	37	0.19	3.8	4.7	82
±SD	313	11.4	0.06	1.3	1.6	17.8

* AUC, $\mu g \times min/ml$; $T_{1/2}$, min; V_{dss}, L/kg; CL_R, $ml/min \times kg$; CL_T, $ml/min \times kg$.

tritional status of the patients or other pharmacotherapy usually administered to the patient (*Pantuck et al. 1984, Bidlack et al, 1986, Anderson et al. 1988*). Our study design also allowed the use of different routes of nutrient intake in the same subject which would be impossible in patients requiring total parenteral nutrition. Thus, our study design eliminated the drawbacks associated with cross-sectional studies in a non-homogeneous population.

Our data showed that the pharmacokinetic profiles of ampicillin were similar regardless of the nutrient regimen. After an intravenous injection of 250 mg of

Table 4.5 Mean ± SD pharmacokinetic parameters of single intravenous dose of ampicillin with different nutrient regimens.

Kinetic Parameters	Nutrient Regimen		
	Enteral	D10W	PN
AUC (μg×min/ml)	892±352	838±246	899±313
$T_{1/2}$ (min)	42±8.4	40±6.5	37±11.4
V_{dss} (L/kg)	0.19±0.05	0.22±0.07	0.19±0.06
CL_R(ml/min×kg)	3.2±1.4	4.3±0.8	3.8±1.3
CL_T(ml/min×kg)	4.7±1.2	4.9±1.6	4.7±1.6
F_u×100%	69±25.7	90±16.8	82±17.8

Values = Mean ± SD of seven subjects. No significant difference among groups in pharmacokinetic parameters for ampicillin, p> 0.05.

ampicillin, the mean values for terminal $T_{\frac{1}{2}}$ of about 0.7 hours, V_{dss} of about 0.20 liters/kg, and 70 to 90% excretion of ampicillin in the urine are comparable to other reported data with subjects receiving unspecified nutrient intake (*Foulds 1986*); to detect a smaller alteration (less than one standard deviation from the mean) in ampicillin clearance among different nutrient regimens would require a larger sample size. This is probably not justified because small alterations in ampicillin clearance have no significant clinical consequence.

Theoretically, the use of PN may affect the stability and pharmacokinetic profile of ampicillin in a number of ways. physiocochemical conditions in PN solution such as pH and concentration of the nutrients are important in the determination of ampicillin stability in vitro (*Feigin et al. 1973, Colding et al. 1978,*

Trissel, 1988). However, any physicochemical interaction between PN and ampicillin was eliminated by our study design of infusing ampicillin through a venous site away from the intravenous nutrient infusion. Contamination from the previous intravenous ampicillin infusion was avoided by carefully flushing the catheter with saline.

Parenteral nutrients may affect protein binding and drug metabolism. However, only about 20% of the circulating ampicillin is protein bound (*Triggs et al. 1980*) and more than 80 % of ampicillin excreted intact in the urine (*Reynolds 1989*). Thus, the metabolic profile of ampicillin is unlikely to be affected to a significant extent with the use of amino acid-containing solutions.

Another possibility for PN to alter the pharmacokinetic profile of ampicillin is with increased GFR during PN, by as much as 100% compared to saline infusion (*Bengoa et al. 1983, Graf et al. 1983*). The majority of ampicillin is normally excreted in the urine (*Foulds 1986*) and its clearance is significantly influenced by altered GFR. In the present study, the rate of amino acid infusion from PN was 75 g per day. This amount of amino acid intake is comparable to the recommended nutrient intakes (RNI) for healthy adults and is about 50% lower than the rate of amino acid infusions which are reported to increase the GFR (*Bengoa et al. 1983, Graf et al. 1983*). The stable GFR as indicated by stable creatinine clearance in our subjects during the study period, at least in part, is related to the normal amino acid/protein intake in our subjects. This is consistent with other reports that amino acid infused at the rate of 1 g/kg/day maintained GFR in the normal

range (*Bengoa et al. 1989*) and that GFR generally is thought to increase by about 20% when protein intake is greater than RNI (*Bosch et al. 1983, Bergstrom et al 1985, Brouhard et al. 1987*).

Theoretically, an increase in renal plasma flow (RPF) during amino acid infusion (*Meyer et al. 1983*) may increase renal tubular secretion of ampicillin and is reflected by an increase in total body ampicillin clearance during PN. The stable total body and renal clearance of ampicillin and the stable creatinine clearances in our subjects would support an absence of increase in RPF during PN.

In the present study, measurement of pharmacokinetic profile of ampicillin beginning at 4 hours after a meal was designed to minimize the alteration in hemodynamic and hormonal changes which may affect GFR and consequently ampicillin clearance (*Bosch et al. 1983, Bergstrom et al. 1985, Brouhard et al. 1987*). This design also avoided any potential hemodynamic changes associated with prolonged severe restriction in protein and caloric intake beyond the "standardized" overnight fast (*Kitt et al. 1989*). Thus the results from the "enteral" regimen may be considered as basal ampicillin clearance.

Glucose and salt overload may increase renal filtration rate, and theoretically ampicillin excretion in urine may also be increased. However, glucose and salt subjects were within the normal limits.

In the present study, there was no glucosuria and the use of D10W, with the same additives as in PN solution, was designed to rule out any volume effect on GFR from intravenous infusion and to rule out the potential effect of other

nutrients on the pharmacokinetic profiles of ampicillin. Calcium and phosphate were not added to the D10W to avoid the risk of precipitation (*Venkataraman et al 1983*). However, calcium and phosphate per se have not been reported to alter ampicillin pharmacokinetics.

The stability of total body and renal clearance of ampicillin with all nutrient regimens also would indicate that under normal circumstances, PN solution is unlikely to affect urinary excretion of ampicillin. We conclude that in the presence of a normal GFR, the use of PN with amino acid content similar to the recommended nutrient intake is unlikely to affect the pharmacokinetics of ampicillin.

4.5 References

Anderson KE., "Influence of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet*, 14, pp.325-346 (1988).

Bengoa JM, Sitrin MD, Wood RJ, Rosenberg IH., "Amino acid-induced hypercalciuria in patients on total parenteral nutrition.", *Am J Clin Nutr*, 38, pp.264-269 (1983).

Bergstrom J, Ahlberg M, Alvestrand A., "Influence of protein intake on renal hemodynamics and plasma hormone concentrations in normal subjects.", *Acta Med Scand*, 217, pp.189-196 (1985).

Bidlack WR, Brown RC, Mohan C., "Nutritional parameters that alter hepatic drug metabolism, conjugation, and toxicity.", *Fed Proc*, 45, pp.142-148 (1986).

Bolton S., "Experimental design in clinical trials.", In: Bolton S., ed. **Pharmaceutical statistics: practical and clinical applications.**, (New York: Marcel Dekker, Inc.) pp.301-333 (1984).

Bosch JP, Saccaggi A, Lauer A, Ronco C, Belledonne M, Glabman S., "Renal functional reserve in humans: effect of protein intake on glomerular filtration rate.", *Am J Med*, pp.943-950 (1983).

Brouhard BH, La Crone LF, Richards GE, Travis LB., "Somatostatin limits rise in glomerular filtration rate after a protein meal.", *J Pediatr*, 110, pp.729-734 (1987).

Colding H, Andersen GE., "Stability of antibiotics and amino acids in two synthetic L-amino acid solutions commonly used for total parenteral nutrition in children.", *Antimicrob Agents Chemother*, 13, pp.555-558 (1978).

Colding H, Moller S, Andersen GE., "Continuous intravenous infusion of ampicillin and gentamicin during parenteral nutrition in 88 newborn infants.", *Arch Dis Child*, 57, pp.602-606 (1982).

Feigin RD, Moss KS, Shackleford PG., "Antibiotic stability in solutions used for intravenous nutrition and fluid therapy.", *Pediatrics*, 51, pp.1016-1026 (1973).

Foulds G., "Pharmacokinetics of sulbactam/ampicillin in human: A review.", *Rev Infect Dis*, 8, pp.S503-S511 (1986).

Garrow JS, Webster J., "Quetelet's index (W/H^2) as a measure of fatness.", *Internat J Obesity*, 9, pp.147-153 (1985).

Gibaldi M, Perrier D., "Noncompartmental analysis based on statistical moment theory.", In:

Gibaldi M, Perrier D., ed. **Pharmacokinetics, 2nd ed.**, (New York: Marcel Dekker, Inc.,) pp.409-419 (1982).

Graf H, Stummvoll HK, Luger A, Prager R., "Effect of amino acid infusion on glomerular filtration rate.", *N Engl J Med*, 308, pp.159-160 (1983).

Hori R, Okumura K, Kamiya A, Nihira H, Nakano H., "Ampicillin and cephalexin in renal insufficiency.", *Clin Pharmacol Ther*, 34, pp.792-798 (1983).

Kitt TM, Park GD, Spector R, Tsalikian E., "Reduced renal clearance of oxypurinol during a 400 calorie protein-free diet.", *J Clin Pharmacol*, 29, pp.65-71 (1989).

Pantuck EJ, Pantuck CB, Weissman C, Askanazi J, Conney AH., "Effects of parenteral nutritional regimens on oxidative drug metabolism.", *Anesthesiology*, 60, pp.534-536 (1984).

Pitts RF., "Clearance and rate of glomerular filtration", In: Pitts RF., ed. **Physiology of the kidney and body fluids, 3rd**, (Chicago: Year Book Med Publ,) pp.60-70 (1974).

Reynolds JEF., **Martindale The extra pharmacopoeia, 29th.**, pp.116-119 (1989).

Stoten A., "A micromethod for creatinine using resin to remove interfering substances.", *J Med Lab Techol*, 25, pp.240-247 (1968).

Triggs EJ, Johnson JM, Learoyd B., "Absorption and disposition of ampicillin in the elderly.", *Eur J Clin Pharmacol*, 18, pp.195-198 (1980).

Trissel LA., "Handbook on injectable drugs, 5th ed. Bethesda MD.", *Amer Soc Hosp Pharm*, pp.54-65 (1988).

Venkataraman PS, Brissie EO, Tsang RC., "Stability of calcium and phosphorus in neonatal parenteral nutrition solutions.", *J Pediatr Gastroenterol Nutr*, 2, pp.640-643 (1983).

Vree TB, Hekster YA, Baars AM, Vander KE., "Rapid determination of amoxycillin (Clamoxyl) and ampicillin (Penbritin) in body fluids by means of high-performance liquid chromatography.", *J Chromatogr Biomed Appl*, 145, pp.496-501 (1978).

CHAPTER 5

THE ACUTE EFFECTS OF PN ON LIDOCAINE PHARMACOKINETICS IN HEALTHY HUMAN VOLUNTEERS[4]

5.1 Introduction

The pharmacokinetics and metabolism of certain drugs in animals and humans have been shown to be influenced by Parenteral nutrition (PN) therapy (*Anderson. 1988*). For example, healthy rats had 24% and 57% lower content and activity, respectively, of hepatic cytochrome P_{450} and 71% lower pentobarbital hepatic clearance after 7 days of PN compared with animals fed with standard laboratory rat chow (*Knodell et al. 1984*). Our preliminary results in minipigs after 7 days of PN showed that the total body clearance (CL_T) and the steady state volume of distribution (V_{dss}) of lidocaine, another drug metabolized by the cytochrome P_{450} enzyme system, were reduced to half of those of the enterally fed controls (*Ke et al. 1987*). In human, the clearance of antipyrine has been used as an indicator of oxidative metabolism by the cytochrome P_{450} enzyme system to assess for nutrient-drug interaction. Antipyrine clearance was decreased by 34% in post-operative patients receiving amino acid-dextrose solution for 7 days (*Burgess et al. 1987*); was increased by 24% in healthy adults receiving intravenous infusion of amino acids for only one day after a 4-day isocaloric infusion of dextrose (*Pantuck et al. 1984*), and increased by 87% in previously malnourished subjects undergoing nutritional repletion with 8 days of PN therapy (*Pantuck et al. 1985*).

[4] A version of this chapter has been published in *Ther Drug Monit* 12: 157-162, (1990)

Preliminary studies in our laboratory (*Tam et al. 1984*) showed that high concentrations of amino acids can competitively inhibit the metabolism of lidocaine in isolated perfused rat livers as another potential mechanism for altering drug metabolism. Thus, it is possible that specific nutrient components of the parenteral solution, the duration of administration and/or the nutritional status of an individual, may significantly affect drug metabolism.

This study was designed to examine in healthy humans, the acute effect of PN, specifically the effect of amino acid infusion on the pharmacokinetics of a model compound, lidocaine. We aim to test the hypothesis that amino acid infusion would result in a decreased metabolism of lidocaine.

5.2 Methods

5.2.1 Subjects

Eight healthy, young adult non-smokers (4 males and 4 females, 20-28 years of age) were recruited for the study. The demographic data for these subjects are listed on Table 4.1. Informed written consent was obtained from each subject. The study was approved by the Ethics Review Committee for Human Experimentation of the University of Alberta and performed at the Clinical Investigations Unit at the University of Alberta Hospital.

5.2.2 Study Design and Sample Collection

Following an overnight fast, each subject received a bolus injection of lidocaine HCl (1 mg/kg, IMS Ltd, Mississauga, Ont, Canada) intravenously in

association with each of three nutrient regimens. The nutrient regimens included: 1) a standard peripheral PN solution in use at the University of Alberta Hospital; each liter of PN solution contains 100 g dextrose, 37.5 g amino acids (Travasol, Baxter-Travenol Lab., Malton, Canada), 50 mmol sodium, 50 mmol potassium, 3 mmol magnesium, 3 mmol calcium, 11 mmol phosphate, 1 ml multiple trace elements (MTE-4, NovaPharm/LyphoMed, Pharmaceutical Co., Markham, Canada) and 10 ml multiple vitamins (MV1-1000, SABEX international Ltd., Montreal, Canada); 2) 10% dextrose water (D10W) without amino acids, containing the same additives as the PN solution except for calcium and phosphate; 3) an enteral meal containing similar fluid volume (1 L), caloric (490 kcal / 2058 kJ), protein (37.5 g) and sodium (66 - 70 mmol/L) content as the PN solution. Water, but no food, was allowed by mouth during the three study periods.

Two peripheral intravenous catheters were inserted in each subject, one in each arm, prior to the experiment. One catheter was used for nutrient infusion and the other was for lidocaine infusion and blood sampling. One litre of a study intravenous solution (PN or D10W) was infused over 12 hour followed immediately by a 12 hour infusion of the other intravenous solution. The order of the infusion of intravenous regimens was randomly assigned. The enteral and parenteral studies were performed at least 28 days apart. The subjects remained at bed rest during each study period.

Lidocaine was infused over a 5 minute period, commencing 2 hours after the initiation of each intravenous nutrient regimen and 4 hours after a standardized

breakfast meal. Three ml of blood from the sampling catheter was discarded immediately prior to each blood sampling. Blood samples (5 ml) were taken at 0, 5, 10, 15, 20, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6 and 8 hours after drug administration. Blood samples were collected into polypropylene tubes and stored at 4°C for one hour prior to centrifugation. After separation, serum was stored at -20°C until analysis.

5.2.3 Sample Analysis

5.2.3.1 Measurement of total lidocaine and its metabolites

The concentration of total lidocaine and its metabolites, N-(N-ethylglycyl)-2,6-xylidine (MEGX) and N-glycyl-2,6-xylidine (GX) in the serum were measured using a slightly modified version of the HPLC method reported by Narang *et al.* (*1978*). Briefly, a 1 ml serum sample was transferred to a 10 ml glass test tube containing 0.1 ml of 10 μg/ml solution of N-ethyl-N-methyl-glycine-2,6-xylidide as internal standard (I.S.), and then 0.1 ml of 1 M NaOH solution and 6 ml of methylene chloride were added. After mixing on a vortex mixer (IKA-VIBRAX-VXR, Terrochem, setting at 1,200), the mixture was centrifuged (1,000 g) for 10 min, and the organic layer was removed and evaporated under a gentle nitrogen stream. The residue was mixed thoroughly with 300 μl of a pH 2.2 HCl solution and then a 100 μl aliquot of this solution was injected onto the HPLC.

The HPLC system (Waters Associates, Mississauga, Ont, Canada) consisted of a M-45 pump, a Model 441 UV detector (set at 214 nm), an automatic sampler

(WISP, Model 710B) and a data processing station (Model 840). Separation of lidocaine and its two metabolites was achieved using a 5 μm C_{18} reversed phase Nova-Pak cartridge (11.5 cm x 8 mm I.D.). No interfering peaks were found in blank serum (Fig. 5.1). The aqueous mobile phase, which consisted of 17% (v/v) acetonitrile, 0.1% (v/v) phosphoric acid and 0.15% (v/v) triethylamine, was pumped at a flow rate of 2 ml/min. The limits of quantification for lidocaine and its two metabolites were 0.05, 0.02 and 0.02 μg/ml, respectively. The examined concentration range was 0.2 - 5 μg/ml for lidocaine and its metabolites in serum and the coefficient of variation over the examined concentration range for the measurement of each compound was <5%.

5.2.3.2 Equilibrium dialysis

Serum protein binding was measured using an equilibrium dialysis technique (*Routledge et al. 1980*). The pH of the stored serum samples was adjusted to pH 7.4 prior to the binding study with 1.0 M phosphoric acid and 0.1 M sodium hydrochloride. A 0.7 ml aliquot of a serum sample was added to a 1 ml Teflon equilibrium dialysis cell (Spectrum, Los Angeles, CA). Dialysis was performed against an equal volume of pH 7.4 isotonic phosphate buffer, containing 0.5% (w/v) sodium chloride and 200 ng/ml ^{14}C lidocaine HCl with specific activity 52.0 mCi/mmol (New England Nuclear, Boston, MA). The buffer and serum compartments were separated by a dialysis membrane (Cellulose tubing, Sigma, St. Louis, MO, molecular size cut off over 2,000) and the cells were rotated for 4 hours in a 37°C water bath. Preliminary studies demonstrated that it required 4 hours for

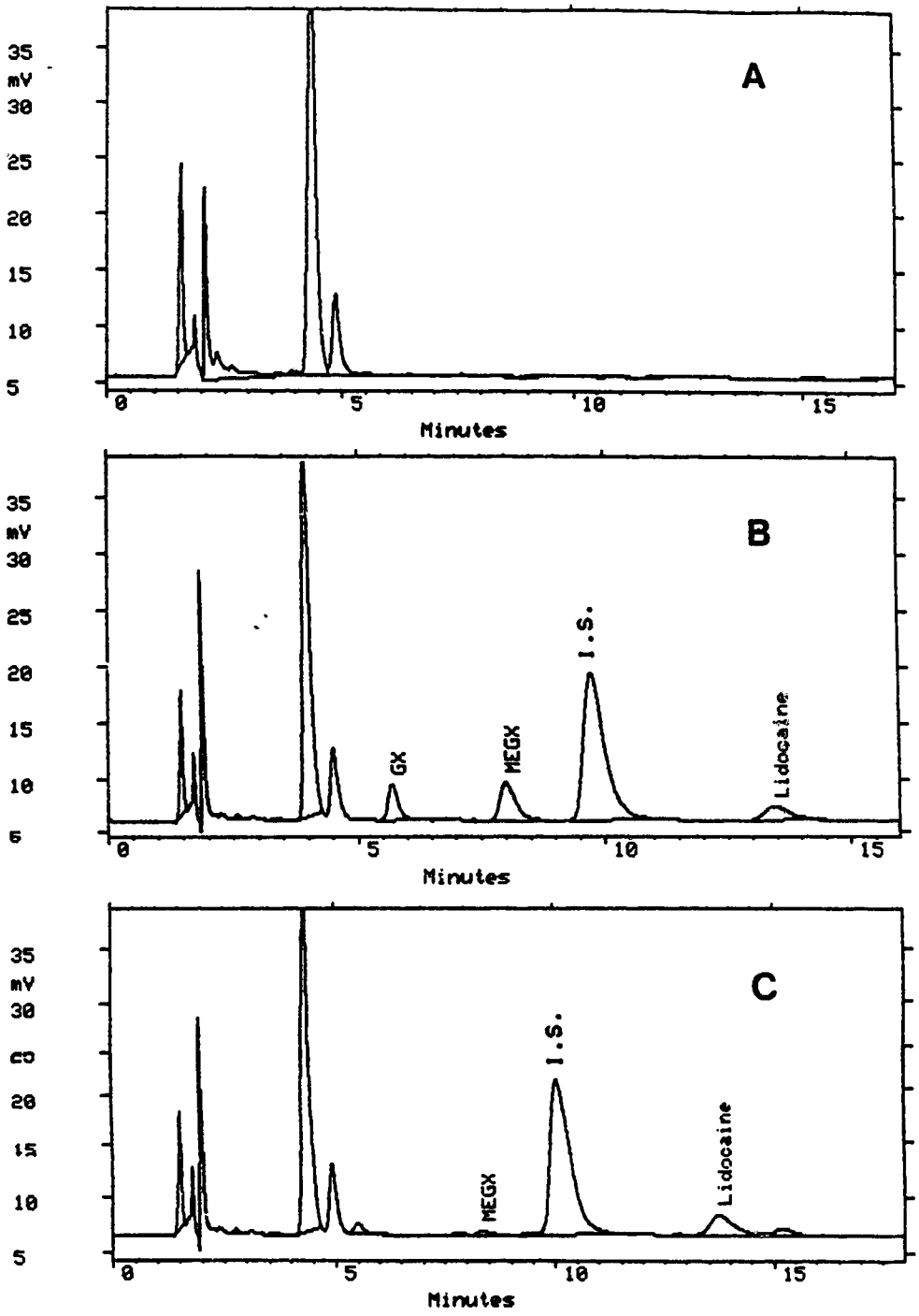

Figure 5.1 Chromatograms obtained from blank (A), spiked (B) and patient serum (C) samples. The concentration of each spiked component was 0.5 μg/ml. The signals were measured in mV.

lidocaine binding to reach equilibrium. The activity of labelled lidocaine in each side of the cell was measured by a liquid scintillation counting method (*Routledge et al. 1980*) using a 1217 PackBeta liquid scintillation counter (LKB WALLAC, Turku, Finland). The fraction of unbound lidocaine in serum (fu) was calculated as the ratio of absolute disintegration rates in buffer and serum. The concentration of unbound lidocaine was calculated by multiplying the HPLC measurements of total lidocaine concentrations by the fraction of unbound lidocaine in serum.

5.2.4 Treatment of Data

5.2.4.1 Pharmacokinetic analyses

Nonlinear regression programs, JANA (*Metzler et al. 1974*) were used to fit the serum drug concentration versus time data. Following single intravenous dose administration, the area under the serum drug concentration curve (AUC) was calculated from the time of administration to infinity by the linear trapezoidal rule, where the area from the last data point (C_t) to infinity was caculated by C_t / β. The apparent elimination rate constant, β, was calculated from the best-fit line passing through the log-linear terminal phase of the curves. Terminal half-life ($T_{\frac{1}{2}}$), total body clearance (CL_T) and volume of distribution at steady state (V_{dss}) were calculated with the following equations (*Gibaldi and Perrier, 1982*):

$$T_{\frac{1}{2}} = 0.693 / \beta$$

$$CL_T = \text{Dose} / \text{AUC}$$

$$V_{dss} = \text{Dose} / \beta \times \text{AUC}$$

Lidocaine pharmacokinetic data analyses were performed using NONLIN software program (Statistical Consultaints Inc., Lexington).

The maximum serum MEGX concentration (C_{max}) and the time to achieve this value (T_{max}) were obtained directly from the concentration versus time data. The AUC of MEGX from time zero to infinity was calculated using a non-compartmental program called LAGRAN (*Rocci and Jusko, 1983*).

5.2.4.2 Statistical methods

Sample size calculation (*Steel and Torrie 1980*) was based on published lidocaine pharmacokinetic data in humans (*Benet and Sheiner. 1985*). The sample size in this study should be adequate to detect a decrease in lidocaine clearance in the intravenous nutrient groups at one standard deviation below the mean of the enteral group with an alpha value of 0.05 and beta value of 0.2. Group comparison for pharmacokinetic parameters and carryover effect from infusion of different intravenous nutrients were performed by repeated measures analysis of variance (*Bolton 1984*). The level of significance was set at p=0.05 and all values were presented as mean ± SD.

5.3 Results

The results for 7 subjects are reported here; one female subject voluntarily withdrew after 1 study period. The concentration of GX in serum was below the quantification limit in all subjects. The time courses for serum concentrations of total lidocaine, free lidocaine and MEGX were not significantly different during

the three nutrient regimens (Fig. 5.2). Pharmacokinetic parameters for lidocaine: CL_T, $T_{\frac{1}{2}}$, V_{dss} and AUC; for MEGX: C_{max}, T_{max} and AUC in individual subjects are shown in Tables 5.1 to 5.3.

Crossover analysis of our data indicate that the sequence of intravenous nutrient infusion had no significant effect on lidocaine disposition. The kinetic parameters of lidocaine (CL_T, $T_{\frac{1}{2}}$, V_{dss} and AUC) and MEGX (C_{max}, T_{max} and AUC) obtained during PN and D10W were not significantly different from each other. Similarly, there were no significant differences between the kinetic results obtained from each of the two intravenous nutritional regimens and the enteral study (Table 5.4). Furthermore, lidocaine serum protein binding remained unchanged [f_u : 0.34± 0.066 (PN) vs. 0.36 ± 0.050 (D10W) vs. 0.33 ± 0.053 (enteral)] during the three nutrient regimens.

5.4 Discussion

The use of PN has increased markedly over the past two decades. At the same time, PN therapy has posed some very fundamental and practical clinical questions regarding the safety and efficacy of concurrent drug therapy. To date only limited data on potential interaction between intravenous nutrients and drugs exist. This study was specifically designed to evaluate for a potentially acute nutrient-drug interaction during PN administration.

In the present study, we used healthy subjects instead of patients already receiving PN to avoid potential interference from underlying disease states, variable

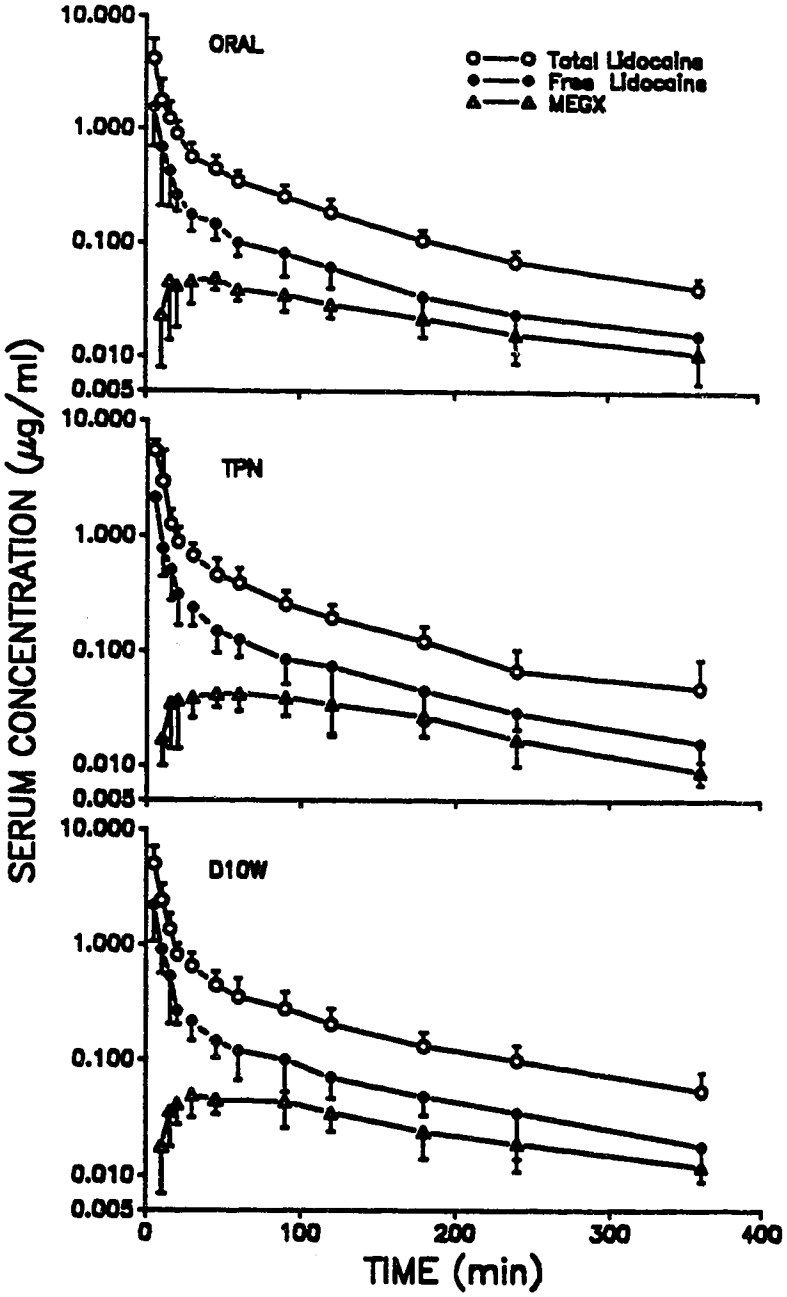

Figure 5.2 Serum concentration-time curves of total lidocaine, free lidocaine and MEGX after an intravenous dose (1 mg/kg) of lidocaine HCl during three different nutrient regimens.

Table 5.1 Pharmacokinetic parameters of lidocaine and MEGX in individual subjects who received single intravenous dose of lidocaine HCl (1 mg/kg) with enteral nutrient.

Subject	Total lidocaine				MEGX		
	CL_T	$T_{1/2}$	V_{dss}	AUC	C_{max}	T_{max}	AUC
MF	6.49	76.8	0.72	150.9	0.049	45	10.9
CE	8.26	51.4	0.61	117.7	0.037	30	7.8
GW	11.55	104.1	1.45	78.5	0.035	45	9.3
GT	10.60	95.7	1.39	88.4	0.057	45	12.0
ED	7.30	61.2	0.65	130.2	0.058	20	9.1
TR	4.87	73.3	0.52	201.3	0.064	30	11.4
GB	5.94	91.9	0.79	160.3	0.049	45	6.5
Mean	7.86	79.2	0.88	132.5	0.050	37.1	9.6
±SD	2.45	19.1	0.38	42.7	0.011	10.35	1.99

CL_T, ml/min×kg; $T_{1/2}$, min; V_{dss}, L/kg; AUC, μg×min/ml; C_{max}, μg/ml; T_{max}, min.

nutritional status or other pharmacotherapy administered concomitantly during PN. This study design also allowed the comparison of the effects of different routes of nutrient intake using the subject as his or her own control. Thus, our study design eliminated some of the drawbacks associated with cross-sectional studies in a non-homogeneous population.

In the enteral feeding group, we chose to infuse lidocaine beginning at 4 hours after a meal to avoid any potential effects from prolonged fasting beyond

Table 5.2 Pharmacokinetic parameters of lidocaine and MEGX in individual subjects who received single intravenous dose of lidocaine HCl (1 mg/kg) with PN.

Subject	Total lidocaine				MEGX		
	CL_T	$T_{1/2}$	V_{dss}	AUC	C_{max}	T_{max}	AUC
MF	6.78	63.5	0.62	144.0	0.027	20	10.6
CE	5.35	142.7	1.10	166.2	0.058	30	7.7
GW	6.60	46.3	0.44	150.0	0.066	20	7.9
GT	9.08	71.3	0.93	108.2	0.046	60	10.2
ED	6.87	80.4	0.80	139.4	0.051	30	13.8
TR	10.86	50.9	0.80	89.6	0.043	45	10.9
GB	8.39	63.5	0.77	117.3	0.054	20	10.1
Mean	7.70	74.0	0.78	130.6	0.049	32.1	10.2
±SD	1.85	32.3	0.21	26.7	0.013	15.23	2.05

CL_T, ml/min×kg; $T_{1/2}$, min; V_{dss}, L/kg; AUC, μg×min/ml; C_{max}, μg/ml; T_{max}, min.

the standardized overnight fast and to minimize the influence of meal-induced alteration in hepatic blood flow (*McLean et al. 1978*) and hepatic drug metabolism (*Liedholm and Melander 1986*). Thus the results from the enteral regimen may be considered as baseline values. In the intravenous nutrient studies, lidocaine administered at 2 hours after the initiation of nutrient infusion. This protocol was based on the assumption that serum concentration of most infused amino acids have reached steady states (*Chami et al. 1978*) and our preliminary rat liver perfusion studies (*Tam et al. 1984*) indicated that competitive interaction

Table 5.3 Pharmacokinetic parameters of lidocaine and MEGX in individual subjects who received single intravenous dose of lidocaine HCl (1 mg/kg) with D10W.

Subject	Total lidocaine				MEGX		
	CL_T	$T_{1/2}$	V_{dss}	AUC	C_{max}	T_{max}	AUC
MF	6.65	98.0	0.94	142.3	0.038	45	8.4
CE	4.54	96.7	0.63	207.3	0.050	30	8.8
GW	8.80	92.9	1.18	109.2	0.072	30	7.7
GT	7.58	82.8	0.91	119.2	0.042	45	11.9
ED	4.19	85.0	0.51	231.7	0.055	30	10.1
TR	5.86	69.2	0.59	163.0	0.046	30	15.9
GB	9.81	103.0	1.46	97.5	0.059	45	8.6
Mean	6.78	89.6	0.89	152.8	0.051	36.4	10.2
±SD	2.10	11.5	0.34	50.8	0.011	8.02	2.86

CL_T, ml/min×kg; $T_{1/2}$, min; V_{dss}, L/kg; AUC, μg×min/ml; C_{max}, μg/ml; T_{max}, min.

for enzyme substrates is demonstrable after 30 minutes of amino acids infusion. Thus, a 12 hour infusion of PN should adequately reflect the acute effects of amino acid infusion on lidocaine pharmacokinetics.

Lidocaine was chosen for this study because its use may reflect a number of potential mechanisms by which PN can induce alterations in drug metabolism. This allows the study of potential competition for a hepatic cytochrome P_{450} enzyme system (*Keenaghan and Boyes 1972, Tam et al. 1987*), because certain

Table 5.4 Mean ± SD pharmacokinetic parameters of lidocaine and MEGX after single intravenous dose of lidocaine HCl (1 mg/kg) with different nutrient regimens.

N.R.	Total lidocaine				MEGX		
	CL_T	$T_{1/2}$	V_{dss}	AUC	C_{max}	T_{max}	AUC
Enteral	7.86±2.45	79.2±19.1	.88±.38	132.5±42.7	.050±.011	37.1±10.35	9.6±1.99
PN	7.70±1.85	74.0±32.3	.78±.21	130.6±20.7	.049±.013	32.1±15.23	10.2 ±2.05
D10W	6.78±2.10	89.6±11.5	.89±.34	152.8±50.8	.051±.011	36.4±8.02	10.2 ±2.86

Values = mean ± SD of seven subjects. No significant differences among the three nutrient regimens in the pharmacokinetic parameters for lidocaine and MEGX were detected ($p > 0.05$).
Abbreviations:
N.R.= Nutrient regimens; CL_T, ml/min×kg; $T_{1/2}$, min; V_{dss}, L/kg; AUC, μg×min/ml; C_{max}, μg/ml; T_{max}, min.

aromatic amino acids in PN have similar chemical structures and undergo the same aromatic ring *p*-hydroxylation pathway in the liver as lidocaine. Alteration of serum protein binding of lidocaine may reflect the potential competition for binding sites from certain aromatic amino acids, such as L-tryptophan and L-phenylalanine. N-deethylation is a major primary hepatic metabolic pathway for lidocaine in human (*Keenaghan and Boyes 1972*) and the measurement of metabolically active deethylation products (*Burney et al. 1974*), MEGX and GX, may increase understanding of the mechanisms involved in nutrient-drug interaction.

There were no significant differences in the pharmacokinetic parameters of lidocaine and MEGX obtained during PN and D10W regimens. Thus, it is unlikely that lidocaine-induced enzyme inactivation occurred when the subjects received

two intravenous injections of lidocaine (1 mg/kg) 12 hours apart. In contrast, previous studies in our laboratory (*Saville et al. 1989*) showed that N-deethylation of lidocaine decreased in the isolated perfused rat livers from the animals that received a pretreatment with an intravenous injection of lidocaine (10 mg/kg). Differences in the dosage of lidocaine used and/or species differences in hepatic metabolism for lidocaine conceivably could account for some of the discrepancies in lidocaine pharmacokinetics between our animal and human data.

The pharmacokinetic data for lidocaine in this study are comparable to that reported in the literature (*Rowland et al. 1971, Nation et al. 1977, Benet et al. 1985*). Based on the composition of our intravenous solutions, it appears that amino acid infusion as part of routine PN does not acutely alter lidocaine metabolism and does not interfere with serum protein binding of lidocaine. The similar MEGX serum concentrations among the three nutrient regimens would further support the contention that routinely used PN probably does not acutely influence lidocaine metabolism. The unquantifiable amount of the active metabolite, GX, in this study probably reflects the small yield of the metabolite from a single dose of lidocaine.

The effect of PN on lidocaine metabolism in our human study differs from that observed using the minipig model (*Ke et al. 1987*). Duration of PN administration may influence lidocaine metabolism and explain the differences observed. Altered liver function (*Merritt 1986*) and hepatic drug metabolism (*Knodell et al. 1980 & 1984*) have been reported even after one week of PN. In our human subjects, lidocaine was co-infused within 2 hours after commencement of PN, whereas

lidocaine was administered after 7 days of PN in the minipigs. In the present study, one standard deviation for lidocaine clearance is about 30% of the mean clearance value, and is similar to the reported value (*Benet et al. 1985*). Thus, to detect smaller differences in pharmacokinetics of lidocaine would require a much larger sample size of healthy volunteers and is probably of no clinical relevance.

The changes in hepatic oxidative metabolism demonstrated by our data using lidocaine as a model compound contrast with the data using antipyrine as a model compound. Both an increase (*Pantuck et al. 1984 & 1985*) and a decrease (*Burgess et al. 1987)* in antipyrine metabolism have been reported. The discrepancies between the reported studies and our study also may be related to a more prolonged period of PN administration (> 1 week) (*Pantuck et al. 1985 and Burgess et al.1987)* or to the higher rate of amino acid infusion (up to 184 g/day) (*Pantuck et al. 1985*) used in other studies. It is also possible that factors responsible for altered oxidative metabolism of antipyrine need not necessarily have the same effect on all other oxidatively metabolized drugs such as lidocaine, since there exist different isoenzymes of cytochrome P_{450} which are under separate regulatory control (*Lu and West 1979*). Differences in the nutritional status and underlying disease state of the subjects, and the theoretical potential for enzyme induction from prolonged PN are other possible confounders for nutrient-drug (including lidocaine) interaction. In the present study, we demonstrated that at the usual rate of amino acid infusion there is no acute effect of PN on lidocaine pharmacokinetics.

5.5 References

Anderson KE., "Influence of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet*, 14, pp.325-346 (1988).

Benet LZ, Sheiner LB., "Design and optimization of dosage regimens; pharmacokinetic data.", In: Gilman AG, Goodman LS, Rall TW, Murad F, ed. **Goodman and Gilman's the pharmacological basis of therapeutics, 7th ed.**, (New York: Macmillan Publishing Company,) pp.1663-1733 (1985).

Bolton S., "Experimental design in clinical trials.", In: Bolton S., ed. **Pharmaceutical statistics: practical and clinical applications.**, (New York: Marcel Dekker, Inc.) pp.301-333 (1984).

Burgess P, Hall RI, Bateman DN, Johnston IDA., "The effect of total parenteral nutrition on hepatic drug oxidation.", *JPEN*, 11, pp.540-543 (1987).

Burney RG, Difazio GA, Peach MJ, Petrie KA, Sylvester MV., "Antiarrhythmic effects of lidocaine metabolites.", *Am Heart J*, 88, pp.765-769 (1974).

Chami J, Reidenberg MM, Wellner D, David DS, Rubin AL, Stenzel KH., "Pharmacokinetics of essential amino acids in chronic dialysis patients.", *Am J Clin Nutr*, 31, pp.1652-1659 (1978).

Gibaldi M, Perrier D., "Non compartmental analysis based on statistical moment theory.", In: Gibaldi M, Perrier D., ed. **Pharmacokinetics, 2nd ed.**, (New York: Marcel Dekker, Inc.,) pp.409-419 (1982).

Ke J, Tam YK, Semple HA, Coutts RT, Gray MR, "Effects of parenteral nutrition on lidocaine pharmacokinetics.", *J Pharm Sci*, 76, pp.S77 (1987).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man.", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Knodell RG, Spector MH, Brooks DA, Keller FX, Kyner WT., "Alterations in pentobarbital pharmacokinetics in response to parenteral and enteral alimentation in rat.", *Gastroenterology*, 79, pp.1211-1216 (1980).

Knodell RG, Steele NM, Cerra FB, Cross JB, Solomon TE., "Effects of parenteral and enteral hyperalimentation on hepatic drug metabolism in the rat", *J Pharmacol Exper Ther*, 229, pp.589-597 (1984).

Liedholm H, Melander A., "Concomitant food intake can increase the bioavailability of propranolol

by transient inhibition of its presystemic primary conjugation.", *Clin Pharmacol Ther*, 40, pp.29-36 (1986).

Lu AYH, West SB., "Multiplicity of mammalian microsomal cytochromes P450.", *Pharmacol Rev*, 31, pp.277-95 (1979).

McLean AJ, McNamara PJ, du Souich P, Gibaldi M, Lalka D., "Food, splanchnic blood flow, and bioavailability of drugs subject to first-pass metabolism.", *Clin Pharmacol Ther*, 24, pp.5-10 (1978).

Merritt RJ., "Cholestasis associated with total parenteral nutrition.", *J Pediatr Gastroenterol Nutr*, 5, pp.9-22 (1986).

Metzler CM, Elfring GL, McEwen AJ., "A package of computer programs for pharmacokinetic modelling.", *Biometrics*, 30, pp.562 (1974).

Narang PK, Crouthamel WG, Carliner NH, Fisher ML, "Lidocaine and its active metabolites.", *Clin Pharmacol Ther*, 24, pp.654-662 (1978).

Nation R, Triggs EJ, Selig M., "Lignocaine kinetics in cardiac patients and aged subjects.", *Br J Clin Pharmacol*, 4, pp.439-448 (1977).

Pantuck EJ, Pantuck CB, Weissman C, Askanazi J, Conney AH., "Effects of parenteral nutritional regimens on oxidative drug metabolism.", *Anesthesiology*, 60, pp.534-536 (1984).

Pantuck EJ, Pantuck CB, Weissman C, Gil KM, Askanazi J., "Stimulation of oxidative drug metabolism by parenteral refeeding of nutritionally depleted patients.", *Gastroenterology*, 89, pp.241-245 (1985).

Rocci ML, Jusko WJ., "Lagran program for area and moments in pharmacokinetic analysis.", *Computer Prog Biomed*, 16, pp.203-216 (1983).

Routledge PA, Barchowsky A, Bjornsson TD, Kitchell BB, Shand DG., "Lidocaine plasma protein binding.", *Clin Pharmacol Ther*, 27, pp.347-351 (1980).

Rowland M, Thomson PD, Guichard A, Melmon KL., "Disposition kinetics of lidocaine in normal subjects.", *Ann NY Acad Sci*, 179, pp.383-398 (1971).

Saville BD, Gray MR, Tam YK., "Evidence for lidocaine-induced enzyme inactivation.", *J Pharm Sci*, 78, pp.1003-1008 (1989).

Steel RGD, Torrie JH., "Comparison involving two sample means", In: Steel RGD, Torrie JH, ed. "Principles and procedures of statistics: a biometrical approach 2nd ed.", (New York:

McGraw-Hill,) pp.113-119. (1980).

Tam YK, Sim HA, Yau ML, Tawfik S., "The effects of Travasol on lidocaine metabolism.", *IUPHAR 9th International Congress of Pharmacology, London, England*, pp.242 (1984).

Tam YK, Tawfik SR, Ke J, Coutts RT, Gray MR, Wyse DG., "HPLC assay of lidocaine and its nine metabolites in human plasma and urine.", *J Chromatogr Biomed Appl*, 423, pp.199-206 (1987).

CHAPTER 6

EFFECTS OF PN ON HEPATIC ELIMINATION OF LIDOCAINE: A STUDY USING ISOLATED RAT LIVER PERFUSION[5]

6.1 Introduction

The use of parenteral nutrition (PN) is an accepted technique to achieve or maintain normal nutritional status in subjects unable to tolerate adequate oral nutrients. However, PN has recognized complications (*Gutcher and Cutz 1986, Merritt 1986, Klein and Nealon 1988*) and hepatic drug clearance may be altered with the use of PN (*Knodell et al. 1980 and 1984, Pantuck et al. 1984 and 1985, Burgess et al. 1987, Anderson, 1988*). For example, pentobarbital hepatic clearance and cytochrome P_{450} content and associated enzyme activities in liver were lower in healthy rats after 7-days of PN than in animals fed standard laboratory chow *ad libitum* (*Knodell et al. 1980 and 1984*). The clearance of antipyrine, another drug widely used as an index of hepatic oxidative drug metabolizing activity, was decreased by 35% in post-operative patients after 7-days of amino acid-dextrose infusion (*Burgess et al. 1987*).

Preliminary data from our laboratory showed that the total body clearance (CL_T) and the steady state volume of distribution (V_{dss}) of lidocaine, a drug extensively metabolized by a hepatic cytochrome P_{450} enzyme system, were reduced by 50% in pigs after 7 days of PN when compared to normally fed control

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animals (*Ke et al. 1987*). However, recent data from this laboratory in healthy human volunteers who received an intravenous bolus of lidocaine HCl, beginning 2 hours after the commencement of a standard PN solution, showed that lidocaine pharmacokinetics remained unchanged in contrast to what occurred in enterally fed controls (*Ke et al. 1990*). Differences in the duration of PN, the rate of its administration, or species differences in hepatic metabolism for lidocaine (*Keenaghan and Boyes, 1972*) could, conceivably, account for some of the discrepancies in lidocaine clearance between our animal and human data.

The present study was designed to define better the role of PN in lidocaine metabolism with the use of the isolated rat liver perfusion model. We aimed to determine the hepatic lidocaine elimination in the isolated perfused liver model during acute infusion of PN and after 7 days of *in vivo* PN administration.

6.2 Methods

6.2.1 Experimental Procedures

A total of 24 male Sprague-Dawley rats weighing 195-240 g were used as the source of isolated livers. Details of isolated liver perfusion technique were reported previously (*Tam et al. 1987a*). The portal vein and inferior vena cava of the isolated liver were cannulated and the liver was perfused with oxygenated (95:5 oxygen: carbon dioxide) Kreb's bicarbonate buffer (*Ross, 1972*), pH 7.4, containing 2 g of dextrose/L at a flow rate of 3-5 ml/min/ g of liver via the portal vein catheter. The viability of the liver was confirmed by monitoring the rate of

oxygen consumption, which was within the normal ranges reported by Bloxam (*1973*) as measured by oxygen monitor (YSI Model 53, Yellow Springs Instrument Co., Inc. Yellow Springs, OH); the same aspartate and alanine aminotransferase levels in perfusate effluent at 0 and at 80 min; the stable concentrations of lidocaine and its metabolites in the perfusate effluent at steady state during continuous lidocaine infusion; and the physical appearance of the liver.

6.2.1.1 Co-infusion study

Livers were isolated from 12 animals that had free access to standard laboratory rat chow (Wayne Rodent Plox 8604-00, Continental Grain Company, Chicago, IL) and water. The "one-pass" perfusion method was used in the study. In 6 livers (the experimental group), lidocaine and PN solution were co-infused via the portal vein catheter (inlet) starting 20 minutes after the isolation and catheterization of each liver. Lidocaine (0.9 mg/ml) was infused at a rate of 0.097 ml/min via a Harvard pump (Model 600, Harvard Apparatus Co., Inc., Dover, Mass) and PN solution was co-infused at 0.123 ml/min using another Harvard pump (Model 940, Harvard Apparatus, South Natick, Mass). In another 6 livers (the control group), lidocaine was infused at the same rate as the experimental group while normal saline was co-infused at 0.123 ml/min. Lidocaine and PN or lidocaine and saline were infused for 80 minutes. This period was shown to be sufficient for the system to approach steady-state conditions for lidocaine and its metabolites (*Tam et al. 1987a*). The inlet concentration of lidocaine (C_{in}) was determined by averaging 3 samples (0.5 ml each) taken from the inlet at 2, 30 and 60 minutes from the beginning of lidocaine infusion.

The concentrations of lidocaine and its metabolites (C_{out}) were measured from effluent taken from the inferior vena cava catheter (outlet) at 0, 1, 3, 5, 7, 10, 15, 20 minutes and then at 10 min interval until 80 min. Each liver was blotted dry after each study and its weight was recorded.

6.2.1.2 Post PN study

Twelve animals received jugular venous cannulation 8 days prior to isolation of the livers. Six animals (experimental group) received PN solution for 7 days at 65 ml per 24 hours by using a volumetric infusion pump (Model 922, IMED Corporation, San Diego, CA). A small harness was attached to the back of each animal and the infusion line was passed through a coiled metal spring mounted on a swivel (Rodent Single Channel Swivel, Alice King Chatham Medical Arts, Los Angeles, CA) to allow the animal to move freely in its cage. Six animals (control group) with jugular venous catheter *in situ* were allowed free access to a standard laboratory rat chow and water for 7 days. Livers from both groups of animals were isolated on the eighth day after jugular venous cannulation. Each liver was infused with the same lidocaine solution and at the same rate as the co-infusion study. Measurements of samples were made at the inlet for lidocaine, and at the outlet for lidocaine and its metabolites, and liver weights were obtained as described in the co-infusion study.

The contents of PN solution were similar to those reported by Popp and Wagner (*1984*), and Knodell *et al.* (*1984*). Each liter of PN solution contained 242 g dextrose, 52 g amino acids (10% Travasol with electrolytes, Baxter-Travenol

Lab., Malton, Canada), 2 ml of the multivitamin preparation (MVI 1,000, Aqueous Multivitamin IV infusion USV Canada Inc.) and 2.25 mmol of calcium gluconate (10% calcium gluconate injection, SQUIBB Canada Inc., Montreal, Canada). The same PN solution was used in *in vivo* and *in vitro* (liver perfusion) studies.

Blood was collected from the portal veins of the 12 animals immediately prior to catheterizing the liver, and the serum was separated immediately after blood clotting. Serum amino acids concentrations were measured with a fluorescence amino acid analysis system which utilized o-phthaldialdehyde as a precolumn derivatizing agent and ethanolamine as internal standard (*Jones and Gilligan 1983*). The quantification of individual amino acid derivatives was reproducible within an average deviation of ±1.5% (*Sedgwick 1987*).

The animal experimentation met the guidelines of the Canadian Council on Animal Care and was approved by the Health Sciences Animal Welfare Committee of the University of Alberta.

6.2.2 HPLC Assay

6.2.2.1 Chemicals and reagents

Samples of lidocaine, N-(N-ethylglycyl)-2,6-xylidine (MEGX), N-glycyl-2,6-xylidine (GX), 3-hydroxy-lidocaine (3-OH-LIDO) and 3-hydroxy-N-(N-ethylglycyl)-2,6-xylidine (3-OH-MEGX) were gifts from Astra pharmaceuticals (Mississauga, Canada). N-(N,N-diethylglycyl)-2-hydroxymethyl- 6-methylaniline (MeOH-LIDO) and N-(N-ethylglycyl)-2-hydroxymethyl-6-methylaniline (MeOH-MEGX)

were synthesized in our laboratory and their chemical structures and purity were confirmed by NMR, elemental analysis and GC/MS (*Coutts et al. 1987*). All solvents and reagents were from commercial sources.

6.2.2.2 Sample preparation

Lidocaine and its six metabolites (identified immediately above) in the rat liver perfusate samples were simultaneously separated and quantified using a modification of our previously reported procedure (*Tam et al. 1987a*). Briefly, 100μl of a 20 μg/ml solution of N-ethyl-N-methyl-glycine-2,6-xylidide in water was added as internal standard (I.S.) to 1 ml aliquot of the rat liver perfusate samples. The solution was adjusted to pH 8.5 with excess potassium bicarbonate (~1 g) and 8 ml of methylene chloride was added. The mixture was shaken with a vortex mixer (IKA-VIBRAX-VXR, JANKE and KUNKEL, setting at 1,200) for 10 minutes and centrifuged at 1,000 g for 10 minutes. Organic phase (6 ml) was removed and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 300 μl of 0.005 M HCl. Between 50 to 200 μl of the sample was injected onto the HPLC column.

6.2.2.3 Standard curves

Standard solutions were prepared by spiking 1 ml volumes of the Kreb's bicarbonate buffer with various concentrations (0.01-5 μg/ml) of lidocaine and its six metabolites. The calibration curves were constructed by plotting the peak area ratio of lidocaine and each of its metabolites to that of the internal standard versus the respective concentration of the drug and its metabolites.

The limit of sensitivity of this assay was 0.05 μg/ml for lidocaine and 0.03 μg/ml for its six metabolites when a 1-ml aliquot of perfusate sample was used. With one exception, the extraction recovery of lidocaine and its metabolites, in the concentration range studied (0.02-5 μg/ml), was in excess 90% from 1 ml aliquots of perfusate samples. Under these conditions GX recovery was about 50%. Between-day variation in analyses of standard samples containing lidocaine and its metabolites (concentration ranged from 0.03 to 3 μg/ml) were <10%. No significant loss of lidocaine or its metabolites was observed after 1 month of storage at -20°C.

6.2.2.4 Apparatus

The HPLC system (Waters Associates, Mississauga, Ont, Canada) consisted of a M-45 pump, a model 441 UV detector (set at 214 nm), an automatic sampler (WISP, Model 721) and a data processing station (Model 840). Separation of lidocaine and its six metabolites was achieved using a 5 μm C_{18} 11.5 cm × 8 mm I.D. reversed-phase Nova-Pak Cartridge (Fig. 6.1). The aqueous mobile phase contained 10% (v/v) acetonitrile, 0.05% (v/v) phosphoric acid and 0.07% (v/v) triethylamine, and was pumped at a flow rate of 2 ml/min.

6.2.3 Pharmacokinetic Analyses

The time to reach steady state (t_{ss}) was statistically determined according to Saville *et al.*, (*1989a*). The steady state concentrations for lidocaine and its metabolites were calculated by averaging the concentration data from t_{ss} to time 80 min.

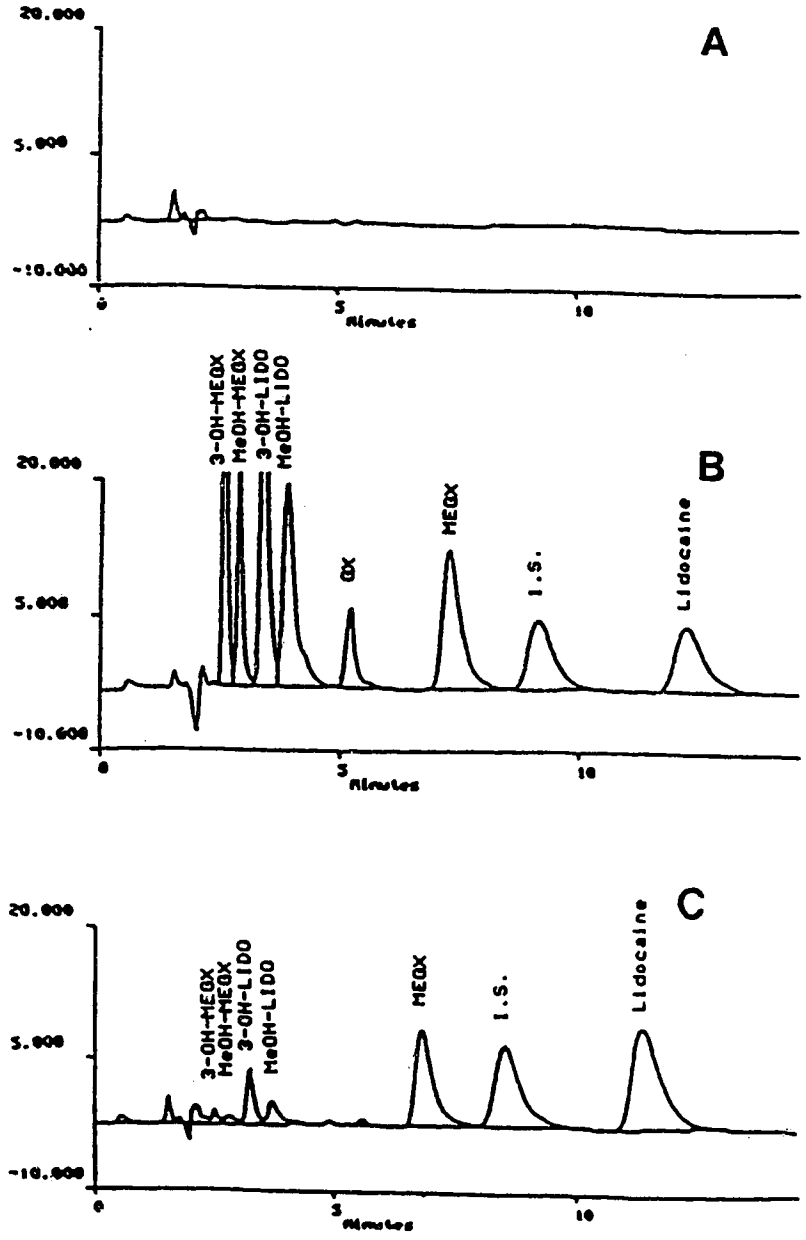

Figure 6.1 Chromatograms obtained from blank perfusate sample (A), perfusate spiked with lidocaine and its six metabolites (B) and a perfusate effluent sample (C) collected at 5 minutes after commencement of continuous portal venous lidocaine infusion (C_{in} = 3.5 μ g/ml). The concentration of each spiked component was 1 μ g/ml. The signals were measured in mV.

The efficiency of the liver in removing lidocaine at steady state was expressed by the following equations (*Gibaldi and Perrier, 1982*):

$$V_{ss} = Q \times (C_{in} - C_{out}) \quad (1)$$

$$E = (C_{in} - C_{out})/C_{in} \quad (2)$$

$$CL_H = Q \times E \quad (3)$$

where, V_{ss} is velocity of drug elimination across the liver at steady state, Q is buffer perfusion rate, C_{in} is inlet drug concentration, C_{out} is effluent drug concentration, E is drug extraction ratio and CL_H is hepatic drug clearance.

6.2.4 Statistical Method

All data are reported as mean ± SD. The two-tailed Student t-test was used, and the level of significance was set at p=0.05.

6.3 Results

6.3.1 Hepatic Lidocaine Elimination with Co-Infusion of PN

There were no significant differences in the concentration versus time profiles for lidocaine and its metabolites between lidocaine-PN or lidocaine-saline co-infusion studies (Fig. 6.2). There were no significant differences between groups in the calculated pharmacokinetic parameters (Table 6.1); or in the total recoveries of lidocaine and its metabolites at the steady state (Table 6.2).

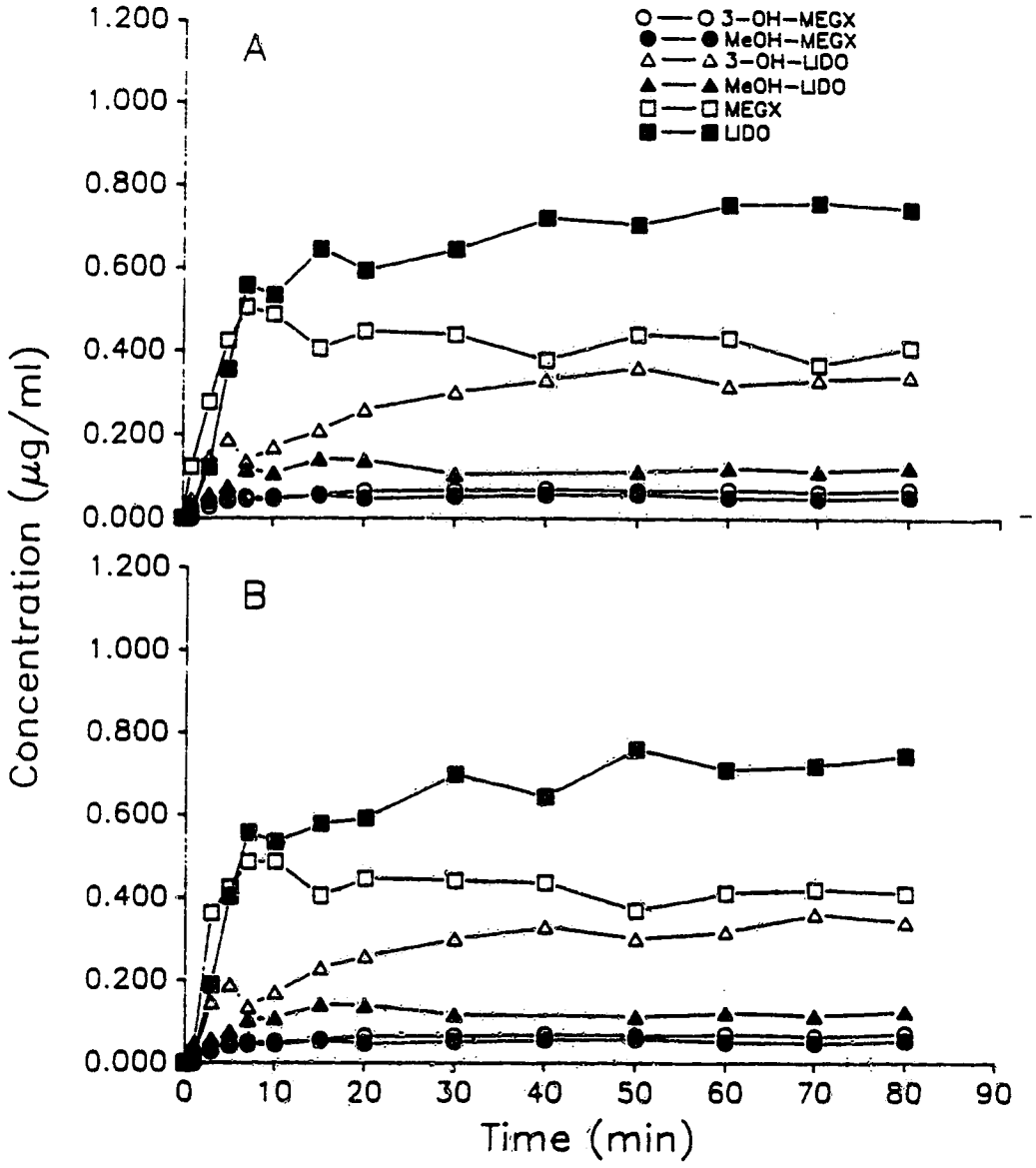

Figure 6.2 Representative concentration vs. time profiles for lidocaine and its metabolites in the perfusion effluent of the lidocaine-PN (A) and lidocaine-saline (B) co-infusion studies.

Table 6.1 Kinetic parameters for lidocaine from perfused rat liver in the co-infusion and the post PN studies.

Kinetic Parameters	Co-infusion		Post PN	
	Lidocaine -PN	Lidocaine -Saline	PN infusion	Sham Operated chow-fed
Q (ml/min)	27.68±1.48	27.49±1.90	26.68±0.17	26.82±0.42
C_{in} (μg/ml)	3.57±0.34	3.48±0.32	3.34±0.15	3.36±0.17
C_{out} (μg/ml)	0.89±0.09	0.84±0.08	1.57±0.17*	0.86±0.10
V_{ss} (μg/min)	73.72±5.22	71.65±3.64	47.63±6.65*	68.09±4.46
E	0.75±0.05	0.76±0.05	0.53±0.07*	0.77±0.06
CL_H (ml/min)	20.74±1.29	20.99±1.34	14.29±1.96*	20.35±2.03

Values = mean ± SD of six isolated liver perfusions in each study group.
* Significantly different from chow-fed group in the post PN study at $p < 0.05$.

6.3.2 Hepatic Lidocaine Elimination After 7 Days of PN

There were no significant differences in the rate of weight gain (5.71 ± 0.87 g/day (PN) vs. 5.18 ± 1.00 g/day (chow-fed)), absolute liver weight (8.16 ± 0.61 g (PN) vs. 8.25 ± 1.30 g (chow-fed)) or the liver weight as percent of body weight (3.46 ± 0.27 % (PN) vs. 3.31 ± 0.33 % (chow-fed)) between animals receiving PN or chow-fed during the 7-day study period.

Portal vein amino acid levels after 7-days of PN or chow-fed are shown in Table 6.3. Serum concentration of citrulline was significantly decreased, whereas phenyalanine, serine, histidine and glycine were significantly increased in the animals that received 7-days of PN when compared to the chow-fed animals.

Table 6.2 Recoveries of lidocaine and its metabolites in effluent perfusate samples at steady state in the co-infusion and the post PN studies.

Compound	Co-infusion (%dose)		Post PN (%dose)	
	Lidocaine -PN	Lidocaine -Saline	PN infusion	Sham Operated chow-fed
Lidocaine	24.92±5.39	23.52±4.83	46.58±7.28*	25.56±5.59
MEGX	12.64±1.59	12.80±2.39	12.13±4.15	13.38±3.28
3-OH-LIDO	7.81±1.37	8.76±1.53	7.43±2.01	9.11±1.90
3-OH-MEGX	1.51±0.43	1.89±0.35	1.28±0.58	1.70±0.31
MeOH-LIDO	2.85±0.68	2.94±0.67	0.21±0.01*	2.75±0.58
MeOH-MEGX	0.41±0.09	0.44±0.07	0.42±0.10	0.43±0.09
GX	-±-	-±-	-±-	-±-
TOTAL	50.41±6.34	51.34±7.32	68.44±5.65*	52.38±5.43

Values = mean ± SD of six isolated liver perfusions in each study group.
* Significantly different from chow-fed group in the post PN study at $p < 0.05$.

Lidocaine concentrations in the effluent (Fig. 6.3) were markedly elevated at the steady state in the post PN group when compared to chow-fed animals (1.57 ± 0.17 μg/ml vs. 0.86 ± 0.10 μg/ml, $p < 0.05$) (Table 6.1). The calculated pharmacokinetic parameters showed that V_{dss}, E and CL_H were lower in the post PN group, even though buffer perfusion rate (Q) and C_{in} were similar between groups (Table 6.1). Recovery at steady state was higher for lidocaine and lower for MeOH-LIDO in the PN group, whereas the recoveries for MEGX, 3-OH-LIDO, 3-OH-MEGX and MeOH-MEGX were similar between groups (Table 6.2). GX

Table 6.3 Portal vein amino acid concentrations in rats after 7-days of parenteral nutrition and in Chow-fed rats.

Compound	Parenteral Nutrition	Chow-fed
Aspartic acid	87.39±35.95	96.28±25.21
Glutamic Acid	284.56±95.87	290.05±69.69
Asparagine	53.07±9.11	69.04±13.00
Serine	517.14±134.65*	331.54±67.55
Glutamine	431.78±90.06	454.15±97.67
Histidine	103.90±28.08*	51.05±24.83
Glycine	1026.18±413.34*	417.65±80.04
Threonine	414.23±145.04	347.92±39.73
Citrulline	43.07±9.24*	74.42±11.44
Arginine	172.77±116.94	119.52±70.73
Taurine	607.68±105.80	412.06±120.99
Alanine	850.58±175.82	744.76±181.25
Tyrosine	96.45±7.15	99.60±8.73
Tryptophan	67.69±24.86	65.21±23.50
Methionine	86.39±38.87	51.93±10.85
Valine	214.40±33.58	184.76±24.74
Phenyalanine	100.24±23.06*	67.18±14.64
Isoleucine	145.51±27.01	138.85±18.73
Leucine	171.51±18.85	167.21±26.28
Lysine	763.84±89.83	785.84±160.77

Value = Mean ± SD in μmol/L for six animals in each group

* Significantly different between groups, $p<0.05$. Two tailed Student-t test.

concentration was too low in the effluent to be accurately quantitated.

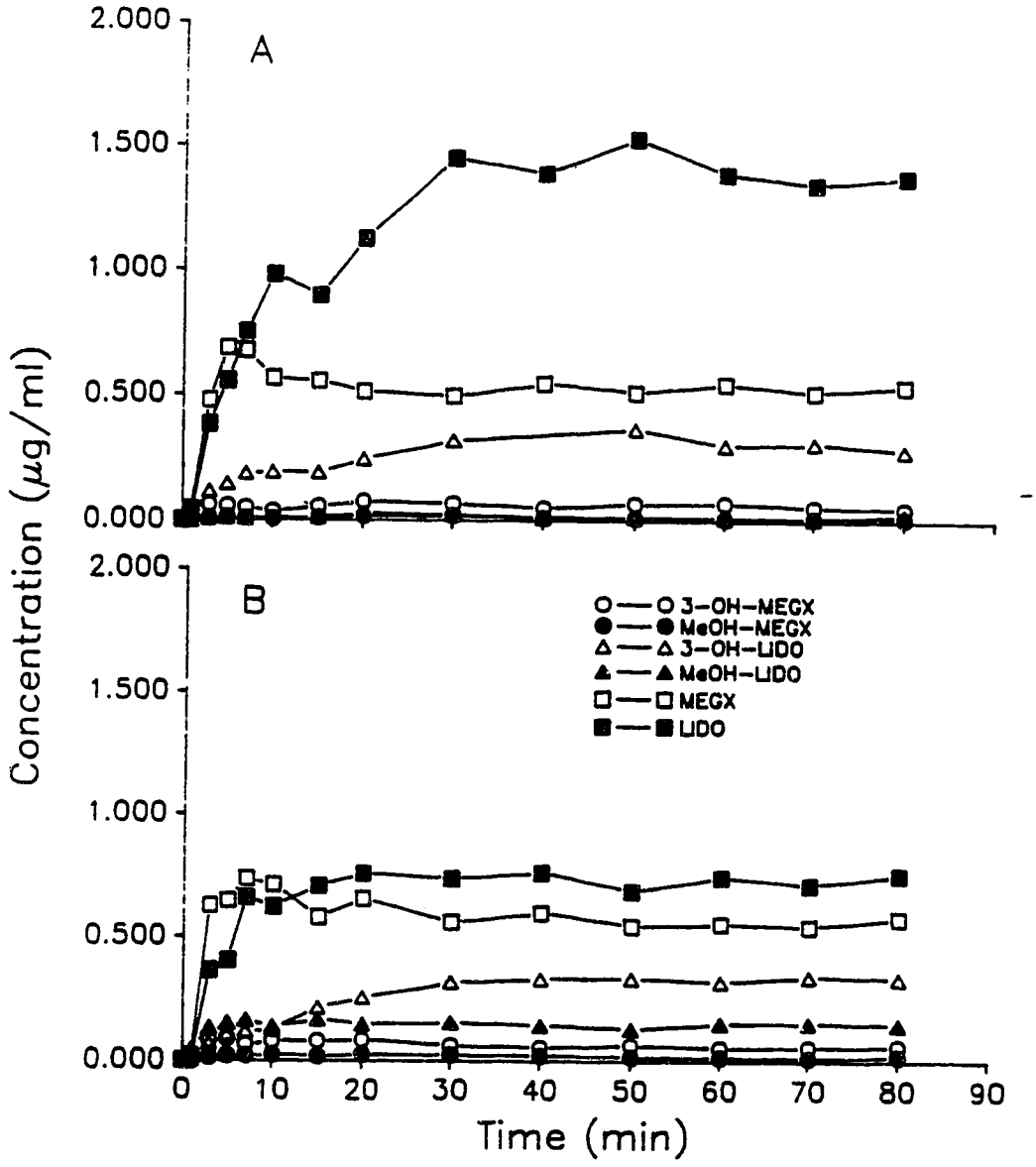

Figure 8.3 Representative concentration vs. time profiles for lidocaine and its metabolites in the perfusion effluent of isolated livers from rats given 7-days of PN (A) or chow-fed (B) animals.

6.4 Discussion

PN may compromise hepatic function (*Klein and Nealon, 1988*) and may affect hepatic drug elimination (*Knodell et al. 1980 and 1984, Anderson, 1988*). However, the mechanisms by which PN affects hepatic drug elimination are not clearly defined. Our experiments using the isolated liver perfusion technique, an acceptable model for the determination of hepatic lidocaine elimination (*Pang and Rowland, 1977 and Tam et al. 1987a*), were designed specifically to determine the competitive inhibition of lidocaine metabolism by parenteral nutrients and the effect on hepatic lidocaine elimination after a period of PN infusion *in vivo*.

Lidocaine was chosen as a model drug for this study because its use may reflect a number of potential mechanisms by which PN can induce alterations in drug kinetics. It is suitable for use in the "one-pass" isolated perfused rat liver model because it is highly extracted (*Pang and Rowland, 1977*) and almost completely metabolized by the liver (*Keenaghan and Boyes 1972, Tam et al. 1987b*). In the rat, the major primary metabolic pathway for lidocaine is via aromatic ring *m*-hydroxylation which is catalyzed by a hepatic cytochrome P_{450} enzyme system. Our experimental model is suitable for the study of potential competition for the P_{450} enzyme system because certain aromatic amino acids in PN have similar chemical structures to lidocaine. Other primary metabolic pathways for lidocaine that occur in the liver are N-deethylation and aryl methyl hydroxylation. Primary metabolites of lidocaine are further metabolized by N-deethylation, ring hydroxylation and/or amide hydrolysis (*Tam et al. 1987b*) or by conjugation with sulfate

or glucuronic acid. Our modified HPLC assay eliminated potential interferences from amino acids in PN solution and allowed simultaneous separation and quantification lidocaine and six of its metabolites: 3 primary metabolites (3-OH-LIDO, MEGX and MeOH-LIDO) and 3 secondary metabolites (3-OH-MEGX, GX and MeOH-MEGX) within 20 minutes. Thus monitoring of the levels of primary and secondary metabolites of lidocaine would further allow the determination of the effects of PN on lidocaine metabolism by various pathways in the liver.

In the co-infusion study, there were no significant differences between the PN and saline groups in the calculated hepatic kinetic parameters: V_{dss}, E and CL_H, for lidocaine elimination, or in the dose recovery for lidocaine at the steady state. These data are consistent with the findings in our human study (*Ke et al. 1990*). In addition, the concentration of parenteral nutrients co-infused with lidocaine into the portal vein was at least three times the concentration of nutrients presented to the liver from our *in vivo* jugular venous infusion. This observation supports the thesis that parenteral nutrients are unlikely to compete directly with lidocaine for hepatic enzymatic sites. Our previous observation (*Tam et al. 1984*) of a competitive inhibition of lidocaine metabolism from infused amino acids in isolated perfused rat liver may be the result of a higher rate of amino acids infusion (100 mg/min in the earlier study vs. 6.4 mg/min in the current study).

In the post PN studies, the C_{out} of lidocaine at the steady state was increased by 82%, and both hepatic clearance and hepatic extraction ratio for lidocaine were reduced by about 30% when compared to the chow-fed animals. Several factors

may be important in the reduction in hepatic lidocaine clearance after 7 days of PN administration. The similarities in the calculated kinetic parameters for and dose recovery of lidocaine at steady states from both the sham-operated group and saline co-infusion group completely ruled out the possible influence from surgical and anesthetic procedures. It may be argued that the PN regimen used in this study was not appropriate to maintain normal growth of the animals, and malnutrition may have lowered hepatic enzyme functions (*Campbell and Hayes, 1976*). However, there was no significant difference in the nutritional status between PN or chow-fed groups in the present study, at least with respect to the rate of body weight gain, absolute liver weight or liver weight as percent of body weight.

Specific nutrients in PN, probably amino acids, may be the principal cause of the reduction in hepatic lidocaine elimination. In humans, antipyrine metabolism was not altered with an increased calorie intake by increasing dextrose concentration from 13% to 25% in the PN solution (*Pantuck et al. 1985*). However, antipyrine metabolism was increased by 24% in healthy adults receiving intravenous infusion of amino acids for only one day after a 4-day isocaloric infusion of dextrose (*Pantuck et al. 1984*). Direct infusion of amino acid solution into the portal vein of rats over 3 days resulted in significantly decreased pentobarbital and mepridine metabolism in hepatic microsome studies (*Knodell et al. 1984*). A high plasma level of glycine is thought to be important in the development of hepatic dysfunction in *in vitro* guinea pig liver explants studies (*Johnson et al. 1975*). In this study, serum levels of several amino acids including glycine were

significantly elevated in rats after 7-days of PN. Therefore, it is plausible that a change in hepatic drug metabolizing functions occurred in our post PN rats.

Unlike our previous observations of *in vivo* metabolism of lidocaine in rats (*Coutts et al. 1987*), 3- and 4-hydroxy-2,6-xylidine were not detected in perfusate samples prior to or after acid hydrolysis. In the present study, conjugates of ring hydroxylated and aryl methyl hydroxylated, and N-deethylated metabolites of lidocaine also were not found in the effluent. GX concentration was below the quantification limitation. The lack of mass balance at steady state is consistent with earlier observations (*Pang et al. 1986, Tam et al. 1987a*) that at low C_{in} (<12 μ g/ml), up to 50% of the infused lidocaine is unaccounted for at steady state. Another explanation of the relatively low material balance noted in this study is that hepatic metabolism of lidocaine involves as yet unidentified metabolic pathway(s). The latter hypothesis is supported by the finding that more than 95% of infused labeled lidocaine was recovered in the effluent of isolated perfused rat liver (*Saville, 1989b*).

In the post PN rats, the material balance at steady state was significantly higher than the chow-fed animals. This increase was mainly attributed to the increase in the recovery of lidocaine in the effluent, suggesting unknown metabolic pathways were inhibited. Similarly, aryl methyl hydroxylation was grossly inhibited as indicated by the dramatic reduction of MeOH-LIDO recovery in the post PN group. Interestingly, the recoveries of the major primary metabolites of lidocaine, such as MEGX and 3-OH-LIDO, were unchanged between the experimental

and the control groups. These observations suggest that the aryl methyl hydroxylation and other unidentified metabolic pathway(s) of lidocaine were selectively inhibited by parenteral nutrients.

It is interesting that the levels of the ring hydroxylated and N-deethylated metabolites of lidocaine are not increased, when other metabolic pathways are selectively inhibited. Since MEGX and 3-OH-LIDO are further metabolized via the same pathways, namely, ring hydroxylation and N-deethylation, respectively, it is postulated that sequential inhibition may be involved in such a way that the levels of primary metabolites are maintained. However, this hypothesis is unlikely to be a real mechanism because the "one-pass" rat liver perfusion model favors primary metabolic pathways. Using the "enzyme-distribution" hypothesis (*Pang et al. 1986*), one may be able to explain why the levels of N-deethylated and ring hydroxylated metabolites of lidocaine remained constant. If lidocaine was exposed to the N-deethylating and ring hydroxylating enzymes prior to the selectively inhibited ones, and if the former enzymes were not inhibited, one would expect that the levels of 3-OH-LIDO, 3-OH-MEGX and MEGX would remain constant. More studies are required to clarify this hypothesis.

We conclude that in the isolated rat liver perfusion model, there is no direct competition for lidocaine metabolism during acute infusion of PN. However reduction of hepatic lidocaine elimination can occur after 7 days of PN. We suggest that the reduction in hepatic lidocaine elimination occurs as result of selective inhibition of hepatic enzyme activities such as those involved in the aryl methyl hydroxylation and unidentified pathway(s).

6.5 References

Anderson KE, "Influences of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet*, 14, pp.325-346 (1988).

Bloxam DL., "Condition and performance of the rat liver perfused with Krebs-Ringer solution, with particular reference to animo acid metabolism", In: Bartosek I, Guaitani A and Miller LL., ed. **Isolated liver perfusion and its applications**, pp.147-153 (New York: Raven Press,) (1973).

Burgess P, Hall RI, Bateman DN, Johnston IDA, "The effect of total parenteral nutrition on hepatic drug oxidation", *JPEN*, 11, pp.540-543 (1987).

Campbell TC, Hayes JR., "The effect of quantity and quality of dietary protein on drug metabolism", *Fed Proc*, 35, pp.2470-2472 (1976).

Coutts RT, Torok-Both GA, Chu LV, Tam YK, Pasutto FM., "In vivo metabolism of lidocaine in the rat. Isolation of urinary metabolites as pentofluorobenzoyl derivatives and their identification by combined gas chromatography-mass spectrometry", *J Chromatogr Biomed Appl*, 421, pp.267-280 (1987).

Gibaldi M, Perrier D., "Clearance concepts", In: Gibaldi M, Perrier D., ed. **Pharmacokinetics, 2nd**, (New York: Marcel Dekker, Inc.,) pp.319-351 (1982).

Gutcher G, Cutz E., "Complication of parenteral nutrition", *Semin Perinatol*, 10, pp.196-207 (1986).

Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA., "Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats", *Gastroenterology*, 68, pp.1177-1183 (1975).

Jones BN, Gilligan JP., "O-phthaldialdehyde precolum derivatization and reversed-phase high-performance liquid chromatography of polypepitide hydrosates and physiological fluids", *J Chromatogr*, 266, pp.471-482 (1983).

Ke J, Tam YK, Semple HA, Coutts RT, Gray MR., "Effects of parenteral nutrition on lidocaine pharmacokinetics", *J Pharm Sci*, 76, pp.S77 (1987).

Ke J, Tam YK, Koo W, Coutts RT, Finegan BA., "Lack of acute effect on lidocaine pharmacokinetics from parenteral nutrition", *Ther Drug Monit*, 12, pp.157-162 (1990).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Klein S, Nealon WH., "Hepatobiliary abnormalities associated with total parenteral nutrition", *Semin Liver Dis*, 8, pp.237-246 (1988).

Knodell RG, Spector MH, Brooks DA, Keller FX, Kyner WT., "Alterations in pentobarbital pharmacokinetics in response to parenteral and enteral alimentation in the rat", *Gastroenterology*, 79, pp.1211-1216 (1980).

Knodell RG, Steele NM, Cerra FB, Gross JB, Solomon TE., "Effects of parenteral and enteral hyperalimentation on hepatic drug metabolism in the rat", *J Pharmacol Exper Ther*, 229, pp.589-597 (1984).

Merritt RJ., "Cholestasis associated with total parenteral nutrition", *J Pediatr Gastrointerol Nutr*, 5, pp.9-22 (1986).

Pang KS, Rowland M., "Hepatic clearance of drug. II. Experimental evidence for acceptance of the "well-stirred" model over the "parallel-tube" model using lidocaine in the perfused rat liver in situ preparation", *J Pharmacokinet Biopharm*, 5, pp.655-699 (1977).

Pang KS, Terrell JA, Nelson SD, Feuer KF, Clements MJ, Endrenyi L., "An enzyme-distributed system for lidocaine metabolism in the perfused rat liver preparation.", *J Pharmacokinet Biopharm*, 14, pp.107-130 (1986).

Pantuck EJ, Pantuck CB, Weissman C, Askanazi J, Conney AH., "Effects of parenteral nutritional regimens on oxidative drug metabolism", *Anesthesiology*, 60, pp.534-536 (1984).

Pantuck EJ, Pantuck CB, Weissman C, Gil KM, Askanzi J., "Stimulation of oxidative drug metabolism by parenteral refeeding of nutritionally depleted patients", *Gastroenterology*, 89, pp.241-245 (1985).

Popp MB, Wagner SC., "Nearly identical oral and intravenous nutritional support in the rat: effects on growth and body composition", *Am J Clin Nutr*, 40, pp.107-115 (1984).

Ross BD., "General principles of organ perfusion", In: Ross BD., ed. **Perfusion techniques in biochemistry**, pp.14-92 (Clarendon Press, Oxford) (1972).

Saville BD, Gray MR, Tam YK., "Evidence for lidocaine-induced enzyme inactivation", *J Pharm Sci*, 78, pp.1003-1008 (1989a).

Saville BD., "The hepatic transport and metabolism of lidocaine", **Ph.D. Thesis, University**

of Alberta, pp.89-100 (1989b).

Sedgwick GW., "Analysis of amino acid by high performance liquid chromatography", (Department of Animal Science, University of Alberta, Edmonton, Canada) (1987).

Stein TP, Buzby GP, Gertner MH., "Effect of parenteral nutrition on protein synthesis and fat metabolism in man", *Am J Physiol*, 239, pp.G280 (1980).

Tam YK, Sim HA, Yau ML, Tawfik S., "The effects of Travasol on lidocaine metabolism.", *IUHAR 9th International Congress of Pharmacology, London, England*, 242, (1984).

Tam YK, Yau M, Berzins R, Montgomery PR, Gray M., "Mechanisms of lidocaine kinetics in the isolated perfused rat liver. I. Effects of continuous infusion", *Drug Metab Dispos*, 15, pp.12-16 (1987a).

Tam YK, Tawfik SR, Ke J, Coutts RT, Wyse GD, Gray MR., "HPLC of lidocaine and nine of its metabolites in human plasma and urine", *J Chromatogr Biomed Appl*, 423, pp.199-206 (1987b).

CHAPTER 7

EFFECTS OF PN AND NUTRITIONAL STATUS ON LIDOCAINE PHARMACOKINETICS: A PILOT STUDY IN PIGS[6]

7.1 Introduction

Numerous studies have shown that diet composition and nutritional status can substantially alter drug disposition in animals and humans (*Campbell and Hayes, 1974, Bidlack et al. 1986 and Anderson 1988*). Altered enzyme activities with changes in nutritional status can influence pharmacokinetics. In humans, the clearance of antipyrine has been used as an index for assessment of the microsomal oxidative capacity of the liver. Studies have shown that a reduction in the metabolism of antipyrine in protein-calorie malnourished children can be reversed within a month of starting a high-protein, high-calorie diet (*Homeida et al. 1979, Narang et al. 1977*). These observations suggest that drug metabolism is dependent upon the nutritional status of a subject.

Parenteral nutrition (PN) may significantly influence drug disposition. For example, antipyrine clearance was decreased by 34% in post-operative patients receiving an amino acid-dextrose solution (*Burgess et al. 1987*); was increased by 24% in healthy adults receiving intravenous infusion of amino acid for only one day after 4 days isocaloric infusion of dextrose (*Pantuck et al. 1984*); and increased by 87% in previously malnourished subjects undergoing nutritional repletion with

[6] Part of the results of this chapter has been published in *J Pharm Sci* 76: S77, (1987)

PN therapy (*Pantuck et al. 1985*). The major difference among these studies is nutritional status of the subjects and these findings imply that the nutritional status and underlying disease state of the subjects are important confounders for PN and drug interaction.

This pilot study was designed specifically to evaluate whether the pig is a good model to examine the effect of PN and different nutritional states on pharmacokinetics of a model compound, lidocaine. We aim to test the hypothesis that the effects of PN on drug disposition could be different in different nutritional states.

7.2 Methods

7.2.1 *in vivo* Pig Model

One male Yucatan miniature pig and two male commercial piglets were used in this pilot study. The Yucatan miniature pig is a good *in vivo* model for studying potential interaction between drug and PN, but its use is more costly compared with the use of commercial piglets. According to the preliminary study in our laboratory, the pharmacokinetics of lidocaine in commercial piglets were similar to those in the Yucatan miniature pig. For this reason, commercial piglets were also used.

Two catheters were surgically implanted into the inferior vena cava. The downstream catheter was used for PN and lidocaine administration. The upstream catheter was used for blood sampling. After surgery, the pigs were given 7-10 days

for recovery. Heparin locks ($\sim$ 0.5 ml, 1,000 units/ml) were used to maintain catheter patency. During experiments, heparin (20 units/ml) in saline was used in between sampling to fill the catheters, as it has been found that this concentration did not interfere with lidocaine serum protein binding.

7.2.2 Experimental Design and Sample Collection

Two protocols were designed in this pilot study. The objective of the first protocol was to delineate the effects of PN and nutritional status on lidocaine pharmacokinetics. Four different nutritional states were tested in sequence, namely normal enterally fed (control), after a 48 hour fast (fast), then initiation of PN infusion (fast+PN) and after a 7-day PN infusion (PN). The pig received a single intravenous infusion of lidocaine HCl (50 μg/kg.min) for 4 hours using a Volumetric infusion pump (model 922, IMED corporation, San Diego, CA) on the morning ($\sim$ 9:00) of day 3 of fast, day 1 and day 8 of commencement of PN administration. PN infusion was continued for 8 days. In the enteral control study, lidocaine HCl was infused after an overnight fast and food was not allowed until 4 hours after the start of lidocaine infusion. Blood samples (3 ml) were taken at appropriate time intervals for 8 hours after drug administration. Blood samples were collected into glass tubes with Teflon lined caps. The serum was separated immediately upon clotting of the blood and was stored at $-20°C$ until analysis. Only one set of data was successfully collected from a commercial piglet (pig # 4) following this protocol.

The second protocol was designed to compare lidocaine pharmacokinetics in pigs after short-term (7 days) PN infusion with enterally fed controls. A crossover

experimental design was adopted to evaluate potential effects of lidocaine-induced enzyme inactivation (*Saville et al. 1989*). When the animal was on PN study first, the pig received regular food for a week prior to the next study. Due to the 72 hours urine collection was scheduled after lidocaine HCl administration, the crossover studies (post PN and control) were performed 10 days apart. The infusion of PN and lidocaine HCl (50-75 μg/kg.min) administration were conducted in exactly the same way as in the first protocol. Blood samples were collected as described in the first protocol. One commercial piglet (# 5) and one Yucatan miniature pig (# 12) were studied using this protocol. Urine samples were also collected from pig #12 before and at 12 hour intervals after drug administration for 72 hours. The volume and pH of urine were measured and an aliquot of 20 ml was stored at $-20°C$ until analysis.

The control animals received a 1.3 kg/day of pig finisher ration (Supplied by the University of Alberta Feed Mill) which provided 182 g protein and 3,100 Kcal/day of digestible energy. The animals on PN infusion received 40 ml/kg.day of PN solution. The PN solution consisted of 25% dextrose and 3.33% Travasol with electrolytes and vitamins (Baxter-Travenol Lab., Malton, Canada).

7.2.3 Sample Analysis

7.2.3.1 Measurement of lidocaine and its metabolites in serum and urine.

The concentration of lidocaine and its nine known and possible metabolites, including *p*-hydroxylated (4-OH-) derivatives of lidocaine (LIDO), N-(N-ethylglycyl)-2,6-xylidine (MEGX), N-glycyl-2,6-xylidine (GX) and 2,6-xylidine (2,

6-XYL); *m*-hydroxylated (3-OH-) LIDO and MEGX; MEGX; GX and 2,6-XYL, in the serum and urine were measured using a HPLC assay recently developed in our laboratory (*Tam et al. 1987*). The major procedures of the assay include acid hydrolysis of conjugates of lidocaine metabolites, aqueous acetylation of the metabolites under mild basic conditions (pH $\sim$ 8.5), then extraction of lidocaine and its derivatized metabolites under basic conditions with ethyl acetate. After extraction, the organic layer was removed and dried under a gentle stream of nitrogen. The residue was reconstituted with HPLC mobile phase and injected onto the HPLC system (Waters Associates, Milford, Mass.) which consisted of a M-45 pump, Model 441 UV detector (set at 214 nm), an automatic sample processor (WISP, Model 721) and data processing station (Model 840). Separation was achieved using a 5 μm C_{18} reversed-phase Nova-Pak cartridge (11.5 cm $\times$ 8 mm ID). The aqueous mobile phase consisted of acetonitrile (12 % v/v), phosphoric acid (0.1 % v/v) and triethylamine (0.15 % v/v), and was pumped at a flow rate of 2 ml/min.

7.2.3.2 Equilibrium dialysis

Lidocaine serum protein binding was measured using an equilibrium dialysis technique (*Routledge et al. 1980*). The pH of the stored serum samples was adjusted to pH 7.4 prior to the binding study. A 0.7 ml aliquot of a serum sample was added to a 1 ml Teflon equilibrium dialysis cell (Spectrum, Los Angeles, CA). Dialysis was performed against an equal volume of pH 7.4 isotonic phosphate buffer, containing 0.5% (w/v) sodium chloride and 200 ng/ml ^{14}C lidocaine HCl

with specific activity 52.0 mCi/mmol (New England Nuclear, Boston, MA). The buffer and serum compartments were separated by a dialysis membrane (Cellulose tubing, Sigma, St. Louis, MO, Molecular size cut off over 2,000) and the cells were rotated for 4 hours in a 37°C water bath. Preliminary studies demonstrated that it required 4 hours for lidocaine binding to reach equilibrium. The activity of labelled lidocaine in each side of the cell was measured by a liquid scintillation counting method (*Routledge et al. 1980*) using a 1217 PackBeta liquid scintillation counter (LKB WALLAC, Turka, Finland). The fraction of unbound lidocaine in serum (fu) was calculated as the ratio of absolute disintegration rates in buffer and serum.

7.2.4 Treatment of Data

Standard methods were used for calculating the non-compartmental kinetic parameters: the area under serum drug concentration versus time curve from time zero to infinity (AUC), total body clearance (CL_T), volume of distribution at steady state (V_{dss}) and mean residence time (MRT) after each intravenous dose (*Gibaldi and Perrier 1982*). Lidocaine pharmacokinetic data analyses were performed using a non-compartmental program called LAGRAN (*Rocci and Jusko, 1983*).

The paired two-tailed Student t-test was used to test for statistical differences in lidocaine pharmacokinetic parameters between the 7-day PN treatment and control studies. The level of significance was set at p=0.05.

7.3 Results

7.3.1 Protocol I

The time courses of serum lidocaine concentration in pig # 4 in four different nutritional states are shown in Fig. 7.1. The steady state level of lidocaine increased by approximately 50% when the animal was starved for 48 hours compared with control. The initiation of PN infusion on the following day did not significantly alter the steady state level of lidocaine compared with the fast study. However, when the animal received PN infusion for 7 days, the steady state level of lidocaine increased more than two-fold when compared with the control state (enteral feed). The calculated pharmacokinetic parameters CL_T, V_{dss} and MRT for lidocaine are shown in Tables 7.1. These results indicate that PN and nutritional states of subjects can significantly influence the disposition of lidocaine. Serum lidocaine protein binding was altered in different nutritional states and lidocaine protein binding was significantly decreased ($\sim$ 10%) after 7 days of PN infusion compared to control (Fig 7.2).

7.3.2 Protocol II

After 7 days of PN infusion, CL_T and V_{dss} were reduced by 54% and 53%, respectively, when compared to those of controls ($p< 0.05$), whereas MRT remained relatively constant (Table 7.2). Urine data in one pig (# 12) echoes the serum data (Table 7.3). In conjunction with the overall reduction in lidocaine clearance, the percentage of intact drug excreted into urine was increased after 7 days of PN

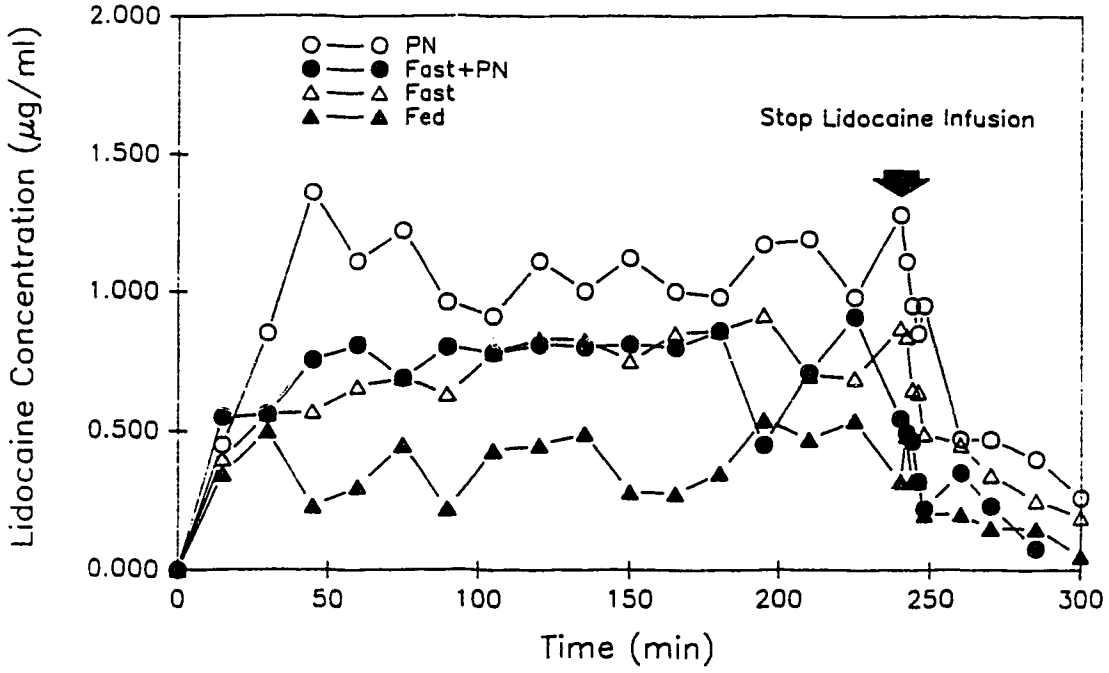

Figure 7.1 Serum lidocaine concentration-time curves during a 4 hour intravenous infusion of lidocaine HCl (50 μg/kg.min) in pig # 4 under different nutritional states.

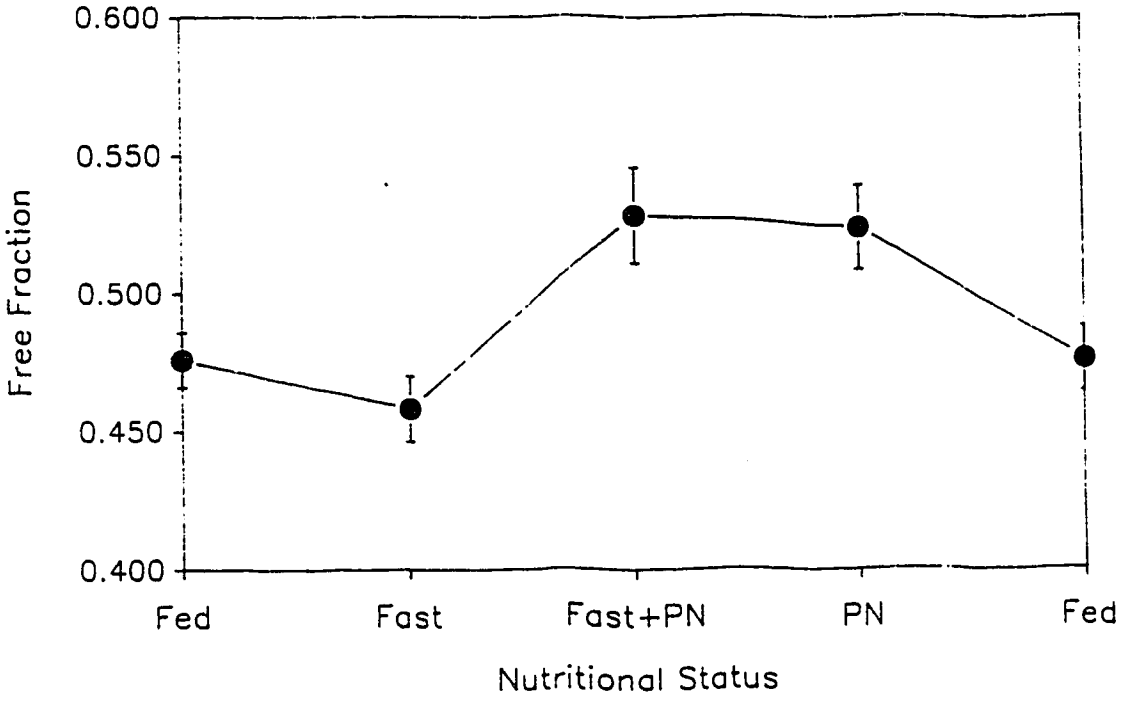

Figure 7.2 Free fraction of lidocaine in serum in pig # 4 under different nutritional states.

Table 7.1 Pharmacokinetic parameters of lidocaine during a 4 hour continuous intravenous infusion of lidocaine HCl (50 μg/kg·min) in pig #4 under different nutritional states

Nutrition	CL_T (L/min)	MRT (min)	V_{dss} (L)
Fed	3.90	150.0	591.2
Fast	1.91	160.0	383.0
Fast-TPN	2.02	140.0	177.8
TPN	1.32	150.0	207.3

infusion, whereas the urinary excretion of *p*-hydroxylated metabolites of lidocaine, 4-OH-GX and 4-OH-XYL, was decreased. In addition, the recovery in urine of the amide hydrolyzed metabolite of lidocaine (2,6-XYL), was also lower. However, the levels of MEGX and GX, N-deethylated metabolites of lidocaine, were higher in the post PN study than in the control. *m*-Hydroxylated metabolites of lidocaine, 3-OH-LIDO and 3-OH-MEGX were relatively constant between the two treatments. No carryover effect was observed. Thus, it is unlikely that potential lidocaine-induced enzyme inactivation has any significant effects on the present study.

Serum amino acid levels of pig # 12 after 7 days of PN or enteral feed are shown in Table 7.4. The levels of alanine and glycine in the serum were increased by 57% and by 79% after 7 days of PN treatment when compared to the enteral feed study.

Table 7.2 Pharmacokinetic parameters of lidocaine in enterally fed and in 7 day PN treated pigs that received a 4 hour intravenous infusion of lidocaine HCl (50-75μg/kg.min).

Pig #	4			5			12		
Body Weight (kg)	26			27			74		
Dose (μg/kg·min)	50			50			75		
	*CTL	PN	%Δ	CTL	PN	%Δ	CTL	PN	%Δ
CL_T (L/min)	3.9	1.3	67	3.5	1.7	51	4.7	2.6	44
V_{dss} (L)	591	207	65	475	280	41	731	340	54
MRT (min)	150	160	7	140	170	21	160	130	19

* CTL = control, PN = parenteral nutrition, %Δ = percent difference between the two conditions.

7.4 Discussion

PN may affect pharmacokinetics (*Anderson 1988, Knodell et al. 1980 and 1984*) and these effects of PN on drug disposition may be different with a change in nutritional status. However, the effects of PN on drug elimination in different nutritional states have yet to be investigated. This pilot study was designed to set up an animal model which allowed us to mimic several different nutritional states, and to delineate the effects of PN and nutritional states on lidocaine pharmacokinetics.

It is not surprising that the lidocaine clearance was reduced by 51% after a

Table 7.3 72 hour urinary recoveries of lidocaine and its metabolites after a 4 hour intravenous infusion of lidocaine HCl (75 μg/kg·min) in pig #12 that received enteral feeding and a 7 day PN regimen.

Compound	Control (%dose)	PN(%dose)
LIDO	23.8	33.7
MEGX	0.4	2.3
GX	1.2	3.1
2,6-XYL	6.6	2.2
4-OH-LIDO	0.2	0.2
4-OH-GX	3.8	2.1
4-OH-XYL	25.5	15.8
3-OH-LIDO	3.0	2.1
3-OH-MEGX	0.4	0.5
TOTAL	64.9	62.0

48 hour fast since fasting, similar to malnutrition, has been shown to lower hepatic enzyme activities (*Yang and Yoo, 1988*). The initiation of PN infusion on the next day did not significantly alter the lidocaine clearance compared to the fasted state. This observation is consistent with the findings in our healthy human experiment (*Ke et al. 1990*) and isolated rat liver co-infusion studies. Thus it is unlikely that nutrients in the PN solution directly compete with lidocaine for hepatic enzyme sites at the present PN infusion rate. However, lidocaine clearance was reduced by more than 50% after 7 days of PN infusion when compared to control, which was in agreement with the results from our post PN study in healthy rats. Therefore, the

Table 7.4 Serum amino acid concentrations in pig #12 after 7-days of PN and after enteral feeding.

Compound	Concentration (μmol/L)		
	PN	Enteral-fed	Change (%)
Methionine	20.10	9.85	↑ 104.1
Alanine	164.06	104.72	↑ 56.7
Lysine	134.00	68.50	↑ 95.6
Isoleucine	98.30	74.13	↑ 32.6
Tyrosine	41.50	65.20	↓ 36.3
Arginine	116.20	75.80	↑ 53.3
Glycine	356.90	[illegible]	↑ 78.5
Asparagine	9.50	15.00	↓ 36.7
Serine	34.00	[illegible]	↓ 28.1
Histidine	37.40	27.90	↑ 34.1
Threonine	60.30	45.30	↑ 33.1
Phenyalanine	52.77	48.68	↑ 8.4
Tryptophan	41.50	65.22	↓ 36.0

preliminary data from the pig combined with the results from our acute and short-term effects of PN studies in healthy humans and/or rats suggest that the extent of interaction between PN and lidocaine is probably independent of the nutritional status of subjects. The CL_T of lidocaine in the control experiments was more than twice the liver blood flow rate, which supports the literature observation that the lung, in addition to the liver, may play an important role in lidocaine elimination in the pig (*Bertler et al. 1978*).

The reduction in lidocaine clearance after 7-days of PN treatment could be caused by several factors. A reduction in hepatic blood flow during PN infusion may cause a decrease in clearance of lidocaine since the elimination of lidocaine is hepatic blood flow rate dependent (*Nation et al. 1977*). Contrarily, according to our preliminary results in anesthetized pigs, the electromagnetically measured hepatic blood flow was increased by approximately 15% when PN was infused. No such increase was found when normal saline was infused at the same rate. However, this small change in hepatic blood flow could not account for such a large change in lidocaine clearance. Moreover, there was no significant change in the clearance of indocyanine green (ICG), an indicator used to measure hepatic blood flow, in patients after 7 days of PN treatment (*Fabri et al. 1987*). These results suggest that alteration of hepatic blood flow during PN infusion could not be a reason for the decreased lidocaine clearance after 7 days of PN infusion.

In humans, prolonged infusion of PN may cause hepatic injury (*Bowyer et al. 1985*), the frequency of which is related to the duration of PN infusion (*Merritt 1986*). In pigs, one week of PN treatment did not result in hepatic abnormalities (*Buckley et al. 1985*). Our preliminary studies in pigs showed that the levels of alanine and aspartate aminotransferase were stable during a course of PN therapy. Furthermore, autopsy performed after our experiments, did not show any gross signs of liver pathology. Thus, all evidence pointed toward a reduction in hepatic lidocaine clearance without obvious liver damage.

Certain amino acids in PN solution may be important for altering drug elimination. For example, in humans, antyprine metabolism was not altered with

an increased calorie intake by increasing dextrose concentration from 13% to 25% in the PN solution. However, antipyrine metabolism was increased by 24% in healthy adults after one day of intravenous infusion of amino acids subsequent to 4 days isocaloric infusion of dextrose (*Pantuck et al. 1984*). Healthy rats had lower hepatic pentobarbital clearance after 7 days of PN compared to animals fed with standard lab chow, and this reduction was directly related to the amino acid concentration in PN solution (*Knodell et al. 1984*). High plasma levels of alanine and glycine are thought to be important in the change of hepatic function in *in vitro* guinea pig liver explants studies (*Johnson et al. 1975*). In the present study, significant elevations in serum levels of alanine (57 %) and glycine (79 %) were found in a pig receiving 7 days of PN infusion as compared to the enterally fed condition. Similar elevation in plasma levels of these two amino acids were also found in rats receiving PN solution for 7 days, which was thought to be partly responsible for the decreased hepatic pentobarbital clearance (*Knodell et al. 1984*). Therefore, PN-induced alteration in drug elimination is probably due to a secondary effect on hepatic function exerted by PN, presumably certain amino acids.

Consistent with a significant reduction in lidocaine clearance, more intact drug was excreted in the urine after 7-days of PN infusion compared to control, whereas the urinary excretion of *p*-hydroxylated metabolites of lidocaine, 4-OH-GX, 4-OH-XYL and amide hydrolyzed metabolite of lidocaine (2,6-XYL) were decreased in urine. These results support the contention that the two metabolic processes, *p*-hydroxylation and amide hydrolysis, were inhibited after 7 days of

PN. Based on the urinary recovery data of lidocaine and its metabolites from one pig, the increased levels of the deethylated metabolites, MEGX and GX, may be a result of reduction of *p*-hydroxylation processes. Meanwhile, PN is unlikely to significantly affect *m*-hydroxylation of lidocaine and MEGX, because the urinary excretion of 3-OH-LIDO and 3-OH-MEGX were not significantly different between PN treatment and controls. These observations are also consistent with the observations from our post PN study in the rat that reduction of lidocaine elimination after 7 days of PN occurs as result of selective inhibition of hepatic enzyme activities. More pig studies are needed to confirm these results.

Information relating to the effects of PN on the binding of drugs to serum proteins and tissues is limited, therefore the effects of PN on the distribution of drugs are not understood. Our data showed that the V_{dss} of lidocaine was reduced significantly after 7 days of PN infusion in pigs. The net result of simultaneous reduction of CL_T and V_{dss} of lidocaine is that the MRT stays relatively constant in different nutritional states (Table 7.1 and 7.2). This observation prompted us to measure the serum protein binding of lidocaine which may partially explain the reduction of V_{dss} during PN administration in pigs.

The preliminary results showed that upon a 48 hour fast, the fraction of unbound lidocaine (f_u) was reduced (Fig. 7.2). This could be due to the increase in the level of α-l-acid glycoprotein (AAG), which binds lidocaine significantly (*Routledge et al. 1980*). A recent study showed that AAG levels increased in malnourished subjects and subsequently propranolol binding was shown to increase

significantly (*Jagadeesan and Krishnaswamy 1985*). The binding of lidocaine decreased once PN was initiated and the serum protein binding was reduced by about 10% after 7 days of PN treatment compared to control.

These observations suggested that the constituents of PN, probably amino acids, compete with lidocaine for protein binding sites. The circulating metabolites of lidocaine were less likely to displace lidocaine from serum protein sites in a course of PN because the lidocaine metabolites were weakly bound to protein (*McNamara et al. 1981*) and their levels were reduced during PN administration. However, the reduction of serum protein binding could not explain why the V_{dss} of lidocaine decreased during PN. It is possible that PN also competes with lidocaine for binding sites at the tissue level. The net result was that the reduction in tissue binding is higher than the reduction in serum protein binding.

We conclude that PN and nutritional status can significantly influence pharmacokinetics and metabolism of lidocaine in pigs. However, the preliminary results from our pilot pig experiments suggest that the extent of PN and drug interaction is independent of the subject's nutritional status. We have also found that the serum protein binding of lidocaine was different in different nutritional states and the serum protein binding of lidocaine was reduced by about 10% after a week of PN treatment compared with the control. We suggest that the reduction in lidocaine clearance after 7 days of PN infusion occurs as a result of selective inhibition of hepatic enzyme activities such as those involved in *p*-hydroxylation and amide hydrolysis pathways.

7.5 References

Anderson KE., "Influence of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet*, 14, pp.325-346 (1988).

Bertler A, Lewis DH, Lofstrom JB, Post C., "*In vivo* Lung uptake of lidocaine in pigs", *Acta Anaes Thesiol Scand*, 22, pp.530-536 (1978).

Bidlack WR, Brown RC, Mohan C., "Nutritional parameters that alter hepatic drug metabolism, conjugation, and toxicity.", *Fed Proc*, 45, pp.142-148 (1986).

Bowyer BA, Fleming CR, Ludwig J, Petz J, McGill DB., "Does long-term home parenteral nutrition in adult patients cause chronic liver disease?", *JPEN*, 9, pp.11-17 (1985).

Buckley DC, Mirtallo JM, Kudsk KA., "Total parenteral nutrition in swine", In: Tumbleson ME., ed. **Swine in biomedical research**, (New York: Plenum Press) pp.1147-1151 (1985).

Burgess P, Hall RI, Bateman DN, Johnston IDA., "The effect of total parenteral nutrition on hepatic drug oxidation.", *JPEN*, 11, pp.540-543 (1987).

Campbell TC, Hayes TR., "Role of nutrition in the drug-metabolizing enzyme system.", *Pharmacol Rev*, 26, pp.171-197 (1974).

Fabri PJ, Deutsch S, Gower W, Mirtallo JM, Staubus A, Love JE., "Parenteral nutrition: Short term effects on hepatic clearance of sodium taurocholate and indocyanine green.", *Clin Biochem*, 20, pp.57-60 (1987).

Gibaldi M, Perrier D., "Noncompartmental analysis based on statistical moment theory.", In: Gibaldi M, Perrier D., ed. **Pharmacokinetics, 2nd ed.**, (New York: Marcel Dekker, Inc.,) pp.409-419 (1982).

Homeida M, Karrar ZA, Roberts JC., "Drug metabolism in malnourished children: a study with antipyrine.", *Arch Dis Child*, 54, pp.299-302 (1979).

Jagadeesan V, Krishnaswamy K., "Drug binding in the undernourished: a study of the binding of propranolol to α-1-acid glycoprotein", *Eur J Clin Pharmacol*, 27, pp.657-659 (1985).

Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA., "Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats", *Gastroenterology*, 68, pp.1177-1183 (1975).

Ke J, Tam YK, Koo W, Coutts RT, Finegan BA., "Lack of acute effect on lidocaine pharmacoki-

netics from parenteral nutrition", *Ther Drug Monit*, 12, pp.157-162 (1990).

Knodell RG, Spector MH, Brooks DA, Keller FX, Kyner WT., "Alterations in pentobarbital pharmacokinetics in response to parenteral and enteral alimentation in the rat", *Gastroenterology*, 79, pp.1211-1216 (1980).

Knodell RG, Steele NM, Cerra FB, Gross JB, Solomon TE., "Effects of parenteral and enteral hyperalimentation on hepatic drug metabolism in the rat", *J Pharmacol Exp Ther*, 229, pp.589-597 (1984).

McNamara PJ, Slaughter RL, Pieper JA, Wyman MG, Lalka D., "Factors influencing serum protein binding of lidocaine in humans.", *Anesth and Analg*, 60, pp.395-400 (1981).

Merritt RJ., "[illegible]steosis associated with total parenteral nutrition.", *J Pediatr Gastrointerol Nutr*, 5, pp.[illegible] (1986).

Narang RK, Mehta S, Mathur VS., "Pharmacokinetic study of antipyrine in malnourished children.", *Am J Clin Nutr*, 30, pp.1979-1982 (1977).

Nation R, Triggs EJ, Selig M., "Lignocaine kinetics in cardiac patients and aged subjects.", *Br J Clin Pharmacol*, 4, pp.439-448 (1977).

Pantuck EJ, Pantuck CB, Weissman C, Askanazi J, Conney AH., "Effects of parenteral nutritional regimens on oxidative drug metabolism.", *Anesthesiology*, 60, pp.534-536 (1984).

Pantuck EJ, Pantuck CB, Weissman C, Gil KM, Askanazi J., "Stimulation of oxidative drug metabolism by parenteral refeeding of nutritionally depleted patients.", *Gastroenterology*, 89, pp.241-245 (1985).

Rocci ML, Jusko WJ., "Lagran program for area and moments in pharmacokinetic analysis.", *Computer Prog Biomed*, 16, pp.203-216 (1983).

Routledge PA, Barchowsky A, Bjornsson TD, Kitchell BB, Shand DG., "Lidocaine plasma protein binding.", *Clin Pharmacol Ther*, 27, pp.347-351 (1980).

Saville BD, Gray MR, Tam YK., "Evidence for lidocaine-induced enzyme inactivation", *J Pharm Sci*, 78, pp.1003-1008 (1989).

Tam YK, Tawfik SR, Ke J, Coutts RT, Gray MR, Wyse DG., "HPLC assay of lidocaine and its nine metabolites in human plasma and urine.", *J Chromatogr Biomed Appl*, 423, pp.199-206 (1987).

Yang CS, Yoo TSH., "Dietary effects on drug metabolism by the mixed-function oxidase system",

Pharmacol Ther, 38, pp.53-72 (1988).

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

Parenteral nutrition (PN) is a widely used medical technique whereby hypertonic solutions of dextrose and amino acids, with or without a lipid component, are delivered intravenously to establish normal nutrition in subjects unable to ingest adequate oral nutrients. However, the effects of PN on pharmacokinetics have to date received little attention (*Corcoran 1986*). The objectives of this study were to consider how and why drug kinetics may be changed by PN therapy and to evaluate various possible mechanisms involved in PN and drug interactions.

It is conceivable that PN administration may cause an instant elevation of concentrations of circulating nutrients in the blood stream. These high concentration of nutrients in blood may compete with drugs for hepatic enzymes sites, if they have similar chemical structure and/or follow metabolic pathways similar to those followed by drugs in the liver; these nutrients may also compete with drugs for protein binding sites, if they share the same binding sites with drugs. Based on this competition hypothesis, PN infusion may acutely influence the disposition of drugs. Amino acid infusion has been shown to double creatinine clearance (CL_{cr}) and increase renal plasma flow (*Graf et al. 1983, Meyer et al. 1983*). This observation implies that renal drug clearance may be altered by PN therapy. Prolonged infusion of PN may compromise hepatic function (*Klein and Nealon, 1988)* and may affect hepatic drug elimination (*Anderson, 1988*). This necessitates studies of

the short-term effects of PN on the pharmacokinetics of drugs. Moreover, difference in the nutritional states of subjects is another possible confounder of PN-drug interactions.

Two drugs were used for this particular project. Ampicillin was chosen as a model compound for the evaluation of potential effects of PN on renal drug excretion. Choosing lidocaine as another model drug may allow the elucidation of the effects of PN on hepatic blood flow, drug metabolism and protein binding.

Lidocaine metabolism has been a subject of interest for a number of years (*Keenaghan and Boyes 1972, Nelson et al. 1977, Coutts et al. 1987*). However, the metabolic pathways of lidocaine are still not fully elucidated. In humans, 4-hydroxy-2,6-xylidine (4-OH-XYL) is the most abundant urinary metabolite, which accounted for more than 70% of an intravenous dose of lidocaine. Some investigators (*Nelson et al., 1977, Mihaly et al. 1978*) have speculated that 4-hydroxylated metabolites of lidocaine may be the intermediates in the metabolism of lidocaine to 4-OH-XYL in addition to 2,6-xylidine (2,6-XYL) and may be excreted in low concentrations in human urine. More recently, 4-hydroxy-N-(N-ethylglycyl-2,6-xylidine (4-OH-MEGX) was detected in a patient's urine (*Torok-Both, 1987*), further supporting these hypotheses. In order to study lidocaine metabolism in detail, and to elucidate mechanisms by which PN may influence lidocaine metabolism, it is imperative to have a HPLC assay available to separate and quantitate lidocaine and all of its known and/or possible metabolites, especially the *p*-hydroxylated precursors of 4-OH-XYL in humans. In this project a new HPLC assay was developed to simultaneously separate and quantitate lidocaine and its nine metabolites

in biological samples. Traces of 4-hydroxy-lidocaine (4-OH-LIDO), 4-OH-MEGX and 4-hydroxy-N-glycyl-2,6-xylidine (4-OH-GX) were identified and quantified in human urine by using this new assay. Furthermore, 4-OH-LIDO and 4-OH-MEGX were experimentally confirmed to be biotransformed to 4-OH-XYL in a pig that received an intravenous injection of 4-OH-LIDO and 4-OH-MEGX on separate occasions. These findings suggest that in addition to N-deethylation, *p*-hydroxylation may also be an important primary pathway in humans.

Potential acute effects of PN on renal ampicillin excretion in human were investigated. There were no significant differences in the glomerular filtration rate (GFR), as indicated by creatinine clearance and ampicillin pharmacokinetic parameters, including area under the serum drug concentration-time curve from time zero to infinity (AUC), terminal elimination half life ($T_{\frac{1}{2}}$), volume of distribution at steady state (V_{dss}), total body clearance (CL_T), renal clearance (CL_R) and the amount of intact drug excreted in urine between PN treatment and control study. These results indicate that the use of PN with amino acid content similar to the recommended nutrient intake does not have any acute effect on the pharmacokinetics of ampicillin in healthy subjects. Furthermore, renal ampicillin clearance is substantially higher than GFR which indicates that active renal secretion occurs. Thus, it is unlikely that PN has any acute effects on active renal secretion of ampicillin, based on the results that both GFR and ampicillin clearance were similar between the experimental and control groups.

Potential acute effects of PN on hepatic lidocaine elimination were studied in humans and in the isolated rat liver perfusion model. Lidocaine pharmacokinetics

including AUC, $T_{\frac{1}{2}}$, V_{dss}, CL_T, serum lidocaine protein binding and concentration-time course of the active metabolite of lidocaine, N-(N-ethylglycyl)-2,6-xylidine were not different between PN treatment and controls. These data suggest that at the usual rate of amino acid infusion there is no acute effect of PN on lidocaine pharmacokinetics in healthy human. Acute effects of PN on hepatic lidocaine elimination were also not observed in the isolated rat liver perfusion model. Thus, it is unlikely that amino acids in the PN solution directly compete with lidocaine for hepatic enzymatic sites at the present rate of amino acid infusion.

A short term effect of PN on hepatic lidocaine elimination was studied using the *in vitro* isolated rat liver perfusion model and an *in vivo* pig model. Hepatic clearance and hepatic extraction ratio for lidocaine were reduced by 29.8% and 31.1% ($p< 0.05$), respectively, in healthy rats receiving 7 days of *in vivo* PN infusion compared with chow-fed animals. In a pilot study in pigs, after 7 days of PN infusion, CL_T and V_{dss} were reduced by 54% and 53% ($p< 0.05$), respectively, when compared with controls. These observations are consistent with reports (*Knodell et al. 1980 and 1984*) that hepatic pentobarbital clearance was lower in healthy rats after 7 days of PN compared to animals fed standard laboratory chow *ad libitum*. Combined with the results from our study of the acute effects of PN, these data support the hypothesis that PN induced alteration of drug elimination is more likely due to a secondary effect on hepatic function from parenteral nutrients, presumably one or more amino acids, rather than an acute and direct competitive effect of nutrients with the drug for metabolic enzymes in liver.

Mass balance in the effluent at steady state showed that recovery was higher for lidocaine (46.6% (PN) vs. 25.6% (chow-fed), $p< 0.05$) and lower for N-(N,N-diethylglycyl)-2-hydroxymethyl-6-methylaniline, an aryl methyl hydroxylation product of lidocaine (0.21% (PN) vs. 2.75% (chow-fed), $p< 0.05$) in rats receiving 7 days of PN compared to chow-fed controls. Recoveries for N-deethylated and *m*-hydroxylated metabolites of lidocaine were similar between PN and chow-fed groups. In pigs, consistent with a significant reduction in lidocaine clearance, more intact drug was excreted in urine after 7 days of PN infusion, whereas the urinary excretion of *p*-hydroxylated metabolites, 4-OH-GX and 4-OH-XYL, and amide hydrolysis metabolite, 2,6-XYL, was decreased. As a result of reductions in *p*-hydroxylation and amide hydrolysis processes for lidocaine metabolism, the levels of N-deethylated metabolites of lidocaine excreted in urine were higher in post PN study than controls. Meanwhile, the urinary excretion of *m*-hydroxylated metabolites of lidocaine were similar between the two treatments, indicating that a short-term of PN infusion did not affect the *m*-hydroxylation pathway in the pig. These findings suggest that reduction of hepatic lidocaine elimination after 7 days of PN occurred as result of selective inhibition of hepatic enzyme activities such as those involved in the aryl methyl hydroxylation pathway in rat, and the *p*-hydroxylation and amide hydrolysis pathways in the pig.

The short term effect of PN on renal function was investigated in rats and in one pig. The creatinine clearance (CL_{Cr}) on day 1 and day 7 after the commencement of PN administration were similar (0.603 $\pm$ 0.17 ml/min (day 1) vs. 0.673 $\pm$ 0.14 ml/min (day 7)) in 6 rats. The preliminary data from one pig also showed

that CL_{Cr} did not change while receiving 7 days of PN treatment compared to the enterally fed condition (37 ml/min (PN) vs. 38 ml/min (Fed)). These results suggest that short term PN infusion did not influence GFR as indicated by the unchanged CL_{Cr} at the present infusion rate of PN.

A pilot study was designed specifically to examine the effects of PN and nutritional states of subjects on lidocaine disposition. An *in vivo* pig model was set up to mimic several nutritional states. After a 48 hour fast lidocaine clearance was reduced by 51% compared with control. The initiation of PN infusion on the next day did not significantly alter lidocaine clearance compared to the fasted state. This observation is consistent with the results from our acute effects of PN studies in healthy humans and in isolated livers from healthy rats in that PN infusion does not acutely affect lidocaine pharmacokinetics. However, after 7 days of PN infusion in one pig that had a 72 hour fast prior to initiation of PN, CL_T and V_{dss} for lidocaine were reduced by 66% and 65% , respectively, when compared to control. These results are also in agreement with the data from our short-term effects of PN study in healthy rats. In addition, we have also found that the serum protein binding of lidocaine was different under different nutritional states of subject. Thus, the preliminary results from the pig experiment suggest that PN and nutritional states can significantly affect lidocaine pharmacokinetics, but the extent of interaction between PN and lidocaine is unlikely to be dependent upon the nutritional status of subjects. More studies are required to confirm this observation.

Furthermore, the combined data from our acute and short-term PN studies in humans and in animals support the contention that the alteration of lidocaine clearance is unlikely to be due to PN-induced changes in hepatic blood flow rate. In the isolated rat liver perfusion study the buffer perfusion rate was maintained the same for all the livers. However, a significant reduction in hepatic lidocaine clearance was observed in rats receiving 7-days of PN infusion compared with the chow-fed animals, suggesting that hepatic blood flow may not be a significant determinant for PN-drug interaction. In addition we also found that serum protein binding of lidocaine was significantly reduced (~ 10%) after a week of PN treatment compared with that of control studies in pigs.

Interactions [illegible] nutrient components in PN regimens were not conducted [illegible] effect of lipid, an essential component in PN regimens [illegible] not evaluated, although it has been reported that the [illegible] antipyrine induced by infusion of amino acid-dextrose sol[illegible]e prevented by the inclusion of lipid calories within the PN regimen (*Burgess et al. 1987*). More studies are required to clarify the lipid effects on drug disposition.

8.1 References

Anderson KE., "Influence of diet and nutrition on clinical pharmacokinetics", *Clin Pharmacokinet*, 14, pp.325-346 (1988).

Burgess P, Hall RI, Bateman DN, Johnston IDA., "The effect of total parenteral nutrition on hepatic drug oxidation.", *JPEN*, 11, pp.540-543 (1987).

Corcoran GB, "Pharmacokinetic considerations during total parenteral nutrition", In: Lebenthal E, ed. **Total Parenteral Nutrition**, (New York: Raven Press) pp.125-140 (1986).

Coutts RT, Torok-Both GA, Tam YK, Chu LV, Pasutto FM., "Acetylation and pentafluorobenzoylation of lidocaine metabolites in aqueous solution and identification of derivatives by combined gas chromatography/mass spectrometry", *Biomed Environ Mass Spectrom*, 14, pp.173-182 (1987).

Graf H, Stummvoll HK, Luger A, Prager R., "Effect of amino acid infusion on glomerular filtration rate.", *N Engl J Med*, 308, pp.159-160 (1983).

Keenaghan JB, Boyes RN., "The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man.", *J Pharmacol Exper Ther*, 180, pp.454-463 (1972).

Klein S, Nealon W., "Hepatobiliary abnormalities associated with total parenteral nutrition", *Semin Liver Dis*, 8, pp.237-246 (1988).

Knodell RG, Spector MH, Brooks DA, Keller FX, Kyner WT., "Alterations in pentobarbital pharmacokinetics in response to parenteral and enteral alimentation in rat.", *Gastroenterology*, 79, pp.1211-1216 (1980).

Knodell RG, Steele NM, Cerra FB, Cross JB, Solomon TE., "Effects of parenteral and enteral hyperalimentation on hepatic drug metabolism in the rat", *J Pharmacol Exper Ther*, 229, pp.589-597 (1984).

Meyer TW, Ichikawa I, Zatz R, Brenner BM., "The renal hemodynamic response to amino acid infusion in the rat.", *Trans Assoc Am Physicians*, 96, pp.76-83 (1983).

Mihaly GW, Moore RG, Thomas J, Triggs EJ, Thomas D, Shanks CA., "The pharmacokinetics and metabolism of the anilide local anaesthetics in neonates.", *Eur J Clin Pharmacol*, 13, pp.143-152 (1978).

Nelson SD, Garland WA, Breck GD, Trager WF., "Quantification of lidocaine and several metabo-

lites utilizing chemical-ionization mass spectrometry and stable isotope labelling.", *J Pharm Sci*, 66, pp.1180-1189 (1977).

Torok-Both G., "Analysis of phenylzine, lidocaine and doxapram: Application to the study of their metabolism and pharmacokinetics.", **Ph.D. Thesis, University of Alberta**, pp.172-177 (1987).

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