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Interaction between Erythromycin and Verapamil

by

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Abstract

A few reports suggest a potentially serious interaction between some macrolide antibiotics and calcium channel blocker, verapamil, resulting in symptoms of verapamil or macrolide toxicity. The mechanism of this interaction is unknown and was speculated to be at the level of pharmacokinetics because both of these drugs can inhibit the metabolism and transport of various drugs. We investigated the interaction between erythromycin and verapamil in the rat. Intravenous erythromycin and verapamil were given alone to rats and their ECG's were recorded. We observed that erythromycin given alone prolongs QT but not PR interval. On the other hand, prolongs PR interval with no effect on QT interval. The coadministration of erythromycin and verapamil did not alter erythromycin-induced prolongation of QT interval but caused a significant increase in the effect of verapamil on PR interval. Indeed, AV node block, a side effect of verapamil, was evident following the combination therapy. This indicates that the undesired effect of the observed interaction stems from an enhanced potency of verapamil not erythromycin. We measured the plasma concentrations of both erythromycin and verapamil when administered alone or together. Neither drug influenced the pharmacokinetics of the other. Drug-free plasma was spiked with erythromycin and verapamil, incubated, and separated by ultrafiltration. The plasma free fractions of the drugs were not influenced by the other. The mechanism behind this interaction remains to be understood. However, an interaction at the pharmacokinetic level can be ruled out in our model since neither drug influenced the concentration-time course of the other, nor did either influence protein binding of the other.

Conclusion: The life-threatening interaction of macrolides and certain cardiovascular drugs appear to be at the pharmacodynamic and not a pharmacokinetic level.

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Abbreviations

AGP	α1-acid glycoprotein
ATP	Adenosine triphosphate
AUC	Area under the time-concentration curve
AUEC	Area under the time-effect curve
AV	Atrioventricular
°C	Degree centigrade
CL_{TB}	Total body clearance
CYP	Cytochrome P-450
ECG	Electrocardiogram
ERY	Erythromycin
f_u	Fraction unbound in plasma
g	Gram
HERG	Human ether a go-go-related gene
HPLC	High performance liquid chromatography
IL	Interleukin
i.p.	Intraperitoneal injection
i.u.	International unit
i.v.	Intravenous injection
kg	Kilogram
M	Molar
mg	Milligram

min	Minutes
ml	Milliliter
mm	Millimeters
MMP	Matrix metalloproteinase
ms	Milliseconds
µg	Micrograms
µl	Microlitre
ng	Nanogram
PGP	P-Glycoprotein
SD	Standard deviation
$t_{1/2}$	Terminal half-life
TNF	Tumor Necrosis Factor
v/v	Volume/Volume
VER	Verapamil

CHAPTER ONE

BACKGROUND

1.1 Introduction

Adverse drug events affect millions of patients each year and are responsible for more than 1 million hospital admissions per year in the US (1, 2). The estimated costs to treat disorders caused by drug related problems in the US increased from $76.6 billion in 1994 to $177.4 billion in 2000 (3). There were more than 100,000 fatal adverse drug reactions in hospitalized patients in 1994, making these reactions about one of the leading causes of death in the US (4).

There are several reasons for adverse drug reactions. These include errors in drug administration, noncompliance, overdose, drug abuse, and therapeutic failures (5). Two important factors contributing to the incidence of adverse drug reactions are the age of patients and the number of medications they take (5, 6). The number of events per patient increases by approximately 10% for each additional medication (2). In Europe, the elderly population uses on average 7.0 drugs per person; 46% had at least one drug combination leading to a drug-drug interaction (3).

Fifty to eighty four percent of adverse drug events are preventable with proper identification and surveillance (7). Drug-drug interactions are a particularly important type of adverse drug events because they are often predictable based on previous reports, clinical studies, and understanding of pharmacological principles (1).

Drugs may interact with each other by pharmacodynamic and/or pharmacokinetic interactions. The pharmacodynamic interactions occur when the pharmacological effects of the object drug are stimulated or inhibited by the precipitant drug. Pharmacokinetic interactions can result from the interference of drug absorption, distribution, metabolism, or elimination of the object drug by the precipitant drug (8, 13).

1.2 Verapamil

Verapamil is a phenylalkylamine calcium channel antagonist that blocks L-type calcium channels. It impedes the inward calcium channel current carried through slow channels (13). Verapamil is also a potent blocker of the human *ether-a go-go-related* gene (HERG) potassium channel in the heart and alveolar epithelial cells (14, 15). Cardiac delayed rectifier K^+ current is composed of two distinct currents, the rapidly and slowly activating components (15). The rapidly activating channel protein is encoded by HERG. Suppression of HERG channels causes action potential and QT interval prolongation.

The pharmacological effects of verapamil result from blocking calcium channels in cardiac or peripheral tissues (10). Cardiac effects of verapamil include slowing cardiac conduction time by depressing atrioventricular (AV) node and decreasing the rate of sinus node discharge, and prolonging AV nodal refractoriness (10, 12). Its depression of AV node results in PR interval prolongation and AV node block (11). The PR interval prolongation is conveniently detectable even after small single doses (16). On the other hand, heart rate is more variable and less reproducible because it is affected by some other factors. Some studies also suggest that verapamil can shorten QT interval at low heart rate, and therefore can be used as protection against *torsades de pointes* (18). Peripheral effects of verapamil include vasodilation of blood vessels by inhibiting the influx of calcium in vascular smooth muscle (10).

Cardiovascular side effects of approved doses of verapamil include hypotension, bradycardia, and heart block (19). Verapamil is contraindicated in patients with heart failure (17). Because of the short half-life of verapamil, it is administered every 6 to 8 hours. Therefore, immediate release verapamil is associated with wide swings in plasma

levels and consequently in blood pressure and heart rate (10, 17). Some studies have also suggested a link between verapamil administration and increased risk of cardiovascular events (17).

Verapamil is used in the treatment of hypertension, stable angina, migraine, narrow QRS supraventricular arrhythmias, and for the potentiation of chemotherapeutic drugs (11, 20). It is bound to plasma proteins including α-acid glycoprotein and is extensively metabolized upon the first-pass through the liver.

Verapamil is a racemic drug with S- and R- enantiomers having significant differences in their pharmacokinetic and pharmacodynamic properties (9). In terms of pharmacodynamics, S- verapamil has been shown to have 5 to 11 times more dromotropic potency than its antipode (13, 21). In terms of pharmacokinetics, there is a difference in the protein binding, and consequently the hepatic metabolism of verapamil enantiomers between humans and rats. In humans, the more potent S isomer is less bound to plasma than its antipode, and is metabolized more efficiently (23, 24). Therefore, lower concentrations of the more active S verapamil are observed in humans (22). On the other hand, in Sprague Dawley rats S verapamil is more highly bound to plasma proteins than its antipode and is metabolized less efficiently. Therefore, higher concentrations of the more active enantiomer S- are observed in rats (25). This difference in protein binding of verapamil between humans and rats is likely due to fact that α1-acid glycoprotein (AGP), the protein that verapamil extensively binds to in plasma, is different between humans and rats. In fact, rat AGP shares only 59% amino acid sequence homology with human AGP (82).

The drug-drug interactions of verapamil are summarized in Table 1.1. Verapamil interacts with other drugs by affecting their pharmacokinetics or pharmacodymics. Verapamil competitively inhibits microsomal drug metabolism through the mixed function oxidase enzyme system (31). In fact, its inhibition of cytochrome P-450 3A4 (CYP3A4) results in many drug interactions. For example, when cyclosporine, a drug that is mainly metabolized by this isozyme, is coadministered with verapamil to humans, up to 6-fold increase in the plasma concentrations of cyclosporin was observed, leading to cases of renal toxicity (26).

Verapamil also inhibits the activity of P-glycoprotein, an ATP-dependent drug transport protein that contributes to the transport of many xenobiotics across biological membranes, in a competitive manner without interrupting the cyclic activity (ATP hydrolysis) of P-glycoprotein (30, 35). Verapamil can increase the plasma concentration of digoxin, a P-glycoprotein substrate, in a dose dependent manner up to 60-90% and thus can cause digoxin intoxication (30).

Verapamil shares similar pharmacological effects with other calcium channel blockers, β-blockers, antiarrhythmic drugs, and digoxin. Coadmininstration of these drugs with verapamil may produce additive or synergistic effects on the prolongation of PR interval, and the reduction of AV nodal conduction and myocardial contractility (26). The coadministration of verapamil and amiodarone, for example, can cause additive reduction in heart rate and myocardial contractility (26).

Table 1.1. Drug-drug interactions of verapamil (26-33).

Class/Drug	Consequences	Mechanism
Simvastatin	Potential for myopathy and rhabdoomyolysis	Inhibition of CYP3A4
Carbamazepine	Carbamazepine toxicity	Inhibition of CYP3A4
Ethanol	Increased Ethanol concentrations and enhanced psychomotor effects	Inhibition of ethanol metabolism
β-blockers (atenolol, metoprolol, propranolol, pidolol)	PR-interval prolongation, shock, bradycardia, heart block, and hypertension	Decreased intracellular calcium concentrations in myocardium
Digoxin	Increased digoxin concentrations, additive reduction in heart rate and myocardial contractility	Inhibition of PGP and pharmacodynamic interaction.
Amiodarone	Additive reduction in heart rate and myocardial contractility	Pharmacodynamic interaction
Cyclosporin	Increased cyclosporin concentrations, renal toxicity	Inhibition of CYP3A4
Quinidine	Increased quinidine concentrations, AV block	Inhibition of CYP3A4

1.3 Erythromycin

Erythromycin is a macrolide antibiotic that is effective against Gram-positive organisms and is used to treat numerous infections, especially those involving the respiratory tract. Erythromycin remains an important alternative to penicillin and tetracycline for a large array of infections (34,35).

In addition to its antimicrobial effects, erythromycin has recently been shown to play a role in the modulation of the immune response, both contributing to the treatment of infective diseases and opening new opportunities for the therapy of other inflammatory conditions (36, 37). Long-term administration of low doses of erythromycin has dramatically increased survival in patients with diffuse panbronchiolitis, a disease with many similarities to cystic fibrosis (38, 39).

Cardiac adverse effects of approved doses of erythromycin include QT interval prolongation and occasional cases of cardiac arrhythmias such as ventricular tachycardia and *torsade de pointes*, especially after intravenous administration (47). Studies have shown that erythromycin and other macrolides can prolong the QT interval by inhibiting the rapid component of the delayed rectifier K^+ current through the block of HERG potassium channels (34, 40).

QT interval prolongation has become a surrogate marker of cardiotoxicity and has received increasing regulatory attention (48). QT prolongation has recently gained clinical importance; primarily because prolongation of this interval can predispose to a potentially fatal ventricular arrhythmia know as *torsades de pointes*. The term "*torsades de pointes*" is often translated as a "twisting of the points," referring to the beat-to-beat changes in QRS axis (41). The vast majority of patients in whom *torsades de pointes*

developed in association with the use of noncardiac drugs had at least one risk factor (41). These risk factors include female sex; cardiac, renal, or liver disease; hypokalemia; a history of the long QT syndrome; and the prescription of a QT prolonging medication in excessive doses or in combination with a second drug that impairs its metabolism or further prolongs the QT interval (42,43). Drugs that prolong QT interval include amiodarone, disopyramide, dofetilide, ibutilide, procainamide quinidine, sotalol, and thioridazine (44). Medications such as cisapride, terfenadine and grepafloxacin have recently been withdrawn from the market because of their risk of causing QT prolongation and fatal arrhythmias such as *torsades de pointes* (43,44). For cisapride and terfenadine, the risk was heightened by the continued co-prescription of medications known to inhibit the clearance of both drugs (44).

The drug-drug interactions of erythromycin are summarized in Table 1.2. Erythromycin interacts with other drugs by affecting their pharmacokinetics or pharmacodynamics. Pharmacokinetic interactions include its inhibition of hepatic and intestinal drug-metabolizing enzymes, such as CYP3A4 and CYP1A2, and its inhibition of P-glycoprotein (49, 50). Inhibition of CYP3A4 by erythromycin is mechanism based: When the drug is metabolized at the isozyme's active site, a reactive metabolite is formed which binds irreversibly to the isozyme, disabling it and thereby reducing the amount of available CYP3A4 (61).

Erythromycin is also a potent inhibitor of P-glycoprotein and some of its interactions are due to the inhibition of the substrate transport. For example, the serum concentrations of cyclosporine, digoxin, carbamazepine, and talinolol are elevated when erythromycin is coadministered (51-53).

Erythromycin has been shown to have electrophysiological effects on the cardiac conducting system similar to those of class IA antiarrhythmic drugs resulting in QT interval prolongation (47). Pharmacodynamic interactions can occur when erythromycin is coadministered with drugs that further delay ventricular repolarization such as cisapride, disopyramide, terfenadine, and astemizole, causing additive or synergestic QT interval prolongation and *torsade de pointes* (48,56,57).

Erythromycin's drug-drug interactions are particularly important in drugs with narrow therapeutic range, such as, carbamazepine, theophylline, cyclosporine, digoxin, and warfarin (52,55).

Table 1.2. Drug-drug interactions of erythromycin (45-61).

Class/Drug	Consequences	Mechanism
Theophylline and aminophylline	Decreased clearance, increased half-life, toxicity	Inhibition of CYP3A4
Carbamazepine	2- to 4-fold increase in serum concentration, toxicity	Inhibition of CYP3A4 and PGP
Cyclosporine	3- to 10-fold increase in serum concentration, renal impairment	Inhibition of PGP
Digoxin	2- to 3-fold increase in serum concentration, toxicity	Inhibition of intestinal PGP
Cisapride, disopyramide, terfenadine, and astemizole	QT interval prolongation and *torsades de pointes*	Additive QT prolongation and inhibition of CYP3A4
Ergot alkaloids	Toxicity	Inhibition of CYP3A4
Quinidine	Cardiac arrhythmias	Inhibition of CYP3A4
Oral anticoagulants	Increased INR, bleeding	Inhibition of CYP3A4
Midazolam and triazolam, phenotoin, clozapine	Increased concentrations, toxicity	Inhibition of CYP3A4
Atorvastatin, lovastatin, cerivastatin, and simvastatin	Increased concentrations	Inhibition of CYP3A4 first pass metabolism
Talinolol	increased bioavailability	Inhibition of PGP

1.4 Inflammation

An important factor that may contribute to drug interactions of erythromycin and verapamil is inflammation. Erythromycin can modulate inflammatory responses and dromotropic activity of verapamil is reduced during inflammation (16,36).

Oral and intravenous erythromycin can reduce the concentrations of interleukin (IL-6) and tumor necrosis factor (TNF-α) induced by heat killed *Streptococcus pneumoniae* in human whole blood *ex-vivo* (62). Erythromycin can also function as an immunomodulator by suppressing the activity of matrix metalloproteinase-9 (MMP-9), an enzyme that degrades various components of extracellular matrix (63, 64). The suppressive effect of erythromycin on MMP-9 activity is one of the anti-inflammatory mechanisms that inhibit the migration of inflammatory cells into the inflammatory site.

Pro-inflammatory mediators have been shown to cause increased plasma concentrations of verapamil and some other cardiac drugs in humans and rats by inhibiting their metabolism. Interestingly, these elevated plasma levels are associated with decreased potency of these drugs in patients and rats (16, 65-67). The mechanism by which inflammation reduces the activity of calcium channel antagonists is by causing a significant reduction in the number of binding sites of the L-type calcium channel acceptor (68).

1.5 Case Reports

There are a few case reports in the literature describing an interaction between macrolides and verapamil (19, 70, 71). One case report suggests that coadministration of verapamil and erythromycin can result in bradycardia (40 beats/min), hypotension (80/40

mm Hg), complete atrioventricular (AV) block, and QTc-interval prolongation (19). These symptoms resolved after the discontinuation of erythromycin. Figure 1.1 shows the ECG of the patient upon admission and two days after admission. Another case report mentions symptoms of severe verapamil overdosage including severe hypotension and bradycardia associated with coadministration of verapamil and clarithromycin, another macrolide that has similar pharmacokinetic and pharmacodynamic properties as erythromycin (70). In a third case report, a patient taking verapamil developed signs of verapamil toxicity 48 hours after the initiation of clarithromycin (71). In the first case report, it was speculated that concomitant administration of verapamil has increased erythromycin absorption as a result of P-glycoprotein and CYP3A4 inhibition. Since both drugs are potent inhibitors of CYP3A4 and P-glycoprotein, pharmacokinetic interaction was assumed to be contributors to this interaction. In the second and third case reports, it was speculated that erythromycin inhibited CYP3A4 metabolism causing increased plasma concentrations of verapamil, leading to symptoms of verapamil toxicity. Since plasma concentrations of verapamil and erythromycin were not measured in these case reports, these hypotheses could not be confirmed.

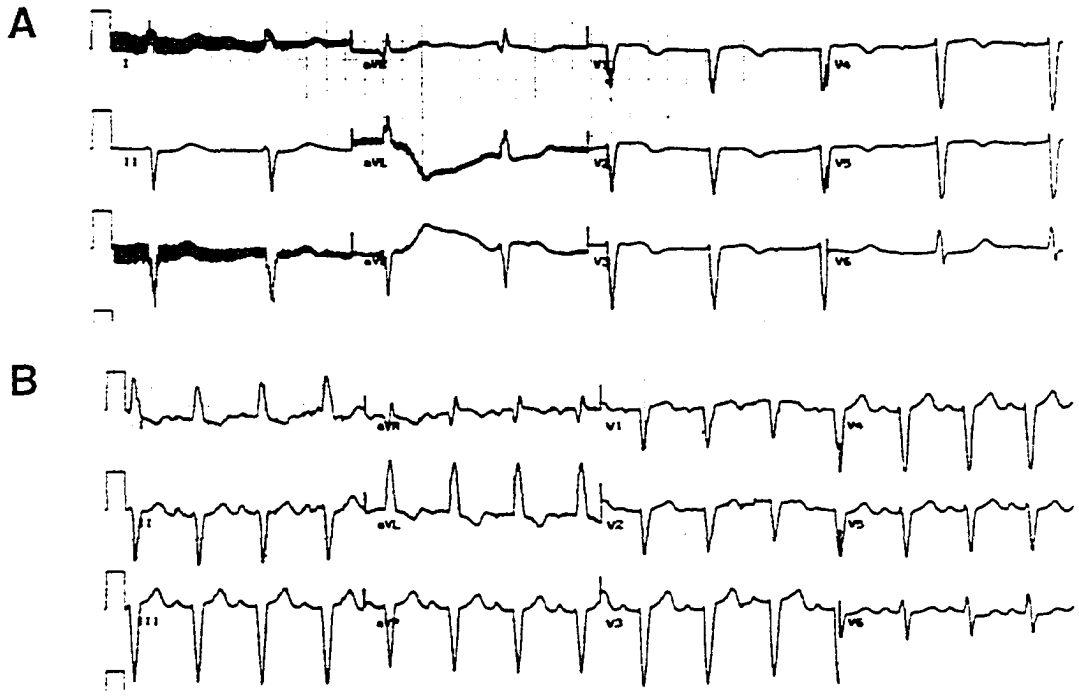

Figure 1.1 A: Electrocardiogram (ECG) performed on admission, demonstrating complete atrioventricular block, escape rhythm of 50 beats/min, QTc prolongation of 583 msec, and pattern of left bundle-branch block. **B:** ECG performed two days following admission demonstrating normal sinus rhythm, with normal PR interval and QTc of 518 msec (Ref 19).

1.6 Rationale

According to a recent study, calcium channel blockers and macrolide antibiotics were among the six most medications prescribed in the emergency department that accounted for most drug interactions (69). Both verapamil and erythromycin have pharmacokinetic interactions resulting from their inhibition of CYP3A4 metabolism and P-glycoprotein transport. Furthermore, their electrophysiological effects on cardiac conducting system give them more potential for drug-drug interactions.

Erythromycin can modulate inflammatory responses (36). Studies suggest that erythromycin can reduce the concentrations of IL-6 and TNF-α, and suppress the activity of matrix metalloproteinase-9 (MMP-9) (62-64).

The interaction between erythromycin and verapamil can be a result of altered pharmacokinetics, pharmacodynamics, or both. Two possible pharmacokinetic interactions are:

- Inhibition of the metabolism or the transport of one of the drugs by the other, causing elevated plasma concentrations of one or both drugs.
- One of the drugs displaces the other from its protein binding positions, resulting in increased plasma free fractions.

There are also three possible pharmacodynamic mechanisms:

- Both erythromycin and verapamil can block HERG potassium channels, so their combination can result in additive or synergistic effect on this acceptor.
- Erythromycin may inhibit cardiac P-glycoprotein, causing increased accumulation of erythromycin in cardiac tissue.

- Erythromycin may reduce pro-inflammatory mediators; hence potentiate the actions of verapamil.

1.6 Hypothesis

The interaction between erythromycin and verapamil is a result of altered pharmacodynamics of one or both drugs.

1.7 Objective

Using the rat as an animal model we set out to study the potential drug interaction between verapamil and erythromycin in order to shed more light on the mechanism of this interaction and to determine if it is at the level of pharmacokinetics or pharmacodynamics.

CHAPTER TWO

METHODS

2.1 Chemicals

- Verapamil Hydrochloride (Sigma Chemical Co. St. Louis, MO, USA).
- Erythromycin lactobionate injectable powder (Novopharm Ltd, Toronto, Canada).
- Sodium Pentobarbital Injection (MTC Pharmaceuticals, Cambridge, Ontario, Canada).
- Heparin sodium injectable 1,000 i.u./mL (Leo Pharma Inc. Thorhill, Ontario, Canada).
- Sodium Chloride 0.9%, injection USP (Astra).
- (+)-Glaucine and Hepatoflurobutanol. (Aldrich, Milwaukee, WI).
- HPLC grade hexane, 2-propanol, and acetonitrile-190. (Caledon Laboratories, Georgetown, Canada).
- Heptane. (Mallinckrodt, Paris, KT).
- 98% Anhydrous ethyl alcohol. (Stanley, Vancouver, Canada).
- Triethylamine (TEA) (Sigma Chemical Co, St. Louis, MO).

2.2 Animals

This investigation was approved by the Animal Ethics Committee of the University of Alberta. Rats were used as an animal model because the verapamil shows similar dromatropic effects in humans and rats. Adult male Sprague–Dawley rats (280-320 g) were housed in standard rodent cages, kept on a 12 h light/dark cycle, and fed a standard diet of Purina rat chow. The procedure of cannulation and insertion of electrodes was performed according to a previously described method (66). Rats were anesthetized using sodium pentobarbital (65 mg/kg i.p.) and a silastic catheter was inserted into the

right jugular vein. Three stainless steel Teflon coated electrodes (Cooner wire, Chatsworth, CA, U.S.A.) were attached to the rats for Lead I monitoring (two electrodes near the right and left axilla regions, and the third at the xiphoid cartilage) and were brought around to each animal's back. Animals were allowed to recover for 24 h before dosing.

2.3 Dosing

Rats were randomly divided into three groups: erythromycin, verapamil, and erythromycin plus verapamil and were dosed as follows:

- Group I (n = 6, weight = 296 ± 25 g) received 1 mg/kg *i.v.* verapamil.
- Group II (n = 6, weight = 299 ± 20 g) received 100 mg/kg *i.v.* erythromycin.
- Group III (n = 6, weight = 294 ± 22 g) received 100 mg/kg *i.v.* erythromycin, and 10 minutes later 1 mg/kg *i.v.* verapamil.

Verapamil solutions were prepared by dissolving verapamil in normal saline (verapamil hydrochloride in sodium chloride 0.9% USP). Erythromycin solutions were prepared by dissolving erythromycin lactobionate injectable powder in injectable water USP. The appropriate volumes of drug solutions were given to rats *via* the jugular vein over approximately 10 seconds. The total injection volume was approximately 500 µl for animal receiving one drug and 1000 µl for animals receiving two drugs. After the administration of the drug, the cannula was flushed with approximately the same volume of normal saline.

2.4 Electrocardiogram Recording

Electrocardiogram (ECG) recording was performed according to a previously published procedure (72). ECG parameters were continually monitored using a Honywell ECG amplifier (Honywell Electronics for Medicine, Edmonton, Canada) and recorded using Acknowledge 3.0 Data Acquisition software (Biopac Systems, Inc., Goleta, CA, U.S.A.). Dosing animals started after establishing a stable baseline of ECG parameters for at least 10 minutes. ECG parameters were recorded at baseline and 1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 300 minutes post verapamil injection.

Figure 2.1 demonstrates a typical rat electrocardiogram. The PR interval represents the time required for an impulse to conduct through the tissues located above the ventricles (i.e., atria, AV node and His bundle). The QT interval, conduction through Purkinjie fibres and ventricular muscle represents drug effect on ventricular depolarization and repolarization, is used as a measure of cardiac potassium channel blocking activity (66). Measurement of PR and QT intervals was blinded. The PR interval was measured as the distance from the crest of the P wave to the crest of the R wave. In the rat, the ST segment of the electrocardiogram cycle forms a plateau that is not seen in a human electrocardiogram. Therefore, in order to quantify the QT interval the distance from the Q dip to the bottom of the ST segment is measured. There are three kinds of atrioventricular (AV) node block. First-degree AV block is when PR-interval is prolonged without missing any QRS complexes. Second-degree AV block is when some P-waves are not followed by QRS complexes. Third degree (complete heart block) AV block is when there is no relationship between P waves and QRS complexes (73).

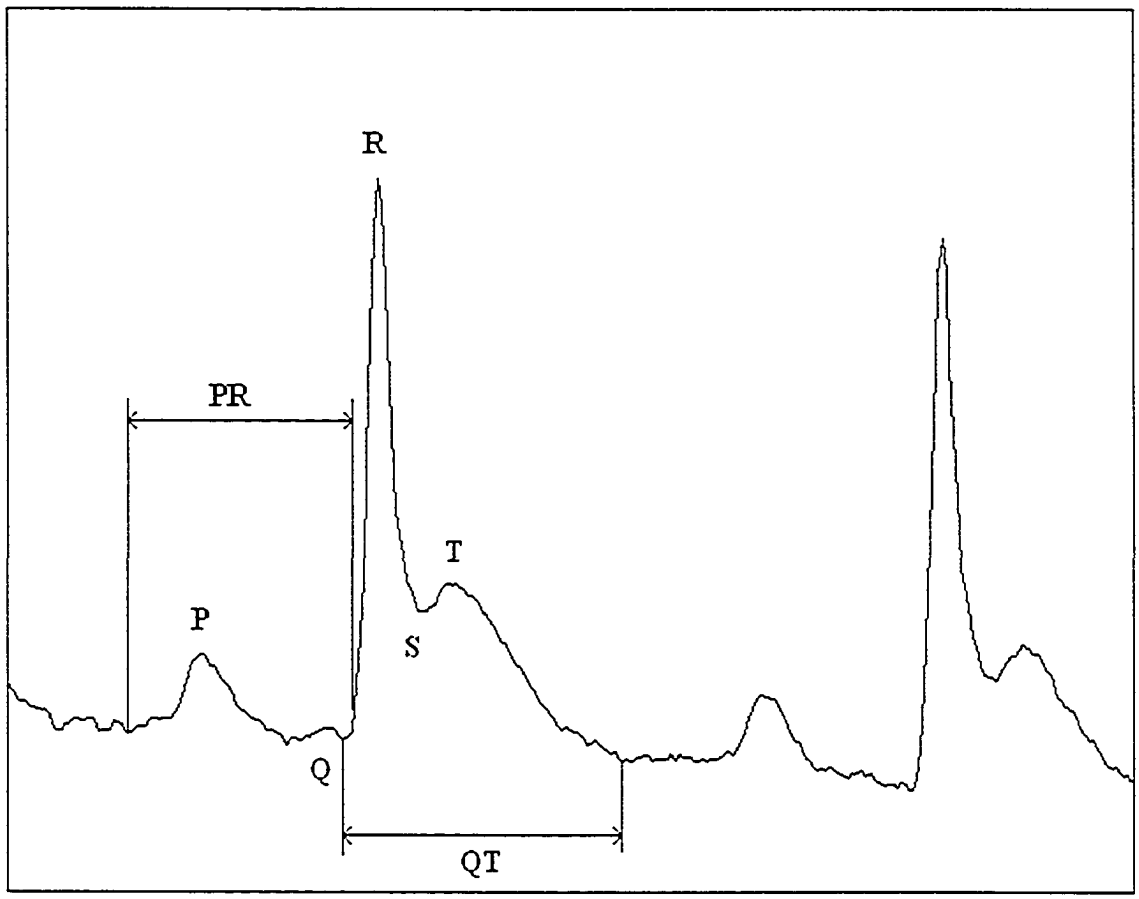

Figure 2.1. Typical rat ECG illustrating P-wave, QRS complex, and T-wave.

2.5 Blood Samples

Pharmacokinetic studies were performed in animals different from those used in the pharmacodynamic experiments in order to avoid any influence of blood sampling on ECG. A 250 μl aliquot (a 500 μl aliquot from animals that received two drugs) of blood was collected from the jugular vein of each rat just prior to drug injection, and at 2, 5, 10, 30, 60, 180, and 300 min in heparinized tubes. After each blood sample collection, a volume of normal saline equal to the volume of blood collected was administered *via* jugular vein cannula as fluid replacement, and the cannula was heparinized (10 i.u./ml). Blood samples were centrifuged at 2000 × *g* for 10 minutes immediately and a 100 μl aliquot of plasma sample (two 100 μl aliquots from animals that received two drugs) was stored in a -20 °C freezer until HPLC analysis.

2.6 *In Vitro* Protein Binding

The protein binding study was performed according to previously published methods (74, 75). The plasma free fraction of both enantiomers of verapamil and as well as erythromycin was determined *in vitro* in the presence and absence of each other by an ultrafiltration method. Drug-free blood was collected through the jugular vein, heparinized, and centrifuged for 10 minutes at 2000 *x g* to separate plasma. Plasma was divided into three groups:

- Group I (n=6), 1 ml plasma each, was spiked with verapamil.
- Group II (n=6), 1 ml plasma each, was spiked with erythromycin.
- Group III (n=6), 1 ml plasma each, was spiked with both verapamil and erythromycin.

In addition, drug solutions were added to 3 tubes containing phosphate buffer (pH = 7.4) to determine the presence of any nonspecific binding or adsorption to the micropartition system. In order to approximate the enantiomeric ratio of verapamil observed in rats *in vivo*, drug free plasma was spiked with 300 ng/ml of R-verapamil and 600 ng/ml of S-verapamil. Plasma was also spiked with 14,000 ng/ml erythromycin solutions in order to approximate the *in vivo* plasma concentration of erythromycin. R and S-verapamil hydrochlorides were dissolved in saline to make a final concentration of 300 and 600 ng/ml, respectively. Erythromycin lactobionate was dissolved in injectable water USP to make a final concentration of 14,000 ng/ml.

The samples were then incubated at 37 °C for one hour, in order to allow the drugs to reach equilibrium with the plasma proteins. A 500-µl aliquot of the sample was put into a microparition chamber (Amicon Division of W.R. Grace & Co, Danvers MA). Thereafter, it was centrifuged at 2000 *x g* for 1 hour to obtain ultrafiltrate. The volumes of both the filtrate and the retained liquid were measured and they were kept in a -20 °C freezer until HPLC analysis. The concentration of R and S verapamil as well as erythromycin in each fraction was determined by HPLC. The fraction unbound f_u was determined as the concentration of the drug in the filtrate divided by total concentration. To ensure concentrations were above the minimum quantifiable limit for verapamil HPLC assay, two micropartitions of the six chambers were pooled allowing for a total of three measurements per group.

2.7 HPLC Analysis

2.7.1 Verapamil HPLC

A previously published stereospecific assay of verapamil was used with one modification (68). The ratio of the components of the mobile (hexane -isopropanol-ethanol-TEA) phase were changed from 85: 7.5: 7.5: 0.1 to 90: 5: 5: 0.1 in order to get better separation of the peaks of S verapamil, R verapamil, and glaucine.

To 100 µl of plasma in a glass test tube were added 75 µl of 400 ng/ml (+)-glaucine (internal standard). Extraction was performed by adding 100 µl 2 M sodium hydroxide, 0.4 ml sodium phosphate buffer (pH 7.0, ionic strength 0.1), and 6 ml heptane: hepatoflurobutanol (99:1). The sample was vortexed for 1 min and then centrifuged at 2000 *x g* for 10 min. The organic layer was transferred to a glass tube and evaporated to dryness in a vacuum centrifuge (Savant Speed Vac, Global Medical Instrumentation Inc., Albertville, Minnesota, USA) at 60° C. The resulting residue was reconstituted in 200 µl of mobile phase, and 100 or 150 µl was injected into the chromatograph.

A Waters (Millipore-Waters, Missassuaga, Canada) chromatograph was used consisting of a twin piston pump, a WISP 710B autosampler, a column oven (31° C) and a 470 fluorescence detector set at excitation of 272, emission of 317 nm and bandwidth at 18 nm. The integrator was a Hewlett-Packard (Avondale, PA) 3390 A model. An achiral column (50 mm × 4.6 mm ID Supelcosil LCSi column, Supelco Inc., Bellefonte PA) was serially attached to a chiral column (250 mm × 4.6 mm i.d. 10 µm Chiralpak AD, Daicel Chemical Ind., Tokyo, Japan). The mobile phase was hexane -isopropanol-ethanol-TEA,

90: 5: 5: 0.1, v/v at 0.5 ml/min. The areas of the chromatographic peaks were used to quantify R and S verapamil.

To construct the calibration curves and check the linearity of the method, seven concentrations (0, 20, 50, 100, 500, 1000, and 2000 ng/ml) of racemic verapamil were prepared by adding 200 µl of the appropriate aqueous solutions of verapamil hydrochloride to drug free plasma. The samples were then extracted and injected into the chromatograph as described above. The calibration curve for plasma was constructed so as to cover a range of S and R verapamil content from 20 to 2000 ng/ml. Standard curves were linear over this range ($R^2 \geq 0.98$). The minimum quantifiable concentration was 20 ng/ml with coefficient of variability (CV) of 7.2% for R and 5.4% for S verapamil.

The HPLC assay of veapamil can also be used to measure the concentrations of norverapamil, a major metabolite of verapamil. Although there was no pure norverapamil to use as a reference in our assay, we campared the peaks of norverapamil in the presence and absence of erythromycin.

2.7.2 Erythromycin HPLC

A previously published erythromycin assay was used (76). To 100 µl of plasma in a glass tube were added 100-µl clarithromycin in acetonitrile (internal standard) to give a final concentration of clarithromycin 20 µg/ml plasma. Extraction was performed by adding 100 µl saturated solution of sodium carbonate, 1 ml distilled water and 5 ml chloroform. Samples were vortexed for 5 min then centrifuged at 2000 x *g* for 10 min. 4.5 ml of the organic solvent was transferred to a clean tube and evaporated to dryness in a vacuum centrifuge at 60 °C.

Erythromycin has a low molecular absorptivity as it lacks a suitable chromophore. (76). Alkali treatment of erythromycin produces an α,β-unsaturated ketone with an absorption peak at 236 nm and a molar extinction coefficient sufficiently high to ensure a good response in the UV band. In order to form a chromophore, the extraction residue was made up with 200 µl of a 1 M aqueous solution of sodium hydroxide and kept at 40 °C for 1 h. 200 µl of a 1 M solution of acetic acid was then added to the tubes to obtain a final pH between 4 and 5. After filtration on 0.45 µm cellulose nitrate filters, the sample was injected into the chromatograph.

A Waters (Millipore-Waters, Missassuaga, Canada) chromatograph was used consisting of a twin piston pump, a WISP 712 autosampler, and a reverse phase column. The detector was Shimadzu (Kyoto, Japan) SPD-6A UV spectrophotometric detector set at wavelength of 236 nm. The integrator was a Shimadzu CR601 model. The mobile phase was acetonitrile–ammonium acetate buffer 0.05 M (16: 84, v/v) at 1.2 ml/min. The heights of the chromatographic peaks were used to quantify erythromycin.

To construct the calibration curves and check the linearity of the method, seven concentrations (0, 100, 500, 1000, 5000, 10,000, 20,000 ng/ml) of erythromycin were prepared by adding 200 µl of the appropriate acetonitrile solutions of erythromycin to drug free plasma. The samples were then extracted and injected into the chromatograph as described above. The calibration curves were constructed so as to cover a range of erythromycin content from 100 to 20,000 ng/ml. Standard curves were linear over this range ($R^2 \geq 0.98$). The minimum quantifiable concentration was 100 ng/ml with coefficient variability (CV) of 4.3%.

2.8 Data Analysis

The variability in the observed values of PR and QT intervals was high. In order to minimize the variability from the baseline values of PR and QT intervals, the percent of change in the intervals were calculated and plotted *vs.* time.

For ECG analysis, the interval measurements were conducted by averaging three cycles. The percent of change of PR and QT intervals was calculated from the differences observed between the baseline and post treatment values. The percent change of PR and QT intervals was plotted *vs.* time and the area under the percent effect-time curve (AUEC) was calculated using the trapezoidal rule.

$$AUEC_{0-last} = \sum AUEC_{i-i+1} = \sum \frac{(E_i + E_{i+1})(t_{i+1} - t_i)}{2}$$

Where E_i is effect at time *i*, t_i is time *i*

2.8.1 Pharmacokinetic analysis

Standard methods were used to calculate pharmacokinetic parameters such as the area under the plasma concentration curve, the total body clearance and terminal half-life (77).

The terminal half-life in each animal was obtained from the slope of the terminal phase (β):

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

The total area under the plasma concentration-time curve from time zero up to the last measured time ($AUC_{0\text{-last}}$) in plasma was calculated in each animal by the trapezoidal

rule extrapolation method, which employs the logarithmic trapezoidal rule for the calculation of the area during the declining plasma level phase.

$$AUC_{0-last} = \sum AUC_{t_i - t_{i+1}} = \sum \frac{(C_i + C_{i+1})(t_{i+1} - t_i)}{2}$$

Where C_i is concentration at time i, t_i is time i

The area from the last data point to time infinity ($AUC_{last-\infty}$) was estimated by dividing the last measured plasma concentration by the terminal rate constant.

$$AUC_{last-\infty} = \frac{C_{last}}{\beta}$$

The total body clearance was calculated in each animal from this equation:

$$CL_{TB} = \frac{Dose}{AUC}$$

2.8.2 Statistical Analysis

A P value of less than 0.05 was considered to be statistically significant using the Student t-test for unpaired data when comparing two means, and the Analysis of Variance (ANOVA) when comparing more than two means. All results are expressed as mean ± standard deviation (SD).

CHAPTER THREE

RESULTS

3.1 Pharmacodynamic Study

The baseline values for ECG parameters in rats were 69.2 ± 5.8 ms (milliseconds) for PR interval and 52.5 ± 11.4 ms for QT interval. There was no significant difference between the three groups in their baseline values of PR and QT intervals and no arrhythmia recorded during the baseline measurement. The ECG parameters obtained in our experiments are presented in three different ways:

- Observed values in milliseconds.
- Percent of change from baseline.
- The area under percentage effect-time curve.

3.1.1 Observed Values

The observed values of PR and QT intervals were plotted *vs.* time. Figures 3.1 and 3.2 show the time-course of PR and QT intervals, respectively.

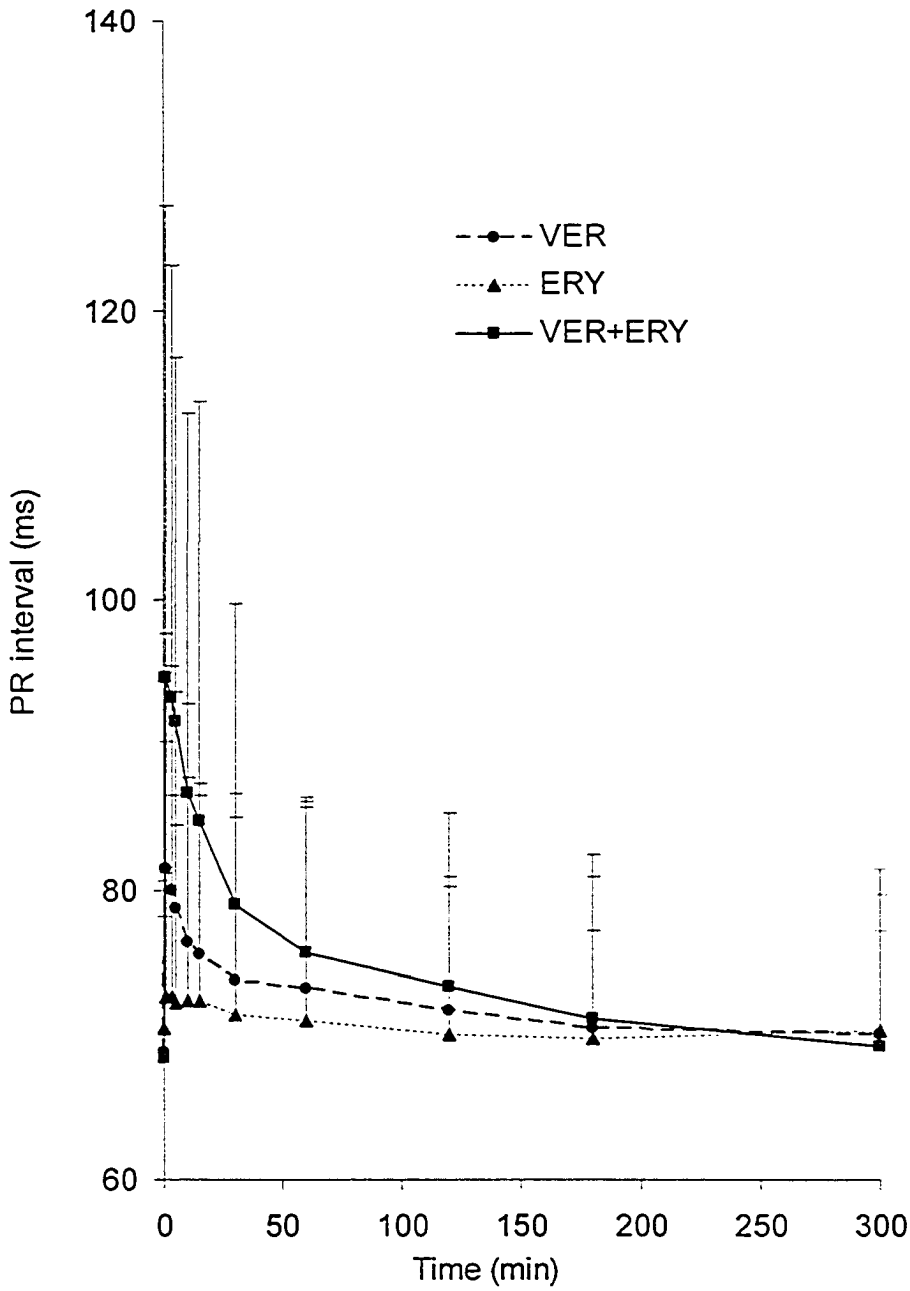

Figure 3.1. The PR interval *vs.* time in three groups of rats: group I was given 100 mg/kg erythromycin *i.v.*, group II was given 1 mg/kg verapamil *i.v.*, and group III was given 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.* Values are means ± SD.

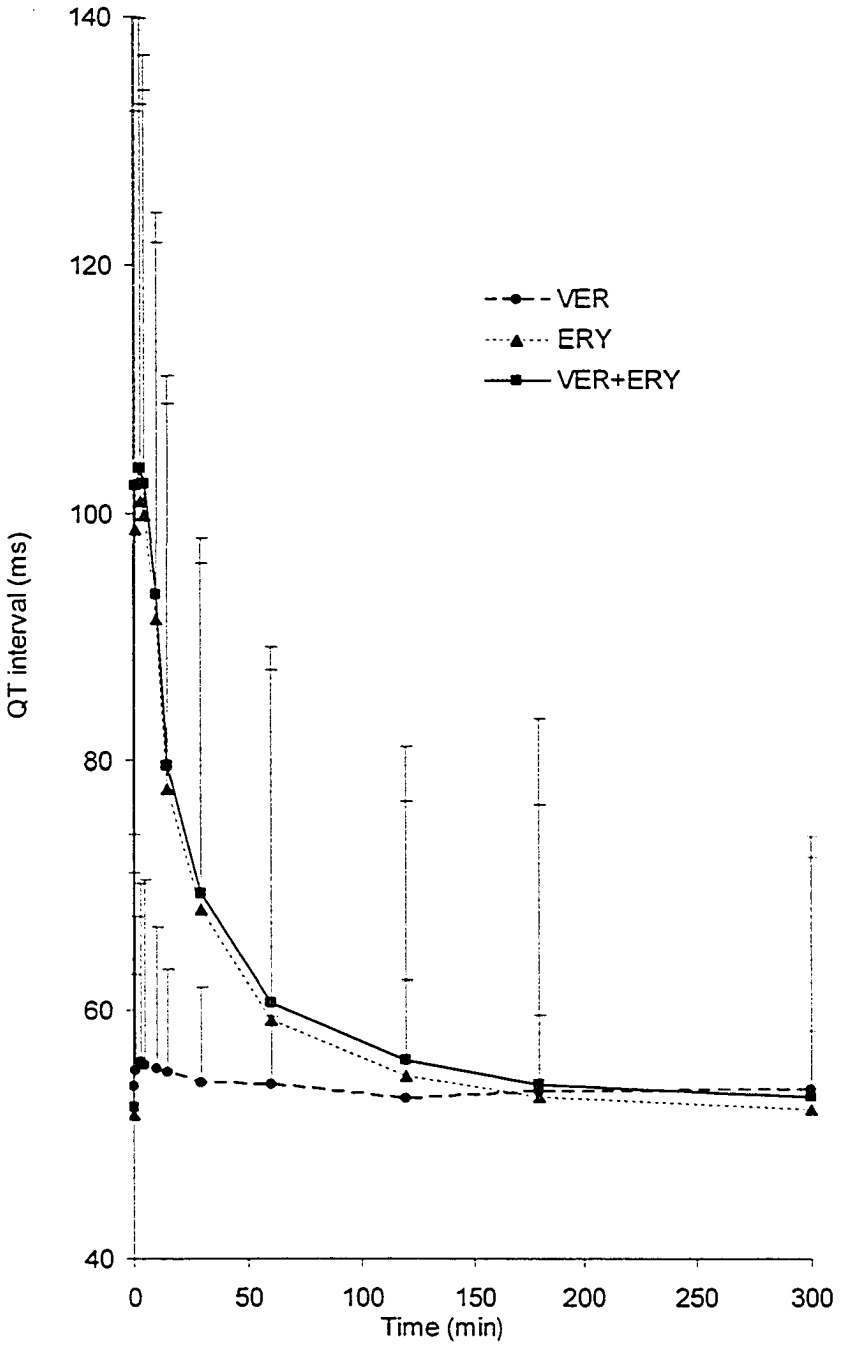

Figure 3.2. QT interval *vs.* time in three groups of rats: group I was given 100 mg/kg erythromycin *i.v.*, group II was given 1 mg/kg verapamil *i.v.*, and group III was given 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.* Values are means ± SD.

3.1.2 Percent of Change from Baseline

The time course of the percent of change in PR and QT intervals from baseline is shown in Figures 3.3 and 3.4, respectively. Erythromycin alone (100 mg/kg intravenously) resulted in a significant ($P < 0.05$) prolongation of QT interval from baseline at times 1, 3, 5, 10, 15, 30, 60, 90, and 120 minutes but had no significant effect on PR interval at any time point . Verapamil alone (1 mg/kg intravenously) resulted in a significant ($P < 0.05$) prolongation PR interval from baseline at times 1, 3, 5, 10, 15, 30, 60, 90, and 120 minutes but had no significant effect on QT interval at any time point. However, the combination of these two drugs caused a significant ($P < 0.05$) increase in PR interval prolongation at times 1, 3, 5, 10, 15, and 30 minutes compared to when verapamil was given alone. This combination had no significant effect on erythromycin induced prolongation of QT interval.

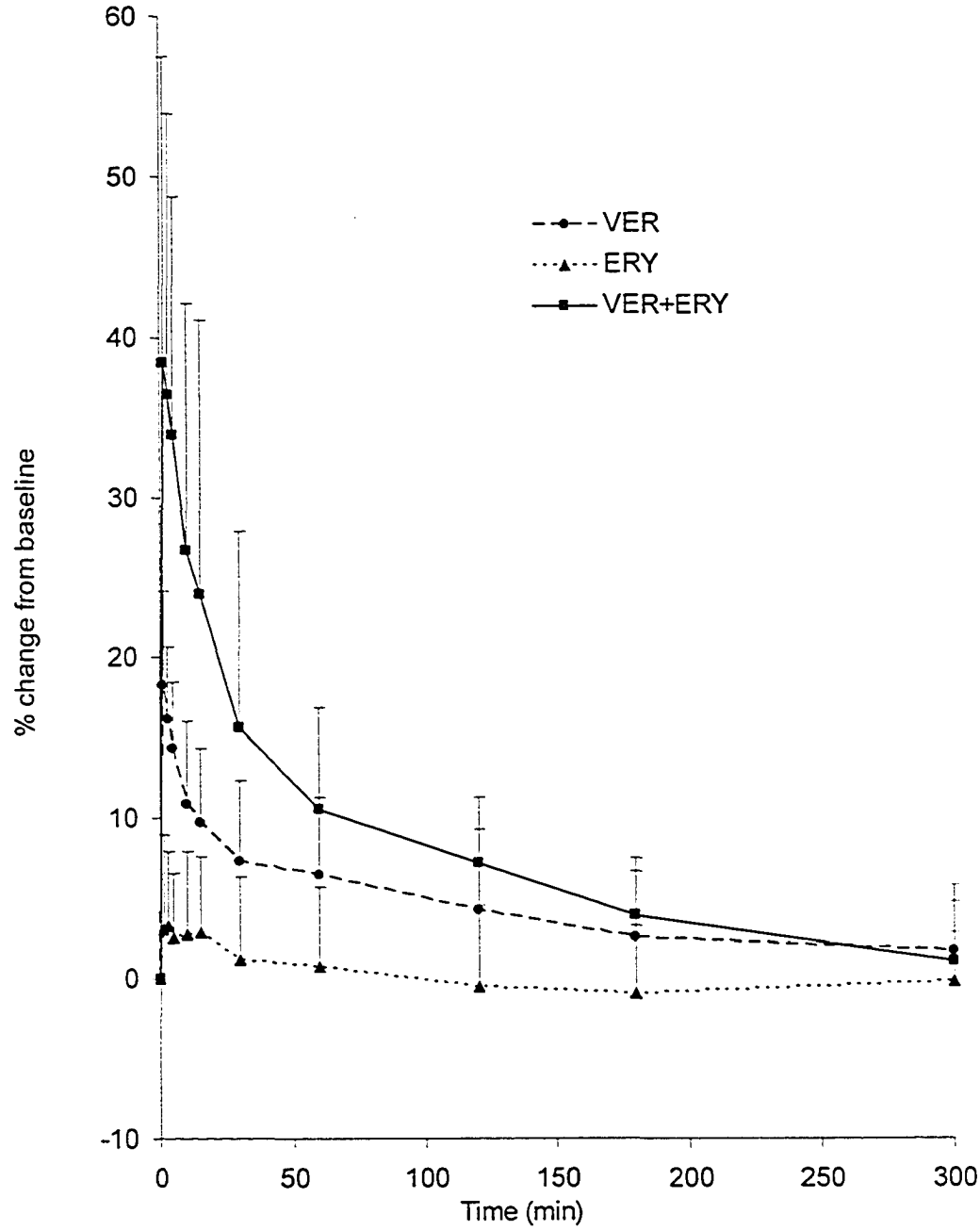

Figure 3.3. The percent of change in PR interval from baseline *vs.* time in three groups of rats: group I was given 100 mg/kg erythromycin *i.v.*, group II was given 1 mg/kg verapamil *i.v.*, and group III was given 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.* Values are means ± SD.

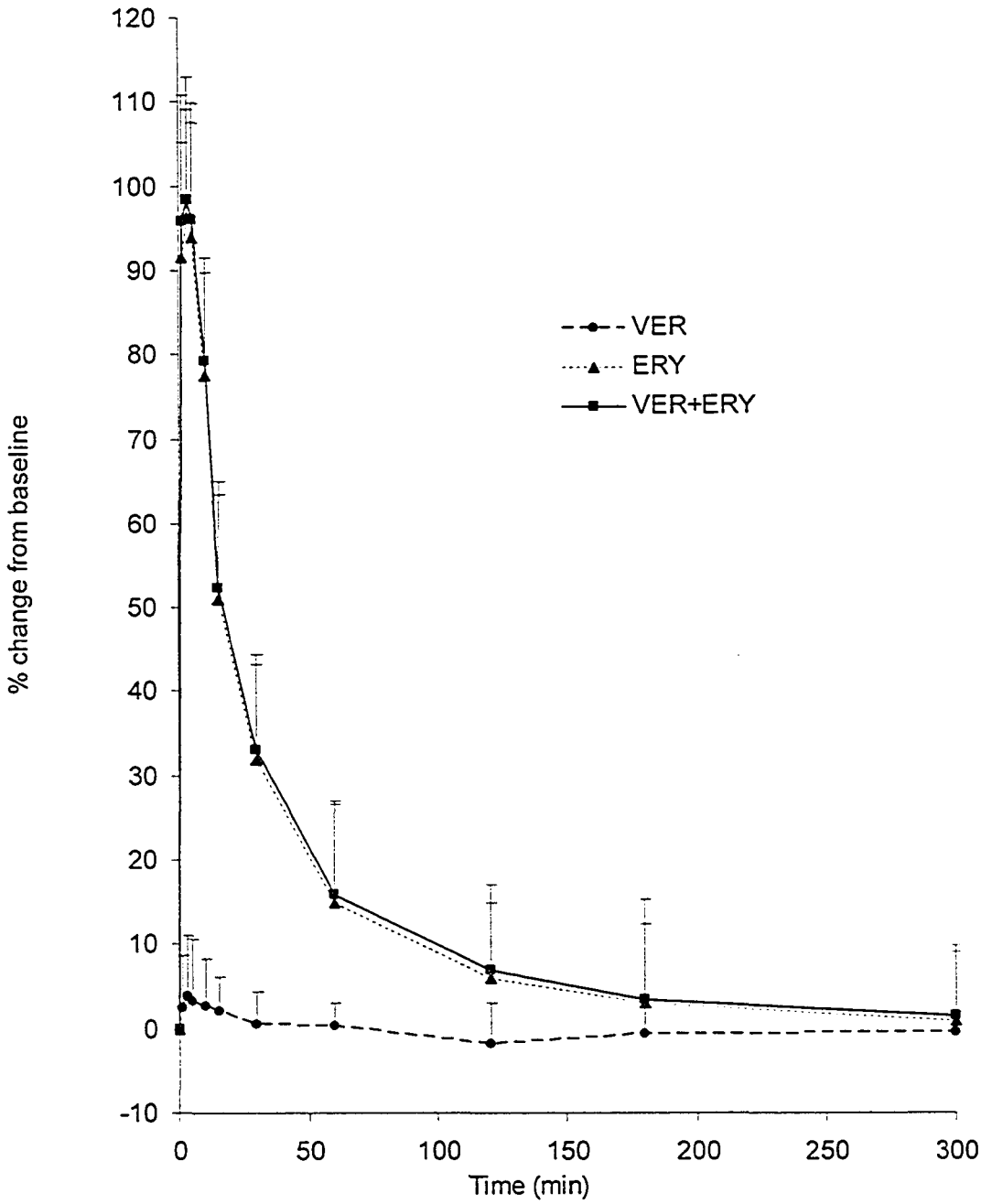

Figure 3.4. The percent of change in QT-interval from baseline *vs.* time in three groups of rats: group I was given 100 mg/kg erythromycin *i.v.*, group II was given 1 mg/kg verapamil *i.v.*, and group III was given 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.* Values are means ± SD.

The maximum changes from baseline in cardiovascular indices were also calculated for each treatment group (Table 3.1). Erythromycin alone resulted in a maximum prolongation of 96.2 ± 12.8 percent in QT interval from baseline but had no significant effect on PR interval. Verapamil alone resulted in a maximum prolongation of 18.3 ± 7.4 percent in PR interval from baseline but had no significant effect on QT interval. The concomitant administration of erythromycin and verapamil to caused a 38.4 ± 19.1 percent increase in PR interval prolongation compared to 18.3 ± 7.4 when verapamil was administered alone and 3.3 ± 4.6 percent when erythromycin was administered alone. This is about a 2-fold synergetic increase in the prolongation of PR interval when both drugs are combined.

Table 3.1. Changes in cardiovascular indices in 3 groups of rats: group I received 100 mg/kg erythromycin *i.v.*, group II received 1 mg/kg verapamil *i.v.*, and group III received 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.* PR and QT interval values represent the percent of maximum change from baseline (Mean ± SD).

Cardiovascular Indices	**Verapamil (n=6)**	**Erythromycin (n= 6)**	**Erythromycin and Verapamil (n=6)**
Maximum percentage PR prolongation	18.3 ± 7.4[a]	3.3 ± 4.6	38.4 ± 19.1[a, b]
Maximum percentage QT prolongation	3.8 ± 7.2	96.2 ± 12.8[a]	98.4 ± 14.5[a]
AV node block	Nil	Nil	5 out of 6[a, b]

[a] Significantly different from baseline; [b] Significantly different from other groups ($\alpha = 0.05$).

In addition to the synergistic increase of PR interval, the combination of verapamil and erythromycin caused an 83% incidence of second-degree atrioventricular (AV) node block. This AV block was not observed when either of these drugs was administered alone. Figure 3.5 shows an example of rat ECG with AV block.

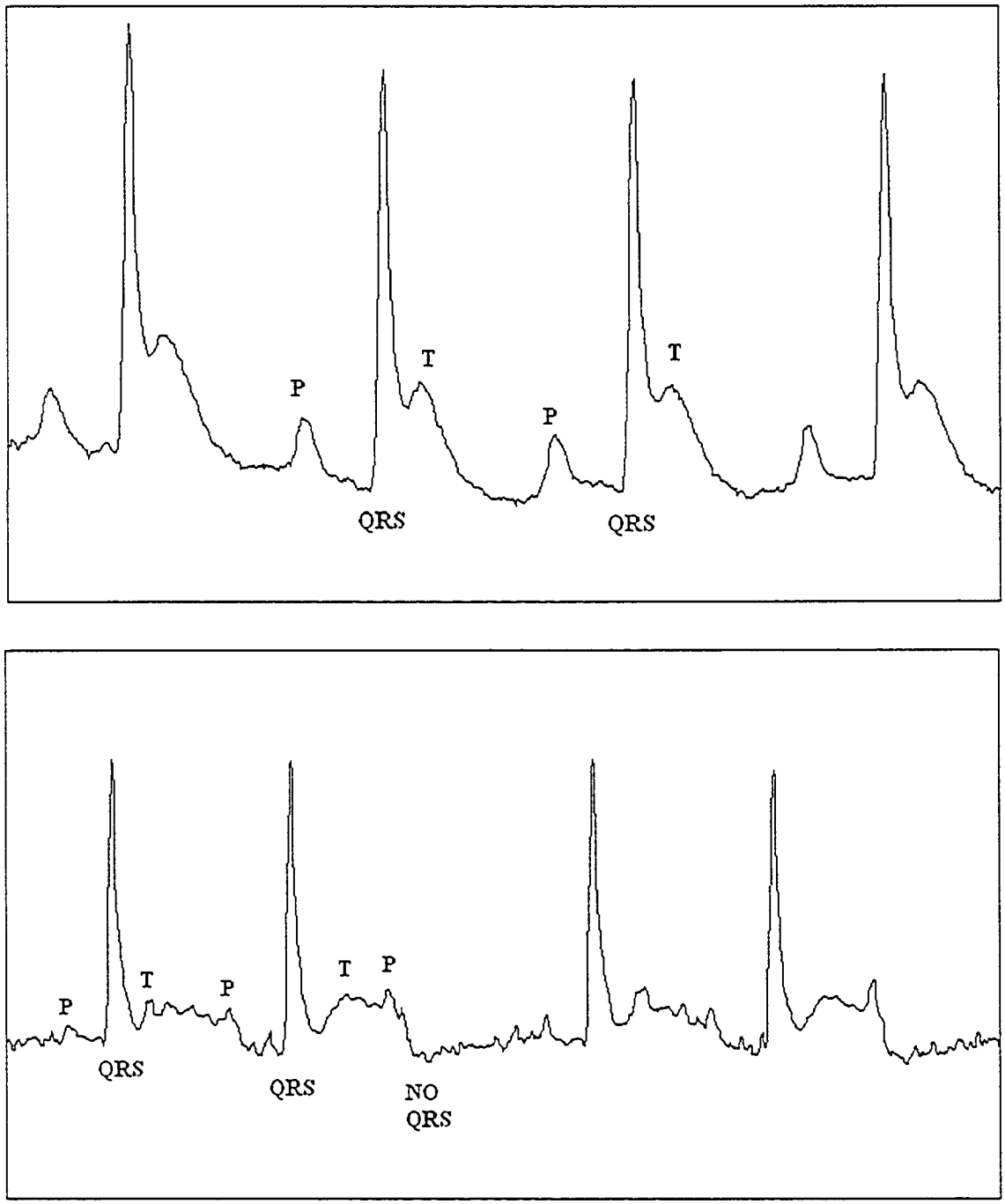

Figure 3.5. Rat Electrocardiogram (ECG): *Top*: Normal ECG, showing P, Q, R, S, and T waves. *Bottom:* AV block, showing some P-waves that were not followed by QRS complexes.

3.1.3 The Area Under percentage-Effect-time Curve (AUEC)

The area under percentage PR and QT prolongation-time curve were calculated for each group. Figure 3.6 compares the area under percentage effect-time curve from time zero to time 300 minutes ($AUEC_{0\text{-}300}$) for PR and QT interval prolongation in the three rat groups. The administration of verapamil caused a significant (21.6 ± 5.8 hours) increase in $AUEC_{0\text{-}300}$ for PR interval but had no significant effect on $AUEC_{0\text{-}300}$ for QT interval. Erythromycin, on the other hand, caused a significant (60.7 ± 15.9 hours) increase in $AUEC_{0\text{-}300}$ for QT interval but had no effect on $AUEC_{0\text{-}300}$ for PR interval. The combination of erythromycin and verapamil caused a synergetic increase (38.3 ± 9.2 hours) in $AUEC_{0\text{-}300}$ for PR interval.

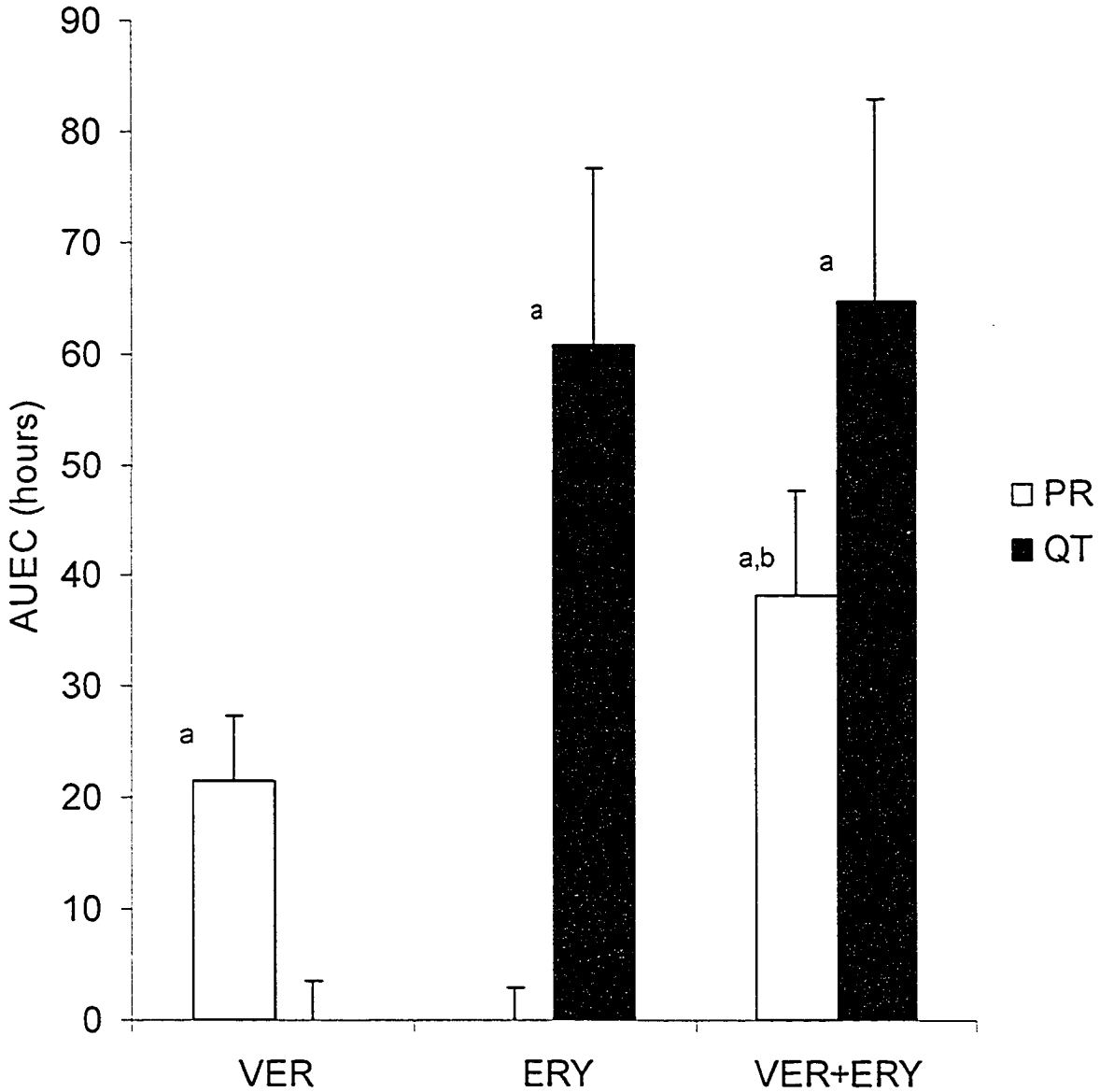

Figure 3.6. The area under percentage PR and QT prolongation-time curve from time zero to 300 min indices in 3 groups of rats: group I received 100 mg/kg erythromycin *i.v.*, group II received 1 mg/kg verapamil *i.v.*, and group III received 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.*

[a] Significantly different from baseline; [b] Significantly different from other groups.

3.2 Pharmacokinetic and Protein Binding Studies.

The mean plasma concentration-time profile of R and S verapamil after the *i.v.* administration of 1 mg/kg racemic verapamil alone or with 100 mg/kg *i.v.* erythromycin are shown in Figures 3.7 and 3.8. There was no significant difference in R or S verapamil concentrations between these two groups at any time point. The plasma concentrations of R and S verapamil at time 300 minute were below the minimum quantifiable concentration of our assay (20 ng/ml). Therefore, these values were not shown in our results.

The mean plasma concentration-time profile of erythromycin after *i.v.* administration of erythromycin alone or erythromycin plus verapamil is shown in Figure 3.9. There was no significant difference in erythromycin concentrations between these two groups at any time point.

The pharmacokinetic parameters of both enantiomers of verapamil after the *i.v.* administration of racemic verapamil alone, or with erythromycin are displayed in Table 3.2. Because there were only two concentrations in the final phase, parameters like half-life and total body clearance could not be calculated for verapamil. There was no significant difference in the area under the curve ($AUC_{0\text{-}180}$) and the fraction unbound (f_u) between the verapamil groups.

For norverapamil, there was no change in the area of its peak between the two groups of rats.

The pharmacokinetic parameters of erythromycin after the *i.v.* administration of erythromycin alone, or with verapamil are displayed in Table 3.3. There was no significant difference in the elimination half-life ($t_{1/2}$), total body clearance (CL_{TB}), the

area under the curve ($AUC_{0-\infty}$), or fraction unbound (f_u) between the two erythromycin groups.

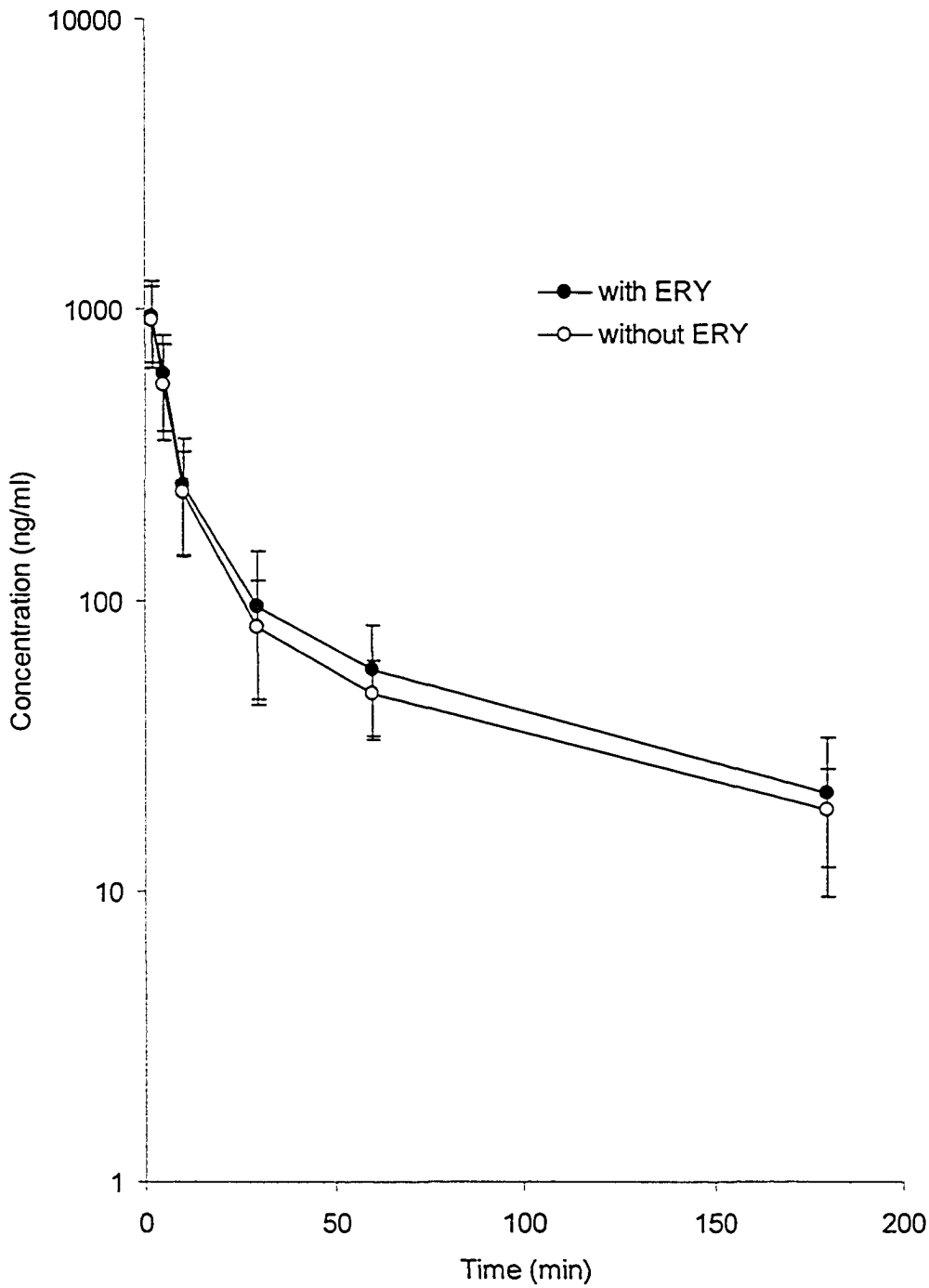

Figure 3.7. The plasma concentration-time profile for S-verapamil in two groups of rats: one received 1 mg/kg verapamil *i.v.* (o), and the other received 100 mg/kg erythromycin *i.v.* and 10 minutes later received 1 mg/kg verapamil *i.v.* (•). Each point is the group mean ± SD.

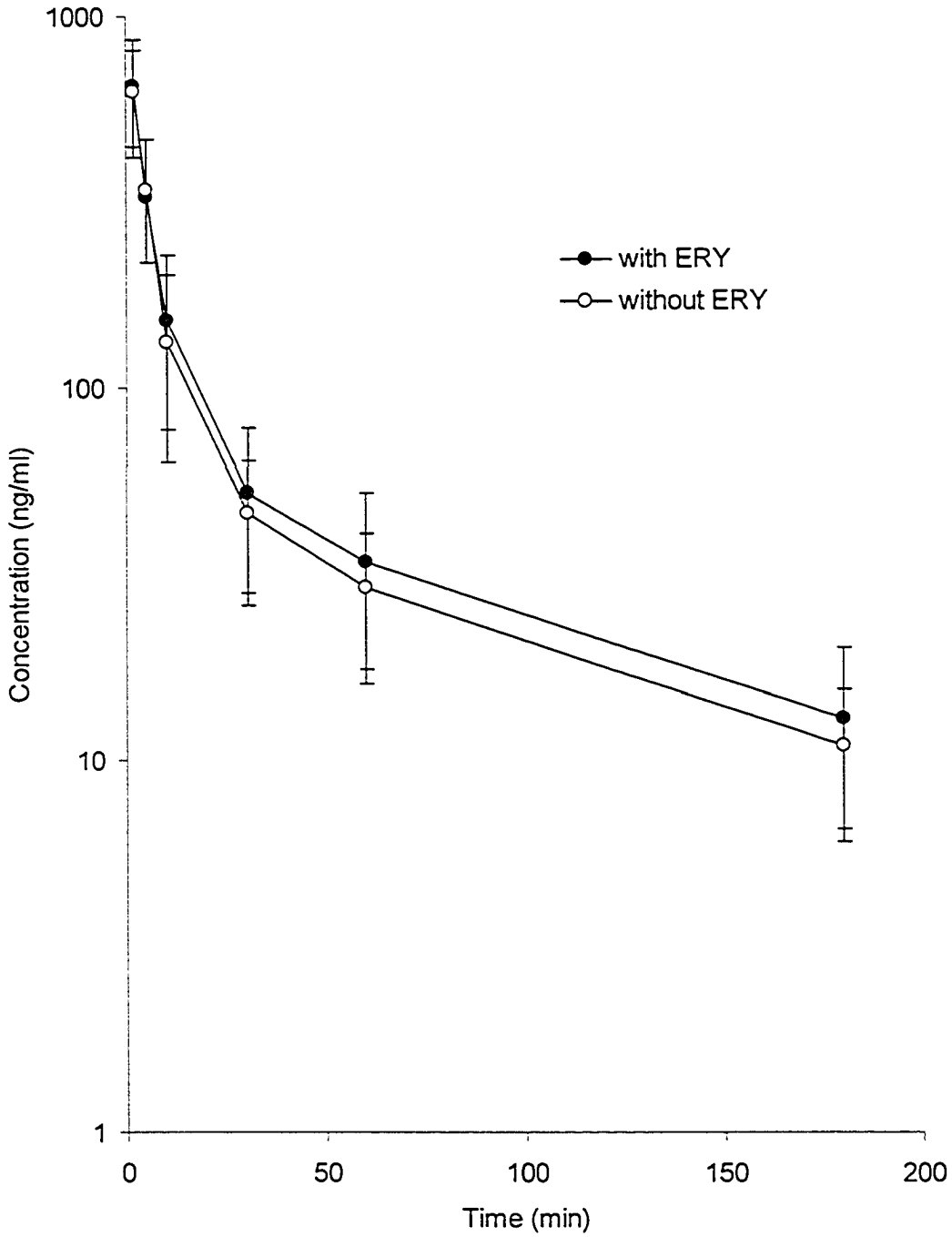

Figure 3.8. The plasma concentration-time profile for R-verapamil in two groups of rats: one received 1 mg/kg verapamil *i.v.* (o), and the other received 100 mg/kg erythromycin *i.v.* and 10 minutes later received 1 mg/kg verapamil *i.v.* (•). Each point is the group mean ± SD.

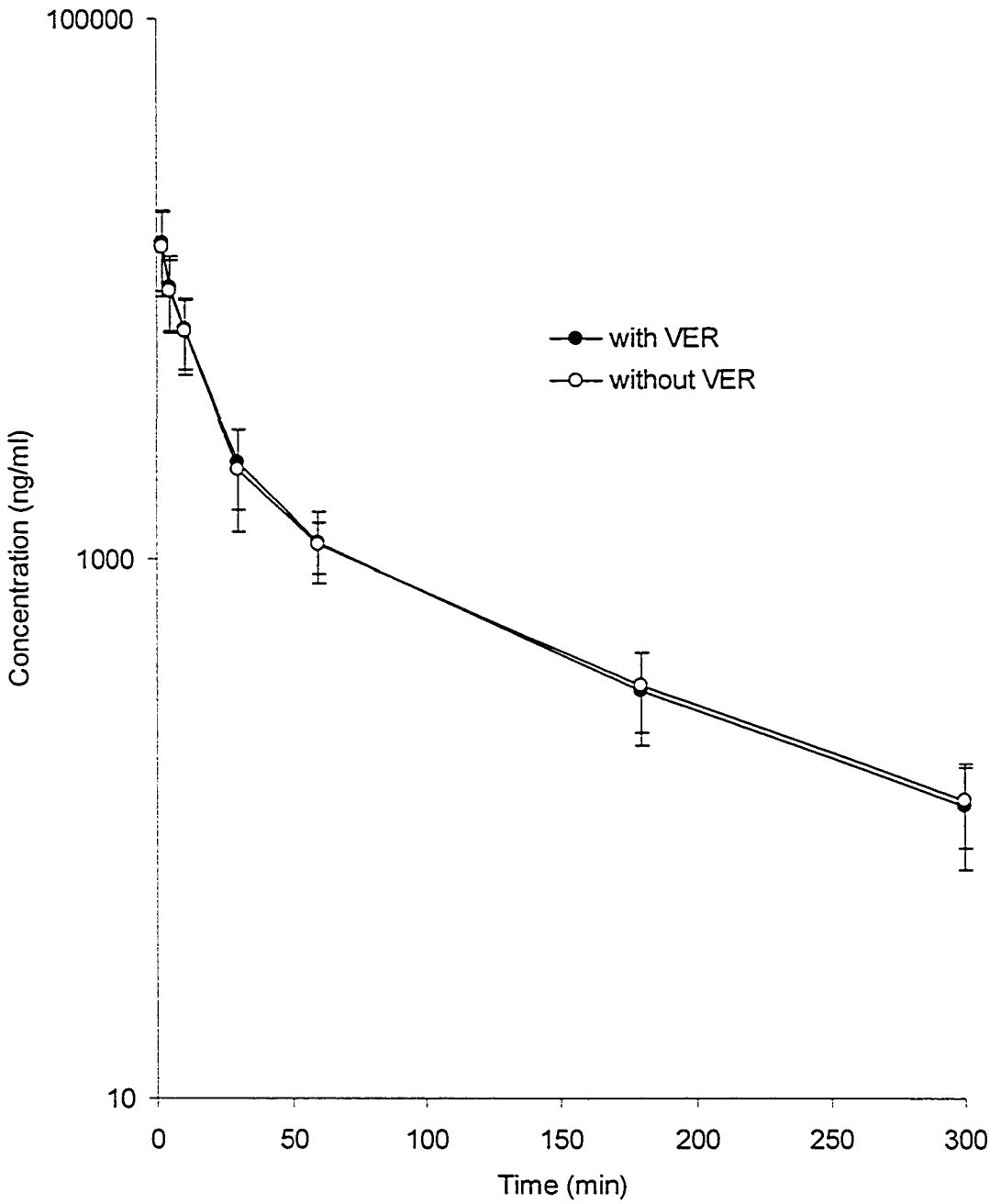

Figure 3.9. The plasma concentration-time profile for erythromycin in two groups of rats: one received 100 mg/kg erythromycin *i.v.* (o), and the other received 100 mg/kg erythromycin *i.v.* and 10 minutes later received 1 mg/kg verapamil *i.v.* (•). Each point is the group means ± SD.

Table 3.2. Pharmacokinetic parameters (mean ± SD) of verapamil enantiomers in two groups of rats: one received 1 mg/kg verapamil *i.v.*, and the other received 100 mg/kg erythromycin *i.v.* and 10 minutes later received 1 mg/kg verapamil *i.v.*

Pharmacokinetic Parameters	S Verapamil		R Verapamil	
	Control	With Erythromycin	Control	With Erythromycin
$AUC_{0\text{-}180}$ (ng.h/ml)	255 ± 87	280 ± 92	158 ± 65	166 ± 73
f_u	0.095 ± 0.052	0.087 ± 0.039	0.201 ± 0.076	0.215 ± 0.091

$AUC_{0\text{-}180}$: the area under the plasma concentration-time curve from time zero to 180 min; f_u: the fraction unbound in plasma.

Table 3.3. Pharmacokinetic parameters (mean ± SD) of erythromycin in two groups of rats: one received 100 mg/kg erythromycin *i.v.*, and the other received 100 mg/kg erythromycin *i.v.* and 10 minutes later 1 mg/kg verapamil *i.v.*

Pharmacokinetic Parameters	Control	With verapamil
$t_{1/2}$ (min)	154 ± 41	149 ± 48
CL_{TB} (ml/min)	79.6 ± 18.1	78.2 ± 22.5
$AUC_{0-\infty}$ (ng.h/ml)	6220 ± 490	6410 ± 570
f_u	0.513 ± 0.161	0.481 ± 0.184

$t_{1/2}$: Terminal half-life.; CL_{TB}: Total body clearance; $AUC_{0-\infty}$: the area under the plasma concentration-time curve from time zero to infinity; f_u: the fraction unbound in plasma.

CHAPTER FOUR

DISCUSSION

4.1 Discussion

There are a few case reports in the literature describing an interaction between macrolide antibiotics and calcium channel blocker, verapamil (1, 70, 71). In two of these cases, a patient taking verapamil for long term developed symptoms of erythromycin or verapamil toxicity 2 to 7 days after the initiation of a macrolide antibiotic (19, 70). In the third report, a patient developed hypotension and bradycardia 48 hours after starting a daily regimen of clarithromycin and verapamil (71). Since verapamil and macrolides are potent inhibitors of CYP3A4 and P-glycoprotein, pharmacokinetic interaction was assumed to be a contributor to this interaction. It was speculated that concomitant administration of these drugs has increased the absorption and reduced the metabolism as a result of P-glycoprotein and CYP3A4 inhibition, leading to elevated plasma concentrations and toxicity. Since plasma concentrations of verapamil and erythromycin were not measured in these case reports, these hypotheses could not be confirmed.

Using the rat as a model, we studied the effect of concomitant administration of verapamil and erythromycin on cardiovascular indices such as PR and QT intervals. While case reports were in patients who received multiple doses of these drugs before developing symptoms of interaction, our study investigated the acute model of the interaction by giving single doses of erythromycin and verapamil. In order to rule out the effect of pre-systemic metabolism and transport, erythromycin and verapamil were administered intravenously.

In the rat, we have observed that a single dose of intravenous erythromycin can prolong QT interval but does not affect PR interval. This was shown by the significant increase in the maximum change in QT interval (Table 3.1) and the AUEC of QT interval

(Figure 3.6). This observation in rats was in accordance of human studies showing that the electrophysiological effects of erythromycin are caused by the blockade of HERG potassium channels, causing prolongation in QT interval and *torsade de pointes*, especially after its intravenous administration (47).

On the other hand, a single dose of intravenous verapamil prolonged PR interval with no significant effect on QT interval in rats. This was shown by the significant increase in the maximum change in PR interval (Table 3.1) and the AUEC of PR interval (Figure 3.6). This is in agreement with human studies showing that the electrophysiological effects of verapamil are caused by the blockade of L-type calcium channels, resulting in slowing cardiac conduction time by depressing atrioventricular (AV) node, which is manifested by PR interval prolongation and, in high dose, AV block (11).

The above studies show that the electrophysiological effects of verapamil and erythromycin in rats are consistent with human data. They also show that verapamil and erythromycin cause different electrophysiological effects in this rat model. Verapamil blocks L-type calcium channels and erythromycin can block HERG potassium channels (10, 34, 40). Therefore, ECG changes caused by erythromycin and verapamil do not interfere with each other. This renders the rat as a suitable model to investigate the interaction of these two drugs.

In the pharmacodynamic study, single doses of intravenous erythromycin and verapamil were administered alone or together to different groups of rats and their ECGs were monitored at baseline and up to 5 hours post drug injection. The combination of these two drugs did not alter erythromycin-induced prolongation of QT interval (Figure

3.4 and Table 3.1). On the other hand, this combination caused a significant increase in the effect of verapamil on PR interval, as shown by the percent of maximum change in PR interval (Table 3.1) and the $AUEC_{0\text{-}300}$ of PR interval (Figure 3.6). In fact, AV node block (Figure 3.5), a side effect of verapamil, was evident following the combination therapy. This indicates that the undesired effect of the observed interaction stems from an enhanced potency of verapamil and not erythromycin.

There are several possible mechanisms to explain the way erythromycin enhances the actions of verapamil. The interaction can be a result of altered pharmacokinetics, pharmacodynamics, or both.

One possible pharmacokinetic mechanism is that erythromycin inhibits the metabolism and/or transport of verapamil, leading to increased plasma concentrations of the later, which results in verapamil toxicity, shown by PR prolongation and AV block. Elevated plasma concentrations of verapamil or erythromycin were proposed in the literature to explain the previous case reports of interaction (19, 70, 71). Erythromycin and verapamil are potent inhibitors of cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein (26). In fact, many drug-drug interactions caused by erythromycin and verapamil are due to increased plasma concentration of drugs that are P-glycoprotein or CYP3A4 substrates (52,59). However, the speculations that the interaction between verapamil and erythromycin were due to a pharmacokinetic alteration were not substantiated with measured plasma concentrations of these drugs.

In our pharmacokinetic study, plasma concentrations of verapamil and erythromycin were measured after they were given alone or concomitantly in rats. The time courses of neither verapamil nor erythromycin were altered when they were

coadministered (Figures 3.7, 3.8, and 3.9). Accordingly, no pharmacokinetic parameter of either drug was affected (Tables 3.2 and 3.3).

An important metabolite of verapamil is norverapamil, which has less dromotropic effect compared to verapamil. So in order for norverapamil to cause such an increase in the dromotropic effect, its concentration must increase dramatically. We used our HPLC assay of veapamil to detect if there are any changes in the concentrations of norverapamil. Norverapamil appeared as very small to negligible in all our samples. This was expected since verapamil was administered intravenously when there is less formation of norverapamil is expected. There was no change in areas of the peaks of norverapamil in the presence and absence of erythromycin. Therefore, norverapamil did not play a role in this interaction.

Another possible mechanism at the level of pharmacokinetics in that erythromycin might displace verapamil from its protein binding positions, causing increased plasma free fraction, and therefore verapamil toxicity. In order to test the possibility of protein binding interaction between verapamil and erythromycin, we performed an *in vitro* protein binding experiment using an ultrafiltration method. Drug-free plasma was spiked with drug concentrations similar to those observed *in vivo*. After incubation, the concentrations of these drugs in the filtrate were measured the free fraction was calculated for each drug. We found no significant difference in the plasma free fraction of verapamil and erythromycin due to their combination (Tables 3.2 and 3.3). Studies have shown that, in humans, verapamil binds extensively to α1-acid-glycoprotein (11). Erythromycin also binds to the same protein but to a lesser extent (74). The fact that the plasma free fraction of both of these drugs did not change as a result of

their combination shows that either these two drugs bind to different positions of α1-acid-glycoprotein or that the concentrations of these drugs was not enough to cause binding saturation and therefore displacement of one of the drugs at the expense of the other.

Verapamil is a drug with high extraction ratio, therefore, its hepatic metabolism is depends mainly on hepatic blood flow. Changes in intrinsic clearance such as inhibition of hepatic metabolizing enzymes are not expected to have significant impact on the clearance of verapamil. This may partially explain the absence of changes in plasma concentrations of verapamil after the coadministration of enzyme inhibitor, erythromycin. However, when verapamil is given orally, its oral bioavailability can be affected by enzyme inhibitors, such as erythromycin. Therefore, it is possible that there were some changes in plasma concentrations in human case reports, because patients were taking verapamil orally.

The route of drug administration in our model was in the intravenous, which might have minimized the effect on drug transport and metabolism by eliminating the first-pass effects. Furthermore, giving a single dose of erythromycin, a drug that inhibits the CYP3A metabolism in a mechanism based manner, might not be enough to cause significant inhibition of the metabolism of verapamil. Since there was a significant interaction after giving single doses of erythromycin and verapamil intravenously, this interaction might have been more severe if these drugs were given in multiple oral doses.

The reported interactions in the literature were in patients receiving multiple oral doses of macrolides and verapamil (19, 70, 71). Therefore, it is possible that in these

case reports there was an inhibition of cytochrome P-450 metabolism and/or P-glycoprotein transport resulting in elevated plasma concentrations of one or both drugs.

The enhanced activity of verapamil in the presence of erythromycin can be explained at the pharmacodynamic level. Both erythromycin and verapamil can block human ether-a-go-go-related gene (HERG) potassium channels. Suppression of HERG channels causes action potential and QT interval prolongation. Erythromycin and other macrolides can block HERG K-channel, resulting in QT prolongation (34,40). Verapamil is also a potent blocker of HERG K-channel in the heart and alveolar epithelial cells (14, 15). However, the *in vitro* inhibition of HERG K-channels by verapamil was at concentrations much higher than those observed *in vivo*. This mechanism cannot explain the interaction in our model because the combination of verapamil and erythromycin did not alter the prolongation of QT interval that is caused by HERG K-channel blockage but it rather potentiated the calcium channel blockage resulting in synergetic PR interval prolongation and AV block. The blockage of HERG K-channels can be used to explain the case report in the literature where QT interval prolongation was observed after concomitant administration of erythromycin and verapamil (19).

A potential role of P-glycoprotein at calcium channel site may explain the enhanced potency of verapamil in the presence of erythromycin. Erythromycin can inhibit P-glycoprotein at the site of the calcium channel protein targets, causing increased accumulation of verapamil in cardiac tissue, resulting in a pharmacodynamic interaction between erythromycin and verapamil. Studies indicate that the phenylalkylamine protein target is located on the intracellular surface of the membrane (21, 75). This implies that a typical phenylalkylamine such as verapamil must enter the transmembrane pore of the

calcium channel in order to bind to the intracellular site occluding the pore. Studies have also shown that P-glycoprotein is present in cardiac tissue and plays a role in limiting the access of some P-glycoprotein substrates to inside the cardiac cells (78, 79). Erythromycin is a potent inhibitor of P-glycoprotein and some of the interactions of erythromycin are due to its inhibition of P-glycoprotein substrates, such as its interaction with digoxin and carbamazepine (52, 53). The inhibition of cardiac P-glycoprotein has been used in the literature to explain some other interactions. Lin used this mechanism to explain the interaction between verapamil and digoxin (46). He suggested that verapamil, a P-glycoprotein inhibitor, might inhibit the cardiac P-glycoprotein, causing increased accumulation of digoxin in cardiac tissue resulting in a pharmacodynamic interaction between verapamil and digoxin (46). However, our current data cannot confirm this theory.

Another possible explanation to the way erythromycin enhances the activity of verapamil at the level of pharmacodynamics is by suppressing inflammation. Giving erythromycin, an inflammatory modulator, to cannulated rats may potentiate the electrophysiological effects of verapamil. Cannulation of jugular veins can increase the levels of pro-inflammatory cytokines. Studies have shown that plasma samples obtained from an intravenous catheter contained increased levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) compared to samples obtained by a simple needle stick (80). In another study, the levels of IL-6 were elevated when blood samples were taken *via* an intravenous catheter starting at 3 hrs after cannulation and reaching a 10-fold high after 24 hours (81).

Erythromycin can modulate inflammatory responses (36). Oral and intravenous erythromycin can reduce the concentrations of IL-6 and TNF-α induced by heat killed *Streptococcus pneumoniae* in human whole blood *ex-vivo* (62). Erythromycin can also function as an immunomodulator by suppressing the activity of matrix metalloproteinase-9 (MMP-9), an enzyme that degrades various components of extracellular matrix (63, 64). The suppressive effect of erythromycin on MMP-9 activity is one of the anti-inflammatory mechanisms that inhibit the migration of inflammatory cells into the inflammatory site.

The dromotropic effect of verapamil, measured by PR interval prolongation, can be reduced during inflammation in humans and rats (16, 68). So, if cannulated rats are treated with erythromycin, they might have reduced levels of pro-inflammtory cytokines, such as IL-6 and TNF-α. Therefore, when these rats are given verapamil, they may show more dromotropic activity than rats not treated with erythromycin. This can explain why giving erythromycin, an inflammatory modulator, to cannulated rats can potentiate the pharmacodynamic effects of verapamil.

In order to extrapolate the above to humans, we observed that patients who have experienced an interaction between verapamil and a macrolide antibiotic had multiple cardiac and other diseases, making them more prone to elevated levels of pro-inflammatory mediators. Therefore, an inflammatory suppressor like erythromycin or clarithromycin may have potentiated the activity of verapamil and resulted in these interactions.

4.2 Conclusions

- The rat is a suitable model to study the interaction between erythromycin and verapamil.
- The undesired effect of the observed interaction stems from an enhanced potency of verapamil and not erythromycin, which is similar to reported human data.
- The interaction of erythromycin and verapamil appears to be a pharmacodynamic not a pharmacokinetics interaction.
- Patients receiving both verapamil and macrolide antibiotics should be monitored, especially those who have multiple conditions.

CHAPTER FIVE

REFERENCES

5.1 References

1. Juurlink DN, Mamdani M, Kopp A, Laupacis A, Redelmeier DA. Drug-drug interactions among elderly patients hospitalized for drug toxicity. JAMA. 2003 Apr 2; 289(13): 1652-8.
2. Gandhi TK, Weingart SN, Borus J, Seger AC, Peterson J, Burdick E, Seger DL, Shu K, Federico F, Leape LL, Bates DW. Adverse drug events in ambulatory care. N Engl J Med. 2003 Apr 17; 348(16): 1556-64.
3. Bjorkman IK, Fastbom J, Schmidt IK, Bernsten CB. Drug-drug interactions in the elderly. Ann Pharmacother. 2002 Nov; 36(11): 1675-81.
4. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA. 1998 Apr 15; 279(15): 1200-5.
5. Routledge PA, O'Mahony MS, Woodhouse KW. Adverse drug reactions in elderly patients. Br J Clin Pharmacol. 2004 Feb; 57(2): 121-6.
6. Peng CC, Glassman PA, Marks IR, Fowler C, Castiglione B, Good CB. Retrospective drug utilization review: incidence of clinically relevant potential drug-drug interactions in a large ambulatory population. J Manag Care Pharm. 2003 Nov-Dec; 9(6): 513-22.
7. Carter BL, Lund BC, Hayase N, Chrischilles E. The extent of potential antihypertensive drug interactions in a Medicaid population. Am J Hypertens. 2002 Nov; 15(11): 953-7.

8. Yamreudeewong W, DeBisschop M, Martin LG, Lower DL. Potentially significant drug interactions of class III antiarrhythmic drugs. Drug Saf. 2003; 26(6): 421-38.
9. He L, Wang S. Pharmacokinetic behavior and tissue distribution of verapamil and its enantiomers in rats by HPLC. Arch Pharm Res. 2003 Sep;26(9):763-7.
10. Katzung BG. Basic and Clinical Pharmacology. 1998. Seventh Edition.
11. Prisant LM. Verapamil revisited: a transition in novel drug delivery systems and outcomes. Heart Disease. 2001; 3(1): 55-62.
12. Satoh K, Yanagisawa T, Taira N. Effects on atrioventricular conduction and blood flow of enantiomers of verapamil and of tetrodotoxin injected into the posterior and the anterior septal artery of the atrioventricular node preparation of the dog. Naunyn Schmiedebergs Arch Pharmacol. 1979 Aug;308(2):89-98.
13. Echizen H, Brecht T, Niedergesass S, Vogelgesang B, Eichelbaum M. The effect of dextro-, levo-, and racemic verapamil on atrioventricular conduction in humans. Am Heart J. 1985 Feb;109(2):210-7.
14. DeCoursey TE. Mechanism of K+ channel block by verapamil and related compounds in rat alveolar epithelial cells. J Gen Physiol. 1995 Oct; 106(4):745-79.
15. Zhang S, Zhou Z, Gong Q, Makielski JC, January CT. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. Circ Res. 1999 May 14; 84(9):989-98.

16. Mayo PR, Skeith K, Russell AS, Jamali F. Decreased dromotropic response to verapamil despite pronounced increased drug concentration in rheumatoid arthritis. Br J Clin Pharmacol. 2000 Dec; 50(6): 605-13.

17. Eisenberg MJ, Brox A, Bestawros AN. Calcium channel blockers: an update. Am J Med. 2004 Jan 1; 116(1):35-43.

18. Fauchier L, Babuty D, Poret P, Autret ML, Cosnay P, Fauchier JP. Effect of verapamil on QT interval dynamicity. Am J Cardiol. 1999 Mar 1;83(5):807-8, A10-1.

19. Goldschmidt N, Azaz-Livshits T, Gotsman, Nir-Paz R, Ben-Yehuda A, Muszkat M. Compound cardiac toxicity of oral erythromycin and verapamil. Ann Pharmacother. 2001 Nov; 35(11): 1396-9.

20. Markham PN. Ellis TM. Tambur AR. Gebel HM. Differential sensitivity of resting and IL-2 activated NK cells to R-verapamil. Transplantation. 1996; 62(12): 1883-8.

21. Hescheler J. Pelzer D. Trube G. Trautwein W. Does the organic calcium channel blocker D600 act from inside or outside on the cardiac cell membrane? Pflugers Archiv - European Journal of Physiology. 1982; 393(4): 287-91.

22. Abernethy D, Wainer I, Longstreth J, Andrawis N. Stereoselective verapamil disposition and dynamics in aging during racemic verapamil administration. *J Phamracol Exp Ther.* 1993; 266: 904-11.

23. Schwartz J, Troconiz I, Veotte D, Liu S, Capili H. Aging effects on stereoselective pharmacokinetics and phamracodynamics of verapamil. *J Pharmacol Exp Ther.* 1993; 265: 690-7.

24. Eichelbaum M, Ende M, Remer G, Schomerus M, Dengler H. The metabolism of DL-[14C] verapamil in man.*Drug Metab Dispos.* 1979; 7(3): 145-8.

25. Bhatti MM. Foster RT. Pharmacokinetics of the enantiomers of verapamil after intravenous and oral administration of racemic verapamil in a rat model. *Biopharmaceutics & Drug Disposition.* 1997; 18(5): 387-96.

26. Anderson JR, Nawarskas JJ. Cardiovascular drug-drug interactions. Cardiol Clin. 2001 May; 19(2): 215-34.

27. Sakurai H, Kei M, Matsubara K, Yokouchi K, Hattori K, Ichihashi R, Hirakawa Y, Tsukamoto H, Saburi Y. Cardiogenic shock triggered by verapamil and atenolol: a case report of therapeutic experience with intravenous calcium. Jpn Circ J. 2000 Nov; 64(11): 893-6.

28. Hofer CA, Smith JK, Tenholder MF. Verapamil intoxication: a literature review of overdoses and discussion of therapeutic options. Am J Med. 1993 Oct; 95(4): 431-8.

29. Kinoshita H, Taniguchi T, Nishiguchi M, Ouchi H, Minami T, Utsumi T, Motomura H, Tsuda T, Ohta T, Aoki S, Komeda M, Kamamoto T, Kubota A, Fuke C, Arao T, Miyazaki T, Hishida S. An autopsy case of combined drug intoxication involving verapamil, metoprolol and digoxin. Forensic Sci Int. 2003 Apr 23; 133(1-2): 107-12.

30. Verschraagen M, Koks CH, Schellens JH, Beijnen JH. P-glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. Pharmacol Res. 1999 Oct; 40 (4): 301-6.

31. Kusus M, Stapleton DD, Lertora JJ, Simon EE, Dreisbach AW. Rhabdomyolysis and acute renal failure in a cardiac transplant recipient due to multiple drug interactions. Am J Med Sci. 2000 Dec; 320(6): 394-7.

32. Rosenthal T, Ezra D. Calcium antagonists. Drug interactions of clinical significance. Drug Saf. 1995 Sep; 13(3): 157-87.

33. Funakoshi S, Murakami T, Yumoto R, Kiribayashi Y, Takano M. Role of P-glycoprotein in pharmacokinetics and drug interactions of digoxin and beta-methyldigoxin in rats. J Pharm Sci. 2003 Jul; 92(7): 1455-63.

34. Volberg WA, Koci BJ, Su W, Lin J, Zhou J. Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. J Pharmacol Exp Ther. 2002 Jul; 302(1): 320-7.

35. Schonfeld W. Kirst HA. Macrolide Antibiotics. 2002. First Edition.

36. Culic O, Erakovic V, Parnham MJ. Anti-inflammatory effects of macrolide antibiotics. Eur J Pharmacol. 2001 Oct 19; 429(1-3):209-29.

37. Hoyt JC, Robbins RA. Macrolide antibiotics and pulmonary inflammation. FEMS Microbiol Lett. 2001 Nov 27;205(1):1-7.

38. Sakito O, Kadota J, Kohno S, Abe K, Shirai R, Hara K. Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. Respiration. 1996;63(1):42-8.

39. Jaffe A, Bush A. Anti-inflammatory effects of macrolides in lung disease. Pediatr Pulmonol. 2001 Jun;31(6):464-73.

40. Abu-Gharbieh E, Vasina V, Poluzzi E, De Ponti F. Antibacterial macrolides: a drug class with a complex pharmacological profile. Pharmacol Res. 2004 Sep;50(3):211-22.

41. Roden DM. Drug-induced prolongation of the QT interval. N Engl J Med. 2004 Mar 4;350(10):1013-22.

42. Viskin S, Justo D, Zeltser D. Drug-induced prolongation of the QT interval. N Engl J Med. 2004 Jun 17; 350(25):2618-21; author reply 2618-21.

43. LaPointe NM, Al-Khatib SM, Kramer JM, Califf RM. Knowledge deficits related to the QT interval could affect patient safety. Ann Noninvasive Electrocardiol. 2003 Apr;8(2):157-60.

44. Al-Khatib SM, LaPointe NM, Kramer JM, Califf RM. What clinicians should know about the QT interval. JAMA. 2003 Apr 23-30;289(16):2120-7.

45. Kantola T, Kivisto KT, Neuvonen PJ. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. Clin Pharmacol Ther. 1998 Aug; 64(2): 177-82.

46. Lin JH. Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. Adv Drug Deliv Rev. 2003 Jan 21; 55(1): 53-81.

47. Rubinstein E. Comparative safety of the different macrolides. Int J Antimicrob Agents. 2001; 18 Suppl 1:S71-6.

48. De Ponti F, Poluzzi E, Cavalli A, Recanatini M, Montanaro N. Safety of non-antiarrhythmic drugs that prolong the QT interval or induce torsade de pointes: an overview. Drug Saf. 2002; 25(4):263-86.

49. von Rosensteil NA, Adam D. Macrolide antibacterials. Drug interactions of clinical significance. Drug Saf. 1995 Aug; 13(2): 105-22.

50. Fisman S, Reniers D, Diaz P. Erythromycin interaction with risperidone or clomipramine in an adolescent. Child Adolesc Psychopharmacol. 1996 Summer; 6(2): 133-8.

51. Schwarz UI, Gramatte T, Krappweis J, Oertel R, Kirch W. P-glycoprotein inhibitor erythromycin increases oral bioavailability of talinolol in humans. Int J Clin Pharmacol Ther. 2000 Apr; 38(4): 161-7.

52. Pauwels O. Factors contributing to carbamazepine-macrolide interactions. Pharmacol Res. 2002 Apr; 45(4): 291-8.

53. Tsutsumi K, Kotegawa T, Kuranari M, Otani Y, Morimoto T, Matsuki S, Nakano S. The effect of erythromycin and clarithromycin on the pharmacokinetics of intravenous digoxin in healthy volunteers. J Clin Pharmacol. 2002 Oct; 42(10): 1159-64.

54. Periti P, Mazzei T, Mini E, Novelli A. Pharmacokinetic drug interactions of macrolides. Clin Pharmacokinet. 1992 Aug; 23(2): 106-31.

55. (no authers) Choosing a macrolide: drug interactions must be taken into account. Prescrire Int. 1999 Dec; 8(44): 183-7.

56. Hanada E, Ohtani H, Kotaki H, Sawada Y, Sato H, Iga T. Pharmacodynamic analysis of the electrocardiographic interaction between disopyramide and erythromycin in rats. J Pharm Sci. 1999 Feb; 88(2): 234-40.

57. Katoh T, Saitoh H, Ohno N, Tateno M, Nakamura T, Dendo I, Kobayashi S, Nagasawa K. Drug interaction between mosapride and erythromycin without electrocardiographic changes. Jpn Heart J. 2003 Mar; 44(2): 225-34.

58. Potschka H, Fedrowitz M, Loscher W. P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. Neuroreport. 2001 Nov 16; 12(16): 3557-60.

59. Gurevitz SL. Erythromycin: drug interactions. J Dent Hyg. 1997 Summer; 71(4): 159-61.

60. Cooper KJ, Martin PD, Dane AL, Warwick MJ, Raza A, Schneck DW. The effect of erythromycin on the pharmacokinetics of rosuvastatin. Eur J Clin Pharmacol. 2003 May; 59(1): 51-6. Epub 2003 Apr 01.

61. Takedomi S, Matsuo H, Yamano K, Ohtani H, Sawada Y. In-vivo kinetics of the interaction between midazolam and erythromycin in rats, taking account of metabolic intermediate complex formation. J Pharm Pharmacol. 2001 May; 53(5): 643-51.

62. Guchelaar HJ, Schultz MJ, van der Poll T, Koopmans RP. Pharmacokinetic-pharmacodynamic modeling of the inhibitory effect of erythromycin on tumour necrosis factor-alpha and interleukin-6 production. Fundam Clin Pharmacol. 2001 Dec; 15(6):419-24.

63. Hashimoto N, Kawabe T, Hara T, Imaizumi K, Wakayama H, Saito H, Shimokata K, Hasegawa Y. Effect of erythromycin on matrix metalloproteinase-9 and cell migration. J Lab Clin Med. 2001 Mar; 137(3):176-83.

64. Romanic AM, Harrison SM, Bao W, Burns-Kurtis CL, Pickering S, Gu J, Grau E, Mao J, Sathe GM, Ohlstein EH, Yue TL. Myocardial protection from ischemia/reperfusion injury by targeted deletion of matrix metalloproteinase-9. Cardiovasc Res. 2002 Jun; 54(3): 549-58.

65. Piquette-Miller M, Jamali F: Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofentreated and untreated adjuvant arthritis. *Drug Metab Dispos* 1995: 23:240-245.

66. Kulmatycki KM, Abouchehade K, Sattari S, Jamali F: Drugdisease interactions: reduced β-adrenergic and potassium channel antagonist activities of sotalol in the presence of acute and chronic inflammatory conditions in the rat. *Br J Pharmacol* 2001: 133:286-294.

67. Guirguis MS, Jamali F: Disease-drug interaction: reduced response to propranolol despite increased concentration in the ratwith inflammation. *J Pharm Sci* 2003: 92:1077-1084.

68. Sattari S, Dryden WF, Eliot LA, Jamali F. Despite increased plasma concentration, inflammation reduces potency of calcium channel antagonists due to lower binding to the rat heart. Br J Pharmacol. 2003 Jul; 139(5):945-54.

69. Heininger-Rothbucher D, Bischinger S, Ulmer H, Pechlaner C, Speer G, Wiedermann CJ. Incidence and risk of potential adverse drug interactions in the emergency room. Resuscitation. 2001 Jun; 49(3): 283-8.

70. Steenbergen JA, Stauffer VL. Potential macrolide interaction with verapamil. Ann Pharmacother. 1998 Mar; 32(3): 387-8.

71. Kaeser YA, Brunner F, Drewe J, Haefeli WE. Severe hypotension and bradycardia associated with verapamil and clarithromycin. Am J Health Syst Pharm. 1998 Nov 15; 55(22): 2417-8.

72. Hanada E, Ohtani H, Kotaki H, Sawada Y, Sato H, Iga T. Pharmacodynamic analysis of the electrocardiographic interaction between disopyramide and erythromycin in rats. J Pharm Sci. 1999 Feb; 88(2):234-40.

73. Houghton AR and Gray D. Making sense of the ECG: a hands-on guide. 2003. Second Edition.

74. Dette GA, Knothe H, Herrmann G. Erythromycin binding to human serum. Biochem Pharmacol. 1982 Mar 15; 31(6):1081-7.

75. Lee KS. Tsien RW. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. Nature. 1983; 302(5911): 790-4.

76. Dreassi E, Corti P, Bezzini F, and Furlanetto S. High-performance liquid chromatographic assay of erythromycin from biological matrix using electrochemical or ultraviolet detection. Analyst, 2000, 125 (6), 1077 – 81.

77. Gibaldi M, and Perrier D. Pharmacokinetics. 1982. Second Edition.

78. Meissner K, Sperker B, Karsten C, Zu Schwabedissen HM, Seeland U, Bohm M, Bien S, Dazert P, Kunert-Keil C, Vogelgesang S, Warzok R, Siegmund W, Cascorbi I, Wendt M, Kroemer HK. Expression and localization of P-glycoprotein in human heart: effects of cardiomyopathy. J Histochem Cytochem. 2002 Oct; 50(10): 1351-6.

79. Weiss M, Kang W. P-glycoprotein inhibitors enhance saturable uptake of idarubicin in rat heart: pharmacokinetic/pharmacodynamic modeling. J Pharmacol Exp Ther. 2002 Feb; 300(2): 688-94.

80. Haack M, Reichenberg A, Kraus T, Schuld A, Yirmiya R, Pollmacher T. Effects of an intravenous catheter on the local production of cytokines and soluble cytokine receptors in healthy men. Cytokine. 2000 Jun; 12(6):694-8.

81. Gudmundsson A, Ershler WB, Goodman B, Lent SJ, Barczi S, Carnes M. Serum concentrations of interleukin-6 are increased when sampled through an indwelling venous catheter. Clin Chem. 1997 Nov; 43(11):2199-201.

82. Fournier T, Medjoubi-N N, Porquet D. Alpha-1-acid glycoprotein. Biochim Biophys Acta. 2000 Oct 18; 1482(1-2):157-71.