Concurrent Administration of Erythromycin and Cimetidine on the Pharmacokinetics of Acetaminophen in Healthy Volunteers

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ชื่อวิทยานิพนธ์	ผลของการบริหารยาอีริโธมัยซินและไซเมทิดีนร่วมกันต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนในอาสาสมัครที่มีสุขภาพปกติ
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บทคัดย่อ

ยาอะเซตามิโนเฟน(พาราเซตามอล) เป็นยาแก้ปวด-ลดไข้ที่ใช้กันอย่างแพร่หลายในปัจจุบัน จึงอาจมีการใช้ร่วมกับยาอีริโธมัยซินและไซเมทิดีน เนื่องจากเป็นยาที่มีการใช้กันมากเช่นกัน ยาอีริโธมัยซินและไซเมทิดีนมีฤทธิ์ในการยับยั้งการทำงานของเอ็นไซม์ไซโตโครมพี - 450 (CYP) โดยอีริโธมัยซินมีผลยับยั้ง CYP3A4 และไซเมทิดีนมีผลยับยั้ง CYP3 (Gonzalez, 1989; Gonzalez and Idle, 1994) ซึ่งCYP3A4 เป็นเอ็นไซม์ที่เกี่ยวข้องในการแปรรูปยาอะเซตามิโนเฟนที่ใช้ในขนาดเพื่อการรักษา (Thummel, et al., 1993) การวิจัยนี้จัดทำขึ้นเพื่อศึกษาผลของการบริหารยาอีริโธมัยซินและไซเมทิดีนร่วมกันต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนในอาสาสมัครสุขภาพปกติจำนวน 7 คน โดยศึกษาค่าเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนหลังจากที่ได้รับประทานยาอะเซตามิโนเฟนขนาด 1,000 มก.ครั้งเดียว ใน 3 ระยะคือ (ก) ได้รับยาอะเซตามิโนเฟนอย่างเดียว (ข) หลังจากรับประทานยาอีริโธมัยซินขนาด 250 มก. วันละ 4 ครั้ง เป็นเวลา 7 วัน และ (ค) หลังจากรับประทานยาอีริโธมัยซินขนาดและระยะเวลาเดียวกันร่วมกับไซเมทิดีน ขนาด 400 มก. วันละ 2 ครั้ง เป็นเวลา 7 วัน ความเข้มข้นของยาอะเซตามิโนเฟนในพลาสมาวัดโดย High Performance Liquid Chromatography (HPLC) ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟน เช่น AUC, t_{max}, C_{max}, etc. คำนวณจากค่าความเข้มข้นของยาในพลาสมา ณ เวลาต่างๆ

ในช่วงเวลา 8 ชั่วโมง หลังรับประทานยา การทดสอบทางสถิติโดยวิธีวิเคราะห์ค่าความแปรปรวน (ANOVA) ผลปรากฏว่าไม่พบความแตกต่างทางสถิติ ($P > 0.05$)ในค่าพารามิเตอร์ใดๆของยาอะเซตามิโนเฟนทั้ง 3 ระยะ จากผลการศึกษาครั้งนี้แสดงให้เห็นว่าการใช้ยาอีริโธมัยซินและไซเมทิดีนร่วมกับอะเซตามิโนเฟนในขนาดที่ใช้เพื่อการรักษานั้น จะไม่ทำให้เกิดปฏิกริยาสัมพันธ์ทางด้านเภสัชจลนศาสตร์ระหว่างยาทั้ง 3 ชนิดนี้

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ABSTRACT

Acetaminophen (paracetamol) is widely used as an analgesic-antipyretic drug and usually prescribed in combination with many drugs including erythromycin or/and cimetidine. Erythromycin and cimetidine have been known to be able to inhibit the cytochrome P-450 (CYP) mixed function oxidases, CYP3A4 and CYP3, respectively (Gonzalez, 1989; Gonzalez and Idle, 1994). These enzymes are involved in the minor metabolic pathway of acetaminophen in therapeutic dosage (Thummel, et al., 1993). The objective of this study is to examine the effect of coadministration of erythromycin and cimetidine on the pharmacokinetics of acetaminophen. The pharmacokinetics of acetaminophen were determined in 7 healthy volunteers after receiving acetaminophen 1000 mg as an oral single-dose in 3 occasions : (a) acetaminophen alone, (b) after pretreatment with erythromycin 250 mg given orally 4 times daily for 7 days, and (c) after pretreatment with erythromycin at the same dose and duration and cimetidine 400 mg given orally twice daily for 7 days. The pharmacokinetic

parameters were determined from plasma acetaminophen concentrations during 8 hours using High Performance Liquid Chromatography (HPLC). Statistical analysis using analysis of variance (ANOVA) indicated that there were no significant difference ($P > 0.05$) in all pharmacokinetic parameters among the 3 studies. Therefore, the results suggest that clinical combination of erythromycin, cimetidine and acetaminophen in therapeutic dosages are not likely to produce pharmacokinetic interactions.

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Duangkhae Rukthai

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LIST OF ABBREVIATIONS

IgG	=	Immunoglobulin G
mM	=	millimolar
Km	=	Michaelis-Menten constant
g	=	gram
ml	=	millilitre
µg	=	microgram
l	=	litre
kg	=	kilogram
mg	=	milligram
r	=	correlation coefficient
hr	=	hour
min	=	minute
wk	=	week
°C	=	degree celcius
%	=	percent
mmol	=	millimole
QID	=	four times daily
LD_{50}	=	lethal dose 50%
®	=	trade name
uv	=	ultraviolet
nm	=	nanometer
mm	=	millimeter

LIST OF ABBREVIATIONS (CONT.)

cm	= centimeter
I.D.	= internal diameter
µm	= micrometer
rpm	= round per minute
µl	= microlitre
C_{max}	= maximal plasma concentration
T_{max}	= time to maximal plasma concentration
Ka	= absorption rate constant
$t_{1/2}$(abs)	= absorption half-life
$t_{1/2}$	= elimination half-life
Ke	= elimination rate constant
Cl/f	= apparent oral clearance
AUC	= area under the concentration-time curve
Vd/f	= apparent volume of distribution
S.D.	= standard deviation
V/V	= volume by volume
mV	= millivolt
F.S.	= full scale
yr	= year
ng	= nanogram
P	= P value

CHAPTER 1

INTRODUCTION

Acetaminophen (Paracetamol; N-acetyl - p - aminophenol) was first used in medicine by Von Mering in 1893. However, it has been gained popularity since 1949, after it was recognized as the major active metabolite of both acetanilide and phenacetin which were proved to be excessively toxic.

Acetaminophen is an effective analgesic - antipyretic drug which has a weak anti-inflammatory action. It is only a weak inhibitor of prostaglandin biosynthesis. Some evidence suggest that it may be more effective against enzymes in the central nervous system than those in the periphery perhaps because of the high concentrations of peroxides that are found in inflammatory lesion (Marshall, 1987).

Single or repeated therapeutic doses of acetaminophen have no effect on neither cardiovascular nor respiratory systems. Acid-base changes do not occur. Acetaminophen does not produce gastric irritation, erosion, or bleeding as occur after administration of salicylates. Moreover, it has no effect on platelets, bleeding time or excretion of uric acid.

Acetaminophen is well tolerated and produces fewer side effects than aspirin. It is available as over the counter drug. Therefore, it has earned a prominent place as a common household analgesic. However, acute overdosage (usually doses greater than 10 to 15 g) and, very rarely, during

long term treatment with higher doses than that of the therapeutic range cause fatal hepatic damage (Bonkowsky, et al.,1978; Johnson and Tolman,1977; Ware, et al.,1978). Because the excessive amount of acetaminophen causes saturation of the glucuronidation and sulphation, large proportions of acetaminophen undergo cytochrome P - 450 mediated N - hydroxylation to form N - acetyl - benzo - quinoneimine, a highly reactive intermediate. This metabolite normally reacts with sulfhydryl groups in glutathione. However, after a large dose of acetaminophen, the metabolite is formed in large amount and can cause depletion of the hepatic glutathione; under these circumstances, reaction with sulfhydryl groups in hepatic proteins is increased and hepatic necrosis can be resulted from acetaminophen hepatotoxicity.

Raucy, et al. (1989) examined the acetaminophen oxidation in human liver microsomes. There were inhibition, mediated anti-P450IIE1 IgG, of the reactive metabolite formed from 10 mM acetaminophen averaging 52% (range 30-78%) and inhibition, mediated anti-P450IA2 IgG, of acetaminophen activation ranging from 30-56% of the total activity. The authors suggested that cytochrome P-450IIE1 and IA2 catalyze nearly all the acetaminophen activity in human liver microsomes.

Thummel, et al. (1993) demonstrated that cytochrome P-450IIIA4 also catalyzed the formation of N-acetyl-p-benzo-quinoneimine, the reactive metabolite of acetaminophen, with a Km of approximately 0.15 mM which correlated with maximal acetaminophen plasma concentration after 1.0 g dose, approximately 0.1 mM, and their result at concentration 10 mM

acetaminophen was similar to those of Raucy, et al. (1989), then the authors suggested that cytochrome P-450IIIA4 contributes appreciably to the formation of the cytotoxic metabolite N-acetyl-p-benzo-quinoneimine at therapeutically relevant concentration of acetaminophen.

The formation of the acetaminophen-reactive metabolite and the severity of hepatotoxicity in laboratory animals are greatly influenced by modification of the activity of the hepatic mixed-function oxidase system. Induction of cytochrome P-450 with phenobarbital, 3 - methylcholanthrene, or ethanol increase hepatotoxicity, (Mitchell 1973; Moldeus 1980) while inhibition of cytochrome P- 450 with piperonyl butoxide, cobalt chloride, or metyrapone marked decreases the sensitivity of laboratory animals to acetaminophen - induced hepatotoxicity (Jollow, et al., 1973; Mitchell, et al., 1973a). In addition, a study of the effects of enzyme inducers (phenytoin and carbamazepine) on acetaminophen metabolism in human volunteers showed that pretreatment with enzyme inducers increased the clearance of acetaminophen and glutathione-derived conjugates. Likewise, some studies showed that propranolol and isoniazid which are enzyme inhibitors, decreased the clearance of acetaminophen and glutathione-derived conjugates (Baraka, et al., 1990; Epstein, et al., 1991). Thus, factors that selectively induce or inhibit the oxidative metabolism of acetaminophen may enhance or reduce the hepatotoxic effects of drugs in man.

The macrolide group of antibiotics includes erythromycin, triacetyloleandomycin, spiramycin and the newer derivatives, josamycin and medicamycin. Among this group, erythromycin and triacetyloleandomycin

are the two most important and most frequently prescribed members (Ludden, 1985). Both erythromycin and triacetyloleandomycin appear to have potential to inhibit drug metabolism in the liver and also drug metabolism by micro - organisms in the gut, either through their antibiotic effect or through complex formation and inactivation of microsomal drug oxidizing enzymes (Danan, et al., 1981; Larrey, et al., 1983a&b; Delaforge, et al., 1983; Pessayre, et al., 1983). Of the two agents, triacetyloleandomycin appears to be the more potent inhibitor of microsomal drug metabolism than erythromycin. Josamycin has seldom been involved in causing drug interactions, while medicamycin and the older derivative spiramycin have not so far been incriminated (Ludden 1985). There were many studies which reported that erythromycin is a specific inhibitor of cytochrome P-450IIIA4 isosyme (Gonzalez,1989; Gonzalez and Idle,1994; Pichard, et al.,1990; Spinler, et al.,1995; Thummel, et al.,1993).

Cimetidine is one of the histamine H_2-receptor antagonists. It is a 4,5-substituted group of imidazole derivative and this part of its structure is important in binding to the heme moiety of cytochrome P-450 and inhibits the activity of microsomal enzyme (Testa and Jenner,1981). Gonzalez (1989) and Gonzalez and Idle (1994) elucidated that cimetidine is able to inhibit cytochrome P-450II and III families in man.

On the basis of the inhibitory action of both erythromycin and cimetidine on the cytochrome P-450 hepatic mixed-function oxidase, the plasma concentration of acetaminophen may be theoretically altered after coadministration with erythromycin alone or in combination use with

cimetidine. Therefore, the present study was undertaken to determine the effect of erythromycin administration alone or in combination with cimetidine on the oral single dose pharmacokinetics of acetaminophen in normal volunteers.

CHAPTER 2

LITERATURE REVIEW

Acetaminophen

Acetaminophen (Paracetamol, N-acetyl-p-aminophenol, 4-hydroxy acetanilide) is a derivative of acetanilide, an aniline-like compound. In 1886, acetanilide was introduced into medical practice and it was soon found to have unacceptable side effects, the most alarming being cyanosis due to methaemoglobinemia. Acetaminophen was first used in medicine by Von Mering in 1893. However, it has been gained popularity since 1949, after it was recognized as the major active metabolite of both acetanilide and phenacetin which proved to be excessively toxic.

Acetaminophen has a molecular weight of 151.2 and is a moderately water and lipid-soluble, weak organic acid with a pKa of 9.5, and is thus largely unionized over the physiological range of pH.

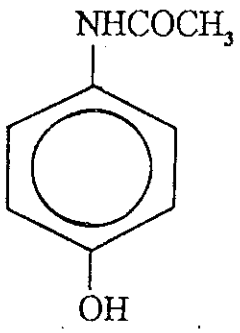

Figure 1 Molecular structure of acetaminophen

The basic pharmacological mechanisms of action of acetaminophen has not received the scientific attention accorded to the salicylates and many explanations for its activity appear to be just speculation. It has analgesic and antipyretic properties which do not differ significantly from those of aspirin but it lacks the potent anti-inflammatory action of aspirin, because it has more effective against enzymes for prostaglandins biosynthesis in the central nervous system than those in the periphery. The explanation of this property is that there might be high concentrations of peroxides which can stimulate the activity of the enzymes for prostaglandins biosynthesis, in inflammatory lesion (Marshall, et al., 1987).

Single or repeated therapeutic doses of acetaminophen does not affect the cardiovascular and respiratory systems. Acid-base balance does not change and the drug does not produce gastric irritation, erosion, or bleeding that may occur after administration of salicylates. It has no effect on platelets, bleeding time, or excretion of uric acid.

1. Pharmacokinetic properties

1.1 Absorption

Acetaminophen is an almost orally administered drug. It is only minimally absorbed from the stomach after taken orally. Absorption is by passive diffusion with first-order kinetics and occurs mainly in the small intestine. The gastric emptying is rate-limiting in the absorption of acetaminophen which Clement, et al. (1978) showed that the value of first-order rate constant for drug transfer from small intestine into systemic

circulation was greater than the apparent first-order rate constant for absorption from gastrointestinal tract. Acetaminophen is rapidly and almost completely absorbed. The systemic bioavailability after oral administration is incomplete because of first-pass metabolism. Some metabolism may occur during absorption. Josting, et al.(1976) studied the glucuronidation of acetaminophen in the rat intestinal loop and found that 8% of the drug was metabolized by the perfused rat intestinal loop during a 30-minutes period. The proportion of a dose reaching the systemic circulation as unchanged acetaminophen appears to depend on the amount of the drug given, decreasing from about 90% after 1 to 2 g to about 70% after 0.5 g (Rawlins, et al.,1977). The concentration in plasma reaches a peak in 30 to 60 minutes and half-life in plasma is about 2 hours after therapeutic doses. The usual therapeutic doses produce plasma concentration of 5 to 20 μg/ml. After 8 hours only small amounts of unchanged acetaminophen are detectable in plasma (Clissold,1986). In absorption phase, acetaminophen absorption seems to be predominantly dependent on the gastric emptying rate. Factors that alter the gastric emptying rate will influence the acetaminophen absorption.

1.1.1 Effect of Sex

Wojcicki, et al.(1979) demonstrated that the maximal plasma acetaminophen concentrations in women in both luteal and follicular phases were significantly higher than in men.

1.1.2 Effect of Food

McGilveray and Muttock (1972) found that in fasting state, the maximal plasma concentration of acetaminophen occured 20 minutes after ingestion, but were delayed to 90 minutes after a high carbohydrate meal, in the same subjects. Similar result was shown by Jaffe, et al. (1971) and they found that high lipid and high protein meals appear to have no such effect. Holt, et al. (1979a) shown that gastric emptying rate and acetaminophen absorption were slower after gel fibre (guar gum and pectin mixed solution) but the total absorption was not significantly reduced. They suggested that viscosity is an important property of dietary fibre to alter gastric emptying rate.

1.1.3 Effect of Posture

Nimmo and Prescott (1978) found that acetaminophen absorption rate were markedly reduced when subjects lay on their left side compared with when ambulant, after acetaminophen ingestion the plasma concentration were 0.18 and 2.8 μg/ml at 15 minutes and 7.8 and 20.8 μg/ml at 30 minutes, respectively. The authors suggested that the alteration of acetaminophen absorption was caused by the changing in the gastric emptying time.

1.1.4 Effect of Drugs

Nimmo, et al (1975) observed that there was marked delayed the rate of gastric emptying and acetaminophen absorption in patients during labour after receiving pethidine, diamorphine, or pentazocine.

Joel, et al. (1995) observed that there is no change in area under concentration-time curve (AUC) of acetaminophen after metoclopramide or propantheline administration, but significant delay in time to reach maximal plasma concentration (T_{max}) being seen on co-administration with etoposide and propantheline.

1.1.5 Effect of Disease

1.1.5.1 Gastrointestinal disease

Gastrointestinal disease may slow gastric emptying and delay the complete absorption of acetaminophen and other inflammatory gastrointestinal disease such as graft-versus-host (GVHD) of the gut, Behcet's syndrome and scleroderma involving the gastrointestinal tract may directly reduced acetaminophen absorption (Gubbins and Bertch 1991).

Holt, et al. (1979b) found that the acetaminophen absorption was slower in the coeliac and Crohn's disease patients, as indicated by later and reduced peak plasma acetaminophen concentration but total absorption was not different.

1.1.5.2 Malnutrition

Bolme, et al. (1982) compared the acetaminophen absorption between the Swedish children and the Ethiopian children. The Ethiopian children were divided in three groups: normal children with adequate nourishment, children with Marasmus (moderate malnutrition) and those with Kwashiochor (severe multrition). The absorption time ($t_{1/2}$(abs) ± S.D.) was similar among 4 groups but the fraction of absorption was reduced in all 3 groups of the Ethiopian children which was related to nutritional status.

1.1.5.3 Pregnancy

Galinsky and Levy (1984) shown that an oral dose of acetaminophen was absorbed much more slowly and incompletely on the last day of pregnancy than 38 days postpartum. The authors suggested that the slowly and incompletely acetaminophen absorption due to decreasing gastric emptying rate in late human pregnancy.

1.1.5.4 Liver disease

El-Azab, et al. (1996) shown that in elderly patients (45-65 years old) with liver cirrhosis from schistosomal infection plasma acetaminophen concentration in early stage, before the time to reach maximal plasma concentration, was significantly higher than younger patients and healthy subjects. The authors suggested that in these patients with higher initial plasma concentration, it is probably due to the development of collateral circulation and reduce the first-pass metabolism of acetaminophen in the liver.

1.2 Distribution

Acetaminophen is rapidly and uniformly distributed throughout most body fluids; the tissue : plasma concentration ratio of drug is unity in most tissues except in fat and cerebrospinal fluid. Generally, the apparent volume of distribution of acetaminophen is about 1 l/kg. Gazzard, et al. (1973) investigated the binding of acetaminophen to human plasma protein by ultrafiltration and equilibrium dialysis and found that at plasma concentrations of less than 60 mg/l, acetaminophen does not apparently bind to plasma proteins; at 90 mg/l protein binding was less than 5%; and after

toxic doses (plasma drug concentrations of up to 280 mg/l) protein binding varied from 8 to 43% with no correlation between binding and plasma acetaminophen concentration, and no binding to red blood cell was observed.

Divoll, et al. (1982) demonstrated that the volume of distribution of acetaminophen (corrected for weight) was larger in men than in women (0.99 and 0.86 l/kg) and declined with age in both sexes. The authors explained that the reduction in volume of distribution of acetaminophen in women and elderly due to increasing fat per kilogram body weight in them and incomplete distribution of nonlipophilic property of acetaminophen into body fat.

Beaulac-Baillargeon and Rochelue (1994) found an inverse correlation of $r = 0.85$ between maximal plasma acetaminophen concentration and the weight of the pregnant women ($P < 0.01$) but not with the weight of the control women. The authors suggested that weight gain in pregnant women due to the expansion of total body water caused by an increase in the plasma volume, extracellular fluid and amniotic fluid. So that increasing in volume of distribution of acetaminophen relate to lower in maximal plasma acetaminophen concentration.

1.3 Elimination

Acetaminophen is excreted unchanged in the urine only 2 to 5% of the therapeutic dose. At therapeutic dosage, it is mainly metabolized (>80%) to the glucuronide and sulphate conjugates in the liver. A less proportion (about 10%) is converted by cytochrome P-450-dependent hepatic

mixed-function oxidase to a highly reactive metabolite, postulated to be N-acetyl-p-benzoquinoneimine (Figure 2). And this metabolite is rapidly inactivated by conjugation with reduced glutathione and excreted in the urine, after further metabolism, as cysteine and mercapturic acid conjugates. Large overdoses of acetaminophen can exhaust stores of glutathione and leave the highly reactive intermediate to bind covalently with vital cell elements, which result in acute hepatic necrosis.

As a moderately lipid-soluble weak organic acid, acetaminophen is filtered by glomerulus with subsequent extensive tubular reabsorption. Excretion of acetaminophen is independent of urinary pH but appears to be weakly correlated with urine flow rate. The highly polar sulphate and glucuronide conjugates of acetaminophen are apparently active secreted by the tubules. There are many reports shown various factors that affect to acetaminophen elimination.

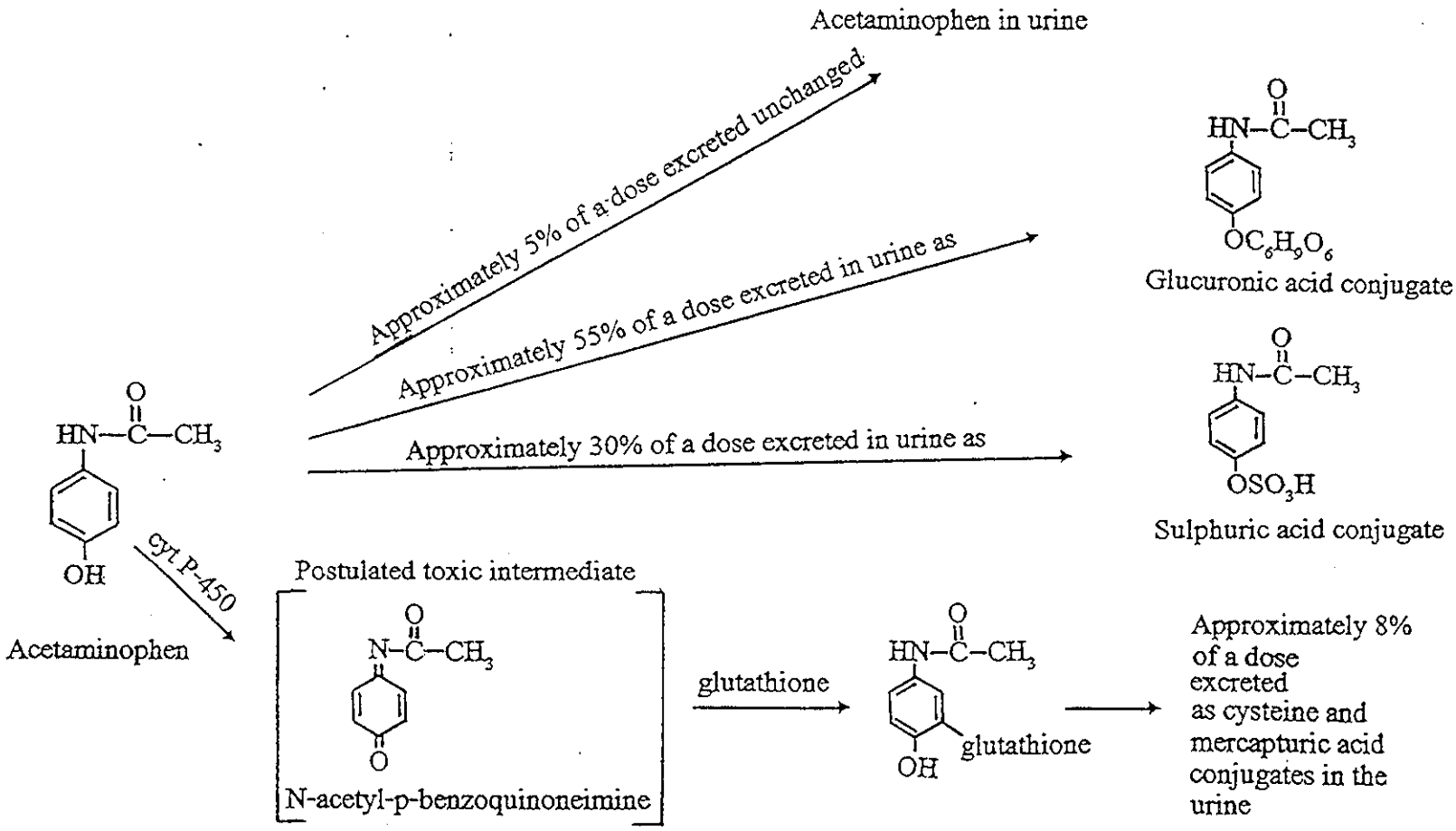

Figure 2 Pathways of acetaminophen metabolism

Source : Clissold, S.P., 1986 : 50.

1.3.1 Effect of Age

Néonates and children aged 3 to 10 years excreted significantly less glucuronide and more sulfate conjugate than children aged 12 years and adults. (Alam, et al., 1977; Levy. et al.,1975; Miller, et al.,1976; Peterson and Rumack. 1978). And the elimination half-life was significantly prolong in neonates.

Divoll, et al. (1982) shown that acetaminophen clearance tended to decline with age in men and women, but difference were of borderline significant.

1.3.2 Effect of Sex, Environmental or Genetic Factors

Miners, et al. (1983) found that acetaminophen clearance was 22% greater in males compared to normal females. This difference was entirely due to increased activity of the glucuronidation pathway in males.

Shively and Vessel (1975) shown that the acetaminophen elimination half-life in healthy males at 6 am was significantly longer (15%) at 2 pm, the plasma clearance was not significantly different at these time. The authors suggested that the difference was presumably due to a change in volume of distribution.

Mucklow, et al. (1980) found that the elimination half-life of acetaminophen was significantly longer and the clearance significantly slower in Asians in London compared with Caucasian subjects.

1.3.3 Effect of Disease

1.3.3.1 Liver Disease

Benson (1983) evaluated the safety of acetaminophen in therapeutic dose in subjects with stable chronic liver disease. The elimination half-life of acetaminophen was prolonged to a mean of 3.42 hr which is 70% higher than in normal subjects (2.04 hr). However, there was no evidence of drug cumulation or hepatotoxicity after receiving acetaminophen 4.0 g daily for 5 days.

Al-Obaidy, et al. (1996) demonstrated that the rate constant of glucuronide formation was higher in the children with liver disease compared to the value reported in healthy children of similar age, while the rate constant of the formation of acetaminophen sulphate was no different from that in normal children. The plasma half-life of acetaminophen was positively related to prothrombin time, and negatively to the serum albumin.

El-Azab, et al. (1996) shown that the elimination of acetaminophen was significantly prolonged in liver cirrhotic patients suffering from schistosomal infection both younger (9-25 years old) and elderly (45-65 years old) groups compared to the normal group. Plasma concentration of acetaminophen-glucuronide formation was greatly decreased in both patient groups, whereas no significant different in the plasma concentration of acetaminophen-sulphate formation compared to those in corresponding healthy subjects.

1.3.3.2 Pregnancy

Beaulac-Baillargeon and Rocheleau (1994) shown that the mean acetaminophen half-life was significantly lower and oral clearance was significantly higher in the first trimester of human pregnancy compared to the normal women.

1.3.3.3 Other Disease

Ismail, et al. (1995) shown that neither during nor after treatment of falciparum malaria affected to acetaminophen disposition in Thai patients.

1.3.4 Effect of Other Drugs

1.3.4.1 Oral Contraceptive Steroids

Miners, et al. (1983) found that acetaminophen clearance in females using oral contraceptive steroids was 49% greater than in the control females. Glucuronide and oxidation metabolism were both induced in the oral contraceptive steroids users but sulphation was not altered.

Mitchell, et al. (1983) examined the effect of low-dose estrogen oral contraceptive steroids on acetaminophen metabolism and elimination. Plasma acetaminophen clearance rose from 287 ± 13 ml/min to 470 ± 51 ml/min in women taking oral contraceptive steroids, whereas the elimination half-life decrease from 2.40 ± 0.14 hr to 1.67 ± 0.16 hr. The fraction clearance and rate of elimination of acetaminophen by glucuronidation increase in women taking oral contraceptive steroids, but the clearance and elimination by sulphation did not differ significantly from values in control subjects. Fractional clearance of the cysteine adduct also increased

significantly, but clearance of acetaminophen mercapturic acid did not change.

1.3.4.2 Sulfinpyrazone

Miners, et al. (1984) demonstrated that pretreatment with 800 mg per day of sulfinpyrazone for 1 wk increased acetaminophen clearance by 23 %. The increase in acetaminophen clearance was a result of induction of acetaminophen glucuronidation and oxidation; clearance of the glucuronic acid conjugate was 26 % greater and clearance of the glutathione-derived conjugates, reflecting the activity of the oxidative pathway, was 43% greater than the values in control group.

1.3.4.3 Anticonvulsant drugs

Miners, et al. (1984) shown that acetaminophen clearance was increased in patients receiving anticonvulsant drugs, phenytoin or carbamazepine, by 46% and acetaminophen half-life was correspondingly decreased. the reduction was a result of an approximately 60% increase in clearance of glucuronic acid conjugate and glutathione-derived conjugates, with clearance of sulphate conjugate unaltered.

1.3.4.4 Propranolol

Baraka, et al. (1990) demonstrated that pretreatment with 160 mg per day of propranolol HCl for 4 days increased the acetaminophen half-life by 25 ± 12% and lower its clearance by 14 ± 3%. Fractional clearance of acetaminophen as glucuronide, cysteine and mercapturate conjugates were significantly reduced but not as sulphate.

1.3.4.5 Isoniazid

Epstein, et al. (1991) investigated the inhibition of the metabolism of acetaminophen by isoniazid. Pretreatment with isoniazid 300 mg daily for 7 days markedly inhibited the formation clearance of the glutathione metabolites by 69.7%. Total acetaminophen clearance was lower by 15.2%. There was no effect of isoniazid on the non-oxidative pathways of acetaminophen elimination. Two days after isoniazid was discontinued, acetaminophen metabolism had returned to pre-isoniazid values. The result shown that isoniazid is a potent reversible inhibitor of the oxidative metabolism of acetaminophen.

1.3.4.6 Probenecid

Kamali (1993) investigated the influence of probenecid on the pharmacokinetics of acetaminophen in healthy volunteers. Pretreatment with probenecid caused a significant decrease in acetaminophen clearance by 54.9%. the urinary excretion of acetaminophen sulphate and glucuronide were significantly reduced, whereas that of acetaminophen was unchanged. The authors suggested that probenecid inhibits the activity of the hepatic enzyme, uridine diphosphate glucuronyl transferase and competes with the active renal excretion of acetaminophen sulphate.

Erythromycin

Erythromycin is the most important of the macrolide antibiotics. It is isolated from a strain of Streptomyces erythreus, an actimomycete first isolated from soil collected in the Philippine Archipelago (Ginburg, 1980).

1. Chemistry

Erythromycin is composed of a macrocyclic lactone nucleus to which various ketones and amino sugars are attached (Figure 2).

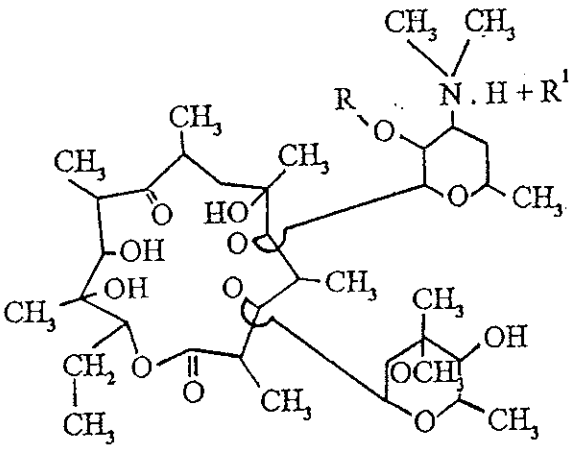

Figure 3 Molecular structure of erythromycin

Erythromycin base has pKa of 8.6 and poorly water-solubility but dissolves readily in organic acid solvent. In solution the drug is relatively stable at 4°C but rapidly losses activity at higher temperatures. Following oral administration, the instability of the drug in gastric secretions leads to erratic absorption and inconsistent serum levels (Ginsburg, 1980). So that enteric-coated tablets were produced for resist to acid in stomach. This approach has resulted in preparations producing blood levels ranging from poor to good, with varying dependence on the relationship between the time of drug administration and food ingestion.

To reduce the inactivated content of drug by gastric acid, various salts and esters of the parent drug have been formulated. At present, three oral forms of erythromycin are commercially available : the stearate (a salt) , the ethylsuccinate (an ester) and the estolate (the salt of an ester) which more resistance than the base.

Following oral administration, the stearate salt immediately dissociates in the intestinal tract to be absorbed as the base. The ethylsuccinate and the estolate esters are absorbed intact and require subsequent hydrolysis to yield the base (Ginsburg, 1980).

2. Mechanism of Action

Erythromycin disrupts the bacterial functional cycle by binding specific ribosome subunit at the level of the 50s bacterial ribosome. The specificity of

its action inhibits protein synthesis by arresting peptide bond formation without causing and alteration in nucleic acid cycle. Organisms with mutational changes in this subunit fail to bind erythromycin and are generally resistant. Erythromycin may inhibit the antimicrobial activity of lincomycin and chloramphenical because of competitive interference for the 50s binding site.

Erythromycin is classed as a bacteriostatic drug, but in vitro it is bacteriostatic in low concentrations, and bacteriocidal in high concentrations (Kuncers and Bennett, 1979 ; 496-506)

3. Spectrum of Action

In the laboratory erythromycin is most active against gram-positive bacteria including most strains of penicillin-resistant staphylococci. While the gram-negative enteric bacilli are generally high resistant, gram-negative coccobacilli show varying degrees of sensitivity, with many strains of *Haemophilus influenzae* and *Neisseria sp.* being susceptible. In vitro, strains of *Mycoplasma*, members of the family *Rickettsiaceae* and *Treponema pallidum* are susceptible at low concentrations. Most anaerobic bacteria including strains of bacteroides, are susceptible in vitro to concentrations of 3.12 μg/ml or less. Strains of *Mycobacterium kansasii, M. scrofulaccum, M. terrae, and M. triviale* are generally susceptible, while nearly all strains of *M.*

fortuitum are resistant. Yeasts, viruses and fungi are resistant to erythromycin (Ginsburg, 1980).

4. Mode of Administration and Dosage

4.1 Oral administration. Erythromycin is most common administered by the oral route. The usual dose is 30-50 mg per kg per day given in three or four divided doses. The usual adult dose is 0.5 g six-hourly. For severe infections higher doses can be used.

4.2 Intramuscular administration. Erythromycin can be administered intramuscularly in the form of erythromycin ethylsuccinate. The usual adult dose is 100 to 200 mg eight-hourly, because these injections are large and painful, so that this route of administration is rarely used.

4.3 Intravenous administration. The drug can be used intravenously for treatment of severe infections either as erythromycin lactobionate or gluceptate. The dose is 300 to 500 mg six-hourly for adult and 30 to 50 mg per kg for children. However a daily intravenous dose as high as 6 g has been given to adult without toxic effect. Erythromycin can be administered by either intermittant intravenous injections or continuous intravenous infusion. By direct injection in to the intravenous tubing, erythromycin lactobionate should be dissolved to a 1 percent solution, and this should be injected slowly over five minutes to avoid pain along the vein.

5. Serum Levels in Relation to Dosage

5.1 Erythromycin base is destroyed by acid in the stomach, and tablets are manufactured with an acid-resistant coating, which subsequently dissolve in the duodenum. Food in the stomach delays its ultimate absorption. Peak concentrations in plasma are only 0.3 to 0.5 μg/ml 4 hours after oral administration of 250 mg of the base and are 0.3 to 1.9 μg/ml after a single dose of 500 mg.

5.2 Erythromycin stearate is less destroyed in the stomach and it dissociated in the duodenum to be absorbed as active erythromycin the peak serum levels after oral administration of commercial preparations of erythromycin base and stearate appear approximately the same, but the absorption of the base may be slightly more delayed.

5.3 Erythromycin estolate is acid-stable and absorbed from the gastro-intestinal tract more completely. It is absorbed mainly as the ester and high serum concentrations are obtained rapidly and remain elevated for a prolong period. Food does not appreciably after the absorption of estolate. A single, oral 250 mg dose of the estolate produces peak concentrations of 4 μg/ml. These peak value include both the ester and the free base, the latter comprising 20 to 35% of the total. Thus, the actual concentration of erythromycin base in plasma may be similar for the three preparations.

5.4 Erythromycin ethlsuccinate is another ester that is adequately absorbed after oral administration, particularly when the stomach is empty.

Peak concentrations in plasma are 1.5 μg/ml (0.5 μg/ml of base) 1 to 2 hours after administration of a 500 mg dose.

5.5 By Intravenous administration, the average serum level in adults is 3.0 μg/ml at 1 hour after 200 mg of erythromycin lactobionate injection.

6. Distribution of the Drug in Body

Erythromycin is concentrated in the liver and spleen and widely distributed in the tissues. It persists in the tissues for longer periods than in the serum. Its antibacterial activity can be achieved at essentially all sites except the brain an the cerebro-spinal fluid. Erythromycin penetrates into prostatic fluid, concentrations are approximately 40 % of those in plasma. Erythromycin traverses the placental barrier and fetal plasma drug concentrations are about 5 to 20 % of those in the maternal circulation.

7. Excretion

Erythromycin is concentrated in the liver and excreted in active form in the bile, which may contain as much as 250 μg/ml when plasma concentrations are very high. Some of the drug may be inactivated by demethylation in the liver. Erythromycin is partly excreted in urine, and only about 2.5 % of an orally administered and 15 % of a parenterally administered dose is recoverable from the urine in the active form. The plasma half-life of erythromycin is approximately 1.6 hours.

8. Therapeutic Uses

8.1 *Mycoplasma pneumoniae* Infections. Erythromycin reduces the duration of fever caused by *M. pneumoniae*. And it may be given orally in doses of 500 mg four times daily, or given intravenously.

8.2 *Legionnaires'* Disease. Erythromycin is currently recommended for the treatment of pneumonia caused by *Legionella pneumophila*, *L. micdadei*, or other *Legionella species*. Erythromycin may be given orally in doses of 0.5 to 1 g four times daily or intravenously in doses of 1 to 4 g per day for 3 weeks.

8.3 *Chlamydia* Infections. *Chlamydia* infections can be treated effectively with erythromycin. It is specifically recommended as an alternative to tetracycline in patients with uncomplicated urethral, endocervical, rectal, or epididymal infections by given 500 mg orally every 6 hours for at least 7 days. During pregnancy, erythromycin is the drug of choice for chlamydial urogenital infections. It is also preferred for chlamydial pneumonia of infancy in the dosage of 50 mg/kg per day in four divided doses for 14 days.

8.4 Diphtheria. Erythromycin is very effective in eradicating the acute or chronic *diphtheria bacillus* carrier state. Erythromycin estolate in the dose of 250 mg four times daily for 7 days, was found to be effective in 90 % of adult. Most of the failures were due to lack of patient compliance.

8.5 Pertussis. Erythromycin may shorten the duration of whooping cough, if administrated early in the course of illness. But not affect to the paroxysmal stage of disease, although it may eliminate the microorganisms from the nasopharynx. And it can prevent whooping cough in susceptible individuals who are exposed to the disease.

8.6 *Streptococcal* Infections, Pharyngitis, scarlet fever, and erysipelas produced by *Strep. pyogens* response to erythromycin. The oral administration of 250 to 500 mg every 6 hours or 20 to 50 mg/kg per days cures these diseases, prevents the appearance of supportive complications, and suppresses the formation of anti-streptococcal antibodies. Erythromycin is an alternative drug for the treatment of streptococcal infections in patients who are allergic to penicillins.

8.7 *Staphylococcal* Infections. The oral administration of 500 mg of erythromycin every 6 hours for 7 to 10 days is effective treatment for staphylococcal infections of the skin or of wounds in patients who are allergic to penicillins and cephalosporins.

8.8 *Campylobactor* Infections. Early treatment of gastroenteritis caused by *Campylobactor jejuni* in children with erythromycin may reduce the duration of symptoms.

8.9 Tetanus. Erythromycin (500 mg orally every 6 hours for 10 days) may be given to eradicate *Clostridium tetani* in patients who are allergic to penicillins.

8.10 Syphilis. Erythromycin in doses of 2 to 4 g per day for 10 to 15 days has been used in the treatment of early syphilis in patients who are allergic to penicillins.

8.11 Gonorrhea. Erythromycin 500 mg of estolate or stearate 6 hourly may be useful for disseminated *gonococcal* disease in pregnant patients who are allergic to beta-lactam antibiotics.

9. Toxicity

9.1 Gastrointestinal side effects. Some of patients who take oral erythromycin will have diarrhea, nausea and vomiting. But these are only occasionally severe.

9.2 Hepatotoxicity. Many studies suggested that hepatotoxicity may occur after administration of erythromycin estolate but not after other erythromycin preparations (Pla, 1980). It appears that the propinyl ester linkage at the $2'$ position confers this property on the estolate, After starting treatment about 10 to 12 days jaundice may occur. In patients who have previously experienced the drug, jaundice may occur within 1 or 2 days. Abdominal pain, fever and pruritus are another major symptoms in these patients. Some of them have hepatic enlargement. Eosinophilia is common. Liver function tests usually indicate cholestasis. The jaundice and other symptoms usually subside rapidly when the drug is stopped, but occasionally jaundice may persist for weeks.

9.3 Skin rashes may rarely occur in patients who have hypersensitivity to drug.

9.4 Ototoxicity. Tinnitus and transient deafness have been described in a small number of patients.

10. Drug Interaction

Over the past 25 years there have been many case reports and controlled studies indicating that erythromycin may interfere with the metabolism of other drugs. The considerable factors that require to evaluate drug interaction may be include (a) dose, formulation, route, time of doses relative to meals, and duration of erythromycin therapy ; (b) dose, formulation, route and serum concentration (relative to possible saturable elimination) of the other drugs ; (c) characteristics of the patients or study subjects (i.e. age, gender, smoking history, pathophysiology, other drugs or environmental factors) (Ludden, 1985).

10.1 Biochemical Studies

10.1.1 Animal studies

Erythromycin is known to affect the cytochrome P-450 drug metabolizing system. Danan, et al. (1981) and Larrey, et al (1983b) found that erythromycin and erythromycin derivatives induce their own formation into metabolites which form an inactive 456-nm absorbing complex with the iron (II) of cytochrome P-450 in rats.

DeLaforge, et al. (1983) also demonstrated that erythromycin derivatives formed stable complexes with cytochrome P-450 both in vitro and in vivo in treated rats. Additionally, these authors studied the structure-activity relationships of erythromycin derivatives and other macrolide antibiotics for complex formation and suggested that the presence of a readily accessible, non-hindered N-dimethylamino group and the hydrophobic character of the molecule are both important for potential inhibition of hepatic drug metabolizing enzymes.

Pessayre, et al. (1983) found that single dose of erythromycin (4 mmol/kg) causes glutathione depletion in the rat. But no reports were found in human.

10.1.2 Human studies

Larrey, et al. (1983a) studied hepatic microsomal enzymes from the untreated patients and 6 patients treated with erythromycin propionate (2 g orally daily for 7 days). The treated patients had increased NADPH-cytocrome C reductase activity and increased total cytochrome P-450 concentrations in the part of the cytochrome P-450 erythromycin metabolite complex. The activity of hexbarbitone hydroxylase was unchanged. Antipyine clearance was measured in 6 additional patients before and after 7 days of treatment with erythromycin propionate 2 g orally daily. There was no statistically significant change in mean antipyrine clearance. The authors concluded that the administration of erythromycin propionate induces

microsomal enzymes and results in the formation of an inactive cytochrome P-450 metabolite complex in human.

11. Effects of Erythromycin of the Pharmacokinetics of Other Drugs

11.1 Carbamazepine

Wong, et al. (1983) studied the effect of erythromycin base 250 mg 6-hourly for 8 days on carbamazepine pharmacokinetics in 89 healthy male volunteers (nonsmokers). A randomized 2-way crossover design with a 4-week washout period was used. A single dose of carbamazepine 400 mg was given in the morning of the 5th day of erythromycin. There was a 19% decrease in oral carbamazepine clearance ($p < 0.05$) in the presence of erythromycin.

Carranco, et al. (1985) described a 41-year-old woman receiving carbamazepine for epilepsy and developed symptoms of carbamazepine toxicity (episodic dizziness, blurred vision, poor balance, nausea and vomiting) within 3 days of receiving erythromycin stearate (500 mg four times daily). Signs and symptoms of toxicity disappeared 24 hours after withdrawal of carbamazepine and erythromycin. And return to use only carbamazepine in the same dose, one day later her carbamazepine level was in therapeutic range and she continued to be asymptomatic. The authors suggested that erythromycin interferes with the metabolism of carbamazepine through competitive binding to cytochrome P-450, a monoxygenase necessary

for the oxidation of carbamazepine, resulting in higher plasma concentrations of carbamazepine and showed its toxicity.

Wroblewski, et al. (1986) studied 4 cases who received carbamazepine and erythromycin concomitantly and had changed in serum carbamazepine concentration. In every case, serum carbamazepine concentration either rose sharply (doubled or tripled previous steady-state concentrations) or dropped precipitously once erythromycin therapy was discontinued. The authors concluded that the combination of erythromycin and carbamazepine represented a clinically significant drug interaction and should be avoided where possible.

Jaster, et al. (1986) described 2 cases of carbamazepine toxicity attributed to interaction with erythromycin. Both of them were given erythromycin while taking carbamaxepine and had increasing of the serum carbamazepine level and noted signs and symptoms of its toxicity (slurred speech, mydriasis, hyperreflexia and severe ataxia). When both erythromycin and carbamazepine were stopped, the carbamazepine level decreased and symptoms subsided.

11.2 Warfarin

Bartle (1980) described the case of a 77-year-old female who had been taking warfarin sodium for approximately 2.5 years before and then received a 1-week course of erythromycin stearate 500 mg 4 times daily for a right

lower lobe infiltration. During that period her prothrombin time rose from about 28 to 64 seconds (control, 11 seconds).

Husserl (1983) described a 69-year-old man was placed on a regimen of 5 mg/day warfarin sodium and maintained his prothrombin time between 17 to 21 seconds. When he received 250 mg of erythromycin stearate 4 times daily, the prothrombin time had increased to 31.3 seconds (control, 11 seconds) in 3 days later. The author concluded that the rapid onset of the interaction suggested a plasma protein binding displacement effect.

Bachmann, et al. (1984) studied 12 healthy volunteers aged 19 to 32 years. Erythromycin base 250 mg administered 6-hourly for 8 days decreased warfarin clearance by a mean of 14% ($p < 0.001$) after a single 1 mg/kg warfarin dose.

11.3 Quinidine

Spinler, et al. (1995) described a 74-year-old man who received 200 mg oral quinidine sulfate every 6 hours. Through steady-state quinidine concentration was 2.8 mg/l. When he received 500 mg intravenous erythromycin lactobionate every 6 hours, the quinidine concentration increased to 4.2 mg/l and quinidine clearance decreased 30 %. The authors suggested that erythromycin inhibited cytochrome P-450IIIA4 which metabolizes quinidine.

11.4 Cyclosporine

Sketris, et al. (1986) described 3 cases of renal transplant recipients who received cyclosporine, an effective immuno-suppressant agent, for long period. During that time they received erythromycin for other infections, then cyclosporine and creatinine serum level increased. The rising of cyclosporine serum level able to be potentially nephrotoxicity. The authors suggested that erythromycin inhibits cyclosporine metabolism by forming a stable P-450 complex.

Freeman, et al. (1991) studied the mechanism of the pharmacokinetic interaction between cyclosporine and erythromycin it the weanling pig. Erythromycin significantly increased maximum concentration (C_{max}) and area under the concentration-time curve (AUC) from 0 to 24 hr cyclosporine in the peripheral circulation and significantly reduced the hepatic extraction ratio calculated from portal and hepatic C_{max} and AUC data. But time to C_{max} (t_{max}) and half life ($t_{1/2}$) of cyclosporine were essentially unchanged. The authors concluded that the mechanism of the cyclosporine and erythromycin interaction is caused by inhibition of hepatic metabolism and the impact of inhibition is greatest during first-pass when cyclosporine concentrations are at their highest.

11.5 Theophylline

The drug interaction between erythromycin and theophylline has been many case reports, studies and controversies.

Zarowitz, et al. (1981) studied in 8 healthy volunteers. Theophylline (4 mg/kg) was given on four separate study days : (1) prior to starting erythromycin, (2) on the third day of erythromycin administration, (3) on the tenth day of erythromycin administration and (4) 2 week after the course of erythromycin (250 mg 6 hourly for 10 days) was completed. A maximum decrease in the theophylline total body clearance was noted by the third study day after erythromycin administration. In the first and fourth group did not significantly change.

LaForce, et al. (1981) studied 15 asthmatic patients and found a 25.8 ± 18.4 % decrease in theophylline clearance and a 40.0 ± 35.3 % increase in theophylline plasma concentration during erythromycin administration.

Hemsworth and Renton (1981) studied the plasma clearance of theophylline in rabbits pretreated with troleandomycin or erythromycin (400 mg/kg/day) over a 10-day period. The elimination of theophylline was significantly impaired after 10 days of antibiotic treatment. No change in theophylline elimination occurred when the antibiotics were given for shorter periods of time. Protein, cytochrome P-450 and cytochrome b_5 levels, aminopyrine N-demethylase and benzo [a] pyrene hydroxylase activities were unchanged in hepatic microsomes prepared from rabbits pretreated with antibiotics for 10 days. Pretreatment of rabbits for 10 days with troleandomycin completely abolished production of 1-methyluric acid from theophylline in isolated hepatic microsomes, but production of 1,3-

dimethyluric acid was unaffected. Troleandomycin, when added in vitro to microsomes, had no direct effect on theophylline metabolism. The authors concluded that long-term treatment with troleandomycin selective blocks or destroys on pathway of theophylline metabolism.

Renton, et al. (1981) studied 12 healthy adults. Single oral doses of theophylline were administered before and after 10 days of erythromycin stearate 250 mg 3 times daily. Theophylline clearance was decreased by 40 % by erythromycin and measurement theophylline metabolites in 3 subjects and the 12-hour excretion of 3-methylxanthinbe and 1,3-dimethyluric acid was found to be decreased. The result differed from the previous study, the authors suggested that there may be either species differences or differences between troleandomycin and erythromycin in the specific pathways affected.

Richer, et al. (1982) studied the effect of erythromycin ethylsuccinate on the theophylline pharmacokinetics using data from 46 patients (15 on erythromycin) with bronchial asthma an 16 patients (8 on erythromycin) with chronic airflow obstruction. Of the patients with bronchial asthma, those who were on erythromycin (15 patients) had a 30 % lower theophylline clearance. However, patients with chronic airflow obstruction exhibited no effect of erythromycin on theophylline clearance. The authors suggested that there may be difference in age, patients with chronic airflow obstruction were approximately 15 year elder than the patients with bronchial asthma, or the relative hyperemia so frequently encountered in patients with chronic airflow

obstruction might be responsible for a decrease in hepatic enzyme activity, or the possible alteration in patients with chronic airflow obstruction leading to decreased free theophylline in plasma.

Maddux, et al. (1982) studied steady-state oral theophylline kinetics in 15 healthy adults before and after 5 days of erythromycin stearate (250 mg 4 times daily). No difference found in theophylline oral clearance or half-life. The authors suggested that a possible explanation for the altered theophylline clearance noted in previous clinical studies was the acute changes in pulmonary function that frequently occur in patients receiving concomitant erythromycin and theophylline.

Reisz, et al. (1983) examined the changing of theophylline pharmacokinetics which were caused by the addition of erythromycin in patients with chronic bronchitis and obstructive pulmonary disease. Erythromycin significantly decreased mean theophylline clearance by 22 % from 4.9 l/min to 3.87 l/min ($p < 0.05$). Mean peak theophylline level increased 28 % from 11.9 μg/ml to 15.3 μg/ml ($p = 0.05$). No change in urine theophylline metabolites was found.

Jonkman, et al. (1983) reviewed articles about the theophylline-erythromycin interaction controversy (Table 1).

Table 1 Summary of Controlled Studies on the Interaction of Theophylline and Oral Erythromycin

Erythromycin Formulation	Dose and Duration	N	Theophylline Cleance Mean % Decrease	Half-Life Mean % Increase	Reference
Interaction Demonstrated					
Uncoated base	250 mg QID x 6 days	6	26	52	7
Ethylsuccinate	250 mg QID x 6 days	6	30	60	7
	23 mg QID x 7 days	15	26	Not measured	4
Uncoated Stearate	250 mg QID x 6 days	6	5	21	7
	250 mg TID x 10 days	12	40	36	5
Coated Base	250 mg QID x 7 days	8	28	21	6
	250 mg QID x 10 days	8	9	15	3
No Interaction					
Uncoated Base or Stearate	500 mg +	9	13 (NS)	30 (NS)	12
Uncoated Stearate	250 mg QID x 1 day				
	250 mg QID x 5 days	13	2(NS)	Not measured	Maddux et al
	500 mg QID x 2 days	8	Not reported	18 (NS)	8
	500 mg QID x 2 days	12	20	11 (NS)	9

source : Jonkman and Handeles, 1983 : 309-310.

NS = not significant.

The authors concluded that the duration of erythromycin therapy and the peak erythromycin serum concentration (a function of dose, extent of absorption, and clearance) are important determinants of this interaction, and variability in these factors between studies accounts for the discrepant results. Furthermore, it is possible that the interaction may not occur with conventional erythromycin doses in patients whose microsomal enzyme system are stimulated by smoking or other drugs.

Cimetidine

Cimetidine is one of the histamine H_2-receptor antagonists. It is an analog of histamine (Figure 4).

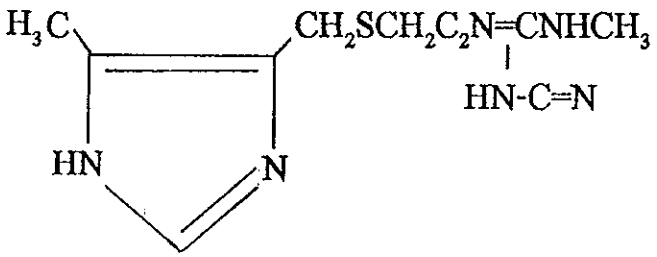

Figure 4 Molecular structure of cimetidine

1. Chemistry

Cimetidine is a 4,5 substituted imidazole derivative. It is a weak base (pKa 6.8) with a high degree of water solubility.

2. Mechanism of Action

Cimetidine reversibly competes with histamine at H_2-receptor sites. This action does not affect H_1-receptor sites. The important effect of histamine H_2 -receptor antagonist is to reduce the gastric acid secretion which is stimulated by histamine, gastrin, cholinomimetic drugs and vagal stimulation.

3. Pharmacokinetic Properties

3.1 Absorption

After taken orally in the fasting state, the 2 peaks are found in the plasma concentration-time profile, the first at about 1 hour and the second after about 3 hours. But this effect does not occur if cimetidine is taken with meal or with metoclopramide (Gugler, et al., 1981). The reason for the 2-peak effect is due to the drug being stored in hepatic parenchymal tissue after absorption, and with the second peak resulting from rapid release of cimetidine and possible conjugates from this tissue with subsequent reabsorption. (Veng Pedersen and Miller, 1980).

By intramuscular administration, cimetidine is absorbed rapidly and completely. The time to maximal concentration in plasma is 15 minutes and bioavailability is 90 to 100 %. Orally absorption by tablet formulation achieved peak plasma concentration between 1 and 2 hours and absolute bioavailability is approximately 60 %.

3.2 Distribution

Cimetidine distributes widely and extensively throughout the majority of body fluids, organs and tissue in human. Because of its high water soluble property, less proportion of cimetidine distributes throughout the fat and the cerebrospinal fluid. The plasma protein binding is about 22 %. The volume of distribution of cimetidine is 1 l/kg body weight.

3.3 Metabolism and Elimination

Cimetidine is metabolized by hepatic microsomal enzyme only 25 to 40 % of the total elimination of cimetidine. The large part of cimetidine is excreted unchanged form in urine. There are 3 known products which are converted from cimetidine by hepatic microsomal enzyme, cimetidine sulphoxide, hydroxymethyl cimetidine and guanylurea cimetidine. The half-time for elimination of cimetidine is 2 to 3 hours.

3.4 Therapeutic Uses.

The clinical use of cimetidine due to the inhibition of gastric acid secretion, especially in patients with peptic ulcer and Zollinger-Ellison Syndrome.

3.5 Toxicity

3.5.1 Central Nervous System Dysfunction. Slurred speech, delirium and confusional states are most common found in elderly patients.

3.5.2 Endocrine Effects. Cimetidine can bind to androgen receptors, so there are antiandrogenic effects such a gynecomastia and impotence to be observed in patients who receive long-term therapy with high doses of cimetidine.

4. Mechanism of the Drug Interaction by Cimetidine to Other Drugs

4.1 Absorption

Charbon, et al. (1980) studied in the anaesthetized dog and found that cimetidine reduced the increasing blood flow from histamine in the left gastric and common hepatic arteries. The high hepatic extraction drugs may be increased in the bioavailability from this effect.

Cimetidine increases gastric pH at least one hour following oral administration, so that the concomitant drugs may be altered in absorption. The weak acid drugs will be increased extent of ionization, dissolution and subsequent absorption. An opposite effect could be expected for weakly basic drugs. And cimetidine appears to have no consistent effect on gastric emptying in man (Heading, et al, 1977).

4.2. Metabolism

Imidazole derivatives have been shown to be potent microsomal enzyme inhibitors (Hajek, et al. 1982). Cimetidine is an imidazole derivative and has the enzyme inhibition property. Its structure is 4,5 - substituted derivative that binds to the heme moiety of cytochrome P-450 and inhibits the activity of microsomal enzyme (Jansen and Gugler, 1985; Pelkonen and Puurunen, 1980; Reilly, et al., 1983). Similarly, Breen, et al. (1982) compared the effects of cimetidine and ranitidine on hepatic drug metabolism and found that the inhibition of drug metabolism by cimetidine is not related to histamine H_2-receptor antagonism property. However, The inhibition does not

affect to conjugation, a reaction not mediated by cytochrome P-450 (Knodell, et al. 1982). Speeg, et al (1981) demonstrated that recovery from inhibition after cimetidine withdrawal is also rapid, occuring within 24 hr and chronic dosing with cimetidine does not result in tolerance to the inhibitory effect.

4.3 Renal excretion

Cimetidine competes with the other cationic drugs for tubular secretion.

5. Effects of Cimetidine on Absorption of the other Drugs.

Cox, et al (1986) studied in 4 normal volunteers each received three intraduodenal infusion of 0.5 mg triazolam solution (a) a pH 2.3 solution, (b) a pH 6.0 solution and (c) a pH 6.0 with cimetidine 1200 mg/d pretreatment. The triazolam pharmacokinetics in treatment (a) and (b) did not differ from each other. Treatment (c) cimetidine reduced the oral clearance and the apparent volume of distribution (Vd/f) of triazolam without affecting on triazolam half-life. The maximal serum concentration and the area under concentration-time curve of triazolam in treatment (c) were significantly increased by cimetidine. The authors suggested that cimetidine reduces intestinal metabolism of triazolam.

6. Effects of Cimetidine on Elimination of other Drugs.

6.1 Low Hepatic Extraction Drugs

6.1.1 Oral Anticoagulants

Serlin, et al. (1979) studied 6 patients receiving anticoagulant therapy [warfarin (4), nicoumalone (1), and phenindione (1)] , and added cimetidine 200 mg 3 times a day and 400 mg at night. The results shown that cimetidine co-administration caused the prolongation of prothrombin times in all patients.

Similar results was reported in a patient case study (Hetzel, et al., 1979), in which cimetidine consistently increased the plasma concentration of warfarin and the prothrombin time ratio. The authors suggested that doses of cimetidine 800-1000 mg dairy increased the prothrombin time ratio by 40-50% while 400 mg at night produced only about 10 % increase.

Staiger, el at. (1982) showed that 10 patients who had had long-term treatment with phenprocoumon, were given 400 mg cimetidine twice daily for 14 days and found that cimetidine did not interfere with phenprocoumon metabolism. The results were contrast to warfarin, even though they are similar in structure.

6.1.2 Theophylline

Roberts, et al (1981) demonstrated that the chronic treatment with cimetidine significantly reduced the plasma clearance and prolonged the elimination half-life of theophylline. Similarly, Lalonde, et al. (1982) studied

the effect of cimetidine on the theophylline pharmacokinetics at steady state in 7 normal volunteers. During cimetidine therapy, the apparent oral total body clearance of theophylline decreased by 29 %, the terminal serum half-life increased from 7.3 ± 1.5 to 10.1 ± 2.1 hr and the mean steady-state serum theophylline concentration was increased 34 %.

Kelly, et al. (1982) studied in 8 healthy volunteers over the age of 65 year and found that the apparent half-life of theophylline was significantly prolonged by treatment with cimetidine. The area under the plasma concentration-time curve for each of the three theophylline metabolites; 3-methyxanthine , 1-methyluric acid, and 1,3-dimethyluric acid, was decreased. The authors suggested that cimetidine appears to depress the metabolism of theophylline in elderly individuals.

One negative result was reported by Ambrose and Harralson (1981). They observed 5 acutely ill elderly (61 to 73 years old) patients on continuous theophylline therapy with various doses of cimetidine (600-1200 mg/d) administered for 3 to 10 days and found that there was no significant difference in the theophylline clearance determined before and during cimetidine therapy. The authors noted that the patients on this study had much lower theophylline clearance than the other group which was affected by cimetidine administration. They suggested that patients with a lower clearance may be less susceptible to the inhibitory effect of cimetidine.

6.1.3 Caffeine

May, et al (1982) investigated the effects of cimetidine on caffeine disposition in 6 smokers and 6 nonsmokers. The total body clearance of caffeine was higher and the elimination half-life was shorter in smokers than nonsmokers. Cimetidine decreased the total body clearance of caffeine by 31 % and by 42 % in smokers and nonsmokers. The increase in the elimination half-life were 45 % and 96 %. The maximal serum caffeine concentration, the time to reach it and the apparent volume of distribution were unaffected by cimetidine in both groups.

6.1.4 Carbamazepine

Telerman-Toppet, et al. (1981) observed a 89-year-old women with trigeminal neuralgia who was well controlled by carbamazepine. After she had been treated by cimetidine (1 g/day) for gastric ulcer without modification of carbamazepine therapy, she developed somnolence, dizziness, nystagmus and involuntary twitchings. At that time carbamazepine blood level increased to 10.5 μg/ml. After cimetidine was withdrawn without any alteration in carbamazepine dose, the concentration of carbamazepine decreased to between 5.7 and 6.8 μg/ml with a rapid improvement in her clinical status.

6.1.5 Phenytoin

Bartle, et al. (1982) investigated the effect of various daily doses of oral cimetidine (400, 1200, and 2400 mg) on the metabolism of a 250 mg

intravenous dose of phenytoin in 8 normal male volunteers. The area under the concentration-time curve of phenytoin was significantly increased at all doses of cimetidine. Plasma phenytoin concentrations (μg/ml) at 48 hr was significantly elevated over control by all cimetidine treatments. All cimetidine treatments significantly decreased plasma clearance of phenytoin.

6.1.6 Cyclosporine

D' Souza, et al (1988) studied the effect of chronic treatment with cimetidine on cyclosporine pharmacokinetics in rabbits. The clearance of cyclosporine was significantly reduced following treatment with cimetidine. There was no significant change in the volume of distribution of cyclosporine. The authors concluded that cimetidine inhibits the metabolism of cyclosporine.

6.1.7 Diazepam

Klotz, et al. (1979) studied the interaction between cimetidine and diazepam in 4 healthy volunteers who received a single intravenous bolus diazepam 0.1 mg/kg before and after cimetidine 200 mg orally 6 hourly for 5 doses. Cimetidine prolonged the elimination half-life and decreased the total plasma clearance of diazepam. The authors suggested that the reduction in the hepatic elimination of diazepam is due to the inhibition of the microsomal drug metabolism by cimetidine.

6.1.8 Chlordiazepoxide

Patwardhan, et al. (1981) studied the time course of inhibition and recovery of cimetidine which inhibited chlordiazepoxide eliminated in 7 healthy volunteers. They received chlordiazepoxide after cimetidine treatment for 1 and 30 days and after cimetidine was stopped for 48 hr. The plasma clearance of chlordiazepoxide was reduced 54 % after 1 day of cimetidine, by 57 % after 30 days of cimetidine and returned to normal (control) value after cimetidine was stopped for 48 hr. The authors suggested that the cimetidine-induced inhibition was rapidly reversible implying absence of morphologic and structural hepatic damage by cimetidine.

6.1.9 Lorazepam and Oxazepam

Patwardhan, et al. (1980) examined the effects of cimetidine in 8 healthy nonsmoking volunteers on drug metabolism via the glucuronidation pathway. Lorazepam and oxazepam are eliminated almost exclusively after conjugation as glucuronide and used to be a model for drug which is metabolism via the glucuronidation pathway. The result showed that cimetidine did not alter the elimination of either lorazepam or oxazepam. The authors suggested that cimetidine spares the glucuronidation of lorazepam and oxazepam.

6.2 High Hepatic Extraction Drugs

6.2.1 Propranolol

Donovan, et al. (1981) observed that a patient who had concomitantly taken cimetidine and a β-blocking drug, developed profound sinus bradycardia and hypotension. They subsequently studied the interaction between cimetidine and propranolol in a 54-year-old man with duodenal ulcer. The results showed that there was a 340 % increasing in the area under concentration-time curve of propranolol during cimetidine therapy. The same result was found in the study in 6 healthy volunteers by Kirch, et al. (1981). Cimetidine increased the mean peak plasma level of propranolol by 95 % and the area under concentration-time curve rose to about the same extent.

6.2.2 Labetalol

Daneshmend and Roberts (1981) studied the effects of cimetidine on labetalol pharmacokinetics. Three normal volunteers received labetalol 200 mg orally or 0.5 mg/kg intravenously before and after cimetidine 400 mg orally 4 times a day for 3 days. The results showed that cimetidine increased the bioavailability of labetalol by 80 %. The authors suggested that cimetidine reduces liver blood flow and caused the reduction of first-pass metabolism of labetalol.

6.2.3 Chlormethiazole

Desmond, et al. (1981) examined the effect of 7 days treatment with cimetidine (1 g/day) on the chlormethiazole pharmacokinetics in 8 healthy

volunteers. Pretreatment with cimetidine increased the maximal concentrations, the area under concentration-time curve and the elimination half-life of chlormethiazole, whereas its clearance was decreased.

6.2.4 Pentopril

Kochak, et al. (1988) examined the effect of cimetidine on the metabolic conversion of pentopril to its acid metabolite in vivo and in vitro. Cimetidine significantly increased the area under concentration-time curve and reduced the clearance of pentopril. In contrast to its acid metabolite, cimetidine decreased the area under concentration-time curve and the maximal concentration of pentopril's acid metabolite. In vitro studied, cimetidine did not inhibit the esterase activity. Base on those results, the authors suggested that cimetidine decreased pentopril hepatic clearance resulting from a reduction in hepatic blood flow rate.

6.2.5 Doxepin

The influence of concurrent cimetidine administration on the disposition of doxepin was evaluated in 10 healthy volunteers by Abernethy and Todd (1986). Cimetidine increased the area under doxepin concentration-time curve (533 to 695 ng/ml.hr) and decreased doxepin oral clearance (4404 to 3278 ml/min). Relative bioavailability during concurrent cimetidine treatment was 123 % of that during the control trial. Plasma protein binding of doxepin was similar between two trials. The authors suggested that doxepin hepatic extraction is impaired by cimetidine after oral administration.

6.3 Renal Excretion

6.3.1 Triamterene

Muirhead, et al. (1986) investigated the effect of cimetidine on renal and hepatic triamterene elimination in 6 healthy volunteers. Cimetidine significantly reduced the hepatic clearance of triamterene by 32 % and the renal clearance of treiamterene by 28 % , with no change in its protein binding. The authors suggested that cimetidine inhibits triamterene metabolism which was mediated cytochrome P-450 enzymes in the liver and also competes with triamterene for renal tubular excretion.

7. Effect of Cimetidine on Acetaminophen Disposition

7.1 Studied in Animals

Mitchell, et al. (1981) studied the protection against acetaminophen hepatotoxicity in cimetidine-treated rats (120 mg/kg) up to 4 hr after intraperitoneal administration of 500 mg/kg of acetaminophen and found that they had less histological damage and lower serum aminotransferase than those treated with acetaminophen alone. The authors showed that cimetidine as effective as N-acetylcysteine in the treatment of acetaminophen overdose, even though the dose of cimetidine given was only $\frac{1}{15}$ th of N-acetylcysteine on a molar basis.

Peterson, et al. (1983) studied the effects of cimetidine co-administration on acetaminophen hepatotoxicity (350 mg/kg) in mice and found the same result in rats that cimetidine reduces acetaminophen induced hepatotoxicity.

7.2 Studied in Humans

Abernethy, et al. (1983) studied the effect of cimetidine on acetaminophen pharmacokinetics in 11 healthy volunteers. All of them received acetaminophen 650 mg intravenously, once before and once during cimetidine treatment (300 mg 6 hourly). Cimetidine did not alter elimination half-life (2.66 vs 2.60 hr) or the clearance (4.8 vs 4.5 ml/min) of acetaminophen. An animal model was used to assess the effect of cimetidine on acetaminophen toxicity. Cimetidine treatment (75 mg/kg) significantly increased the LD_{50} of acetaminophen from 480 to 1020 mg/kg in rats.

Chen and Lee (1985) investigated the effect of single-dose and multiple-dose cimetidine administration on acetaminophen pharmacokinetics in 4 healthy volunteers. Each subject ingested 750 mg of acetaminophen as a single dose alone, co-administration with 200 mg of cimetidine, and following a one-week pretreatment with daily cimetidine (200 mg 6 hourly and 400 mg before retiring). Cimetidine did not significantly affect any pharmacokinetic parameters of acetaminophen.

Slattery, et al. (1989) observed approximately 80 % inhibition of the formation of reactive metabolites of acetaminophen in human liver microsomes by 1.5 mmol/l cimetidine. Though this effect did not occur at 0.02 mmol/l

cimetidine, which is a concentration 5 to 10 times the concentration required for 50 % inhibition of acid secretion in human beings (0.002 to 0.005 mmol/l). Studying in human volunteers, the result showed that cimetidine has no effect on the formation clearance of any acetaminophen metabolite. The authors concluded that cimetidine, at least in therapeutic doses, will not protect against acetaminophen-induced hepatotoxicity in normal human beings and a substantially higher dose is effective in rats and mice may be effective in human beings.

Cytochrome P-450

1. Introduction

The metabolizing foreign compounds process in human uses a large number of enzymes and almost occurs in the liver. Most of the enzymes have been classified as belonging to phase I or phase II pathways of metabolism. Phase I enzymes include reductases, oxidases, and hydrolases. Phase II enzymes are all transferases. These reaction serves transform a hydrophobic compound into a form that is more water soluble and can be easily eliminated from the organism through urine or bile.

In phase I pathway, cytochrome P-450 (P450s) are the most active among drug-metabolizing enzymes. These enzymes are also principally responsible for activation of procarcinogens and promutagens. Most clinically used drugs are metabolized to some degree by P450s.

P450s represent a superfamily of enzymes. They are found in animals, plants, yeast and bacteria. In mammals, some P450s are involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. However, the vast majority of these enzymes, xenobiotic-metabolizing P450s, appear to oxidise chemicals that are not normal constituents of the body. These enzymes are located in the endoplasmic reticulum of the cell and are believed to be partially embedded in the lipid bilayer with the bulk of protein facing the cytoplasmic, as opposed to luminal face of the endoplasmic reticulum (Figure 5).

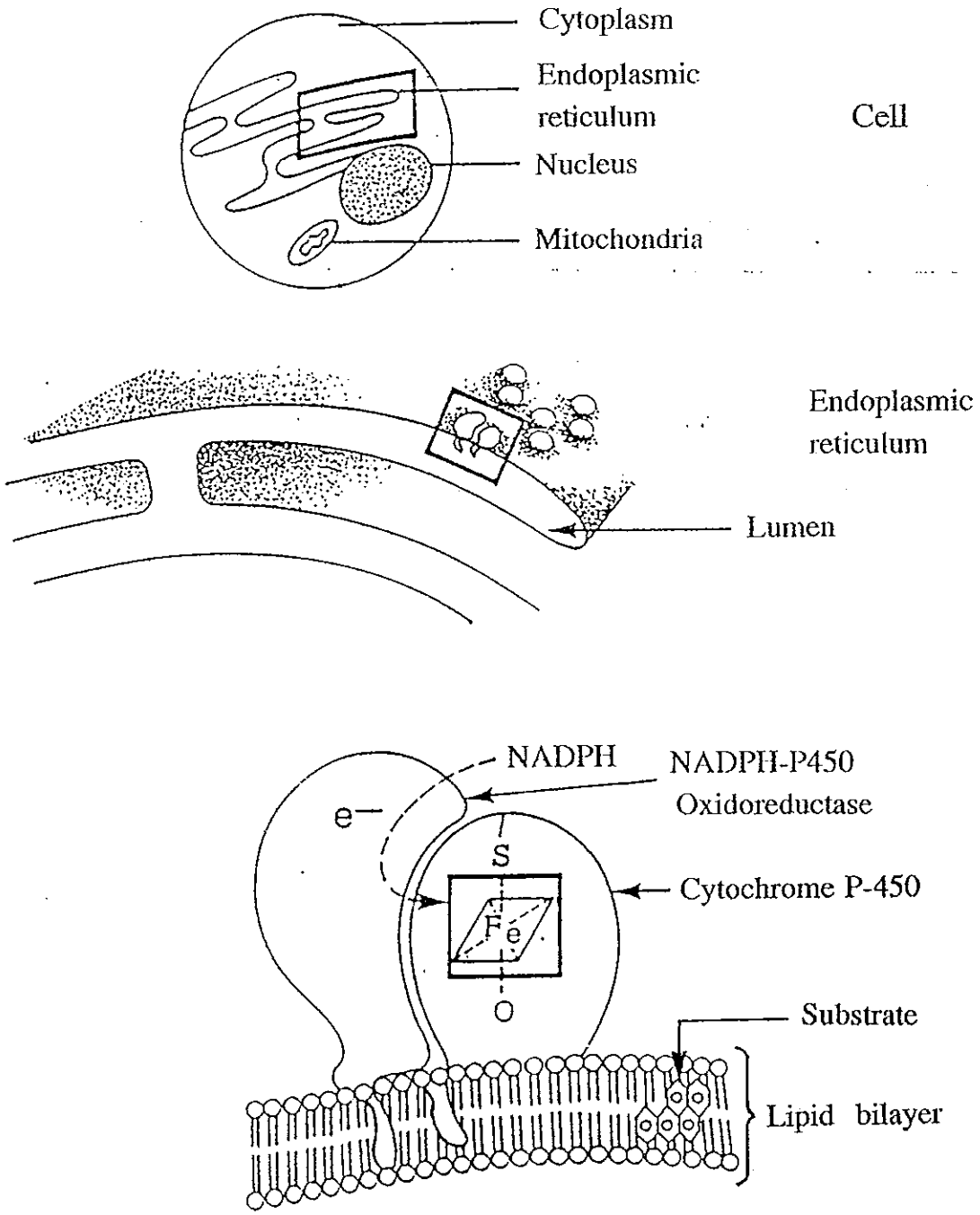

Figure 5 The location of cytochrome P-450 in the endoplasmic reticulum of the cell

Source : Gonzalez, F.G., 1994 : 62.

This membrane localization is ideally suited for the function of P450s in metabolizing hydrophobic chemicals. These P450s have been referred to as mixed function monooxygenases because they add an atom of oxygen to numerous structurally-diverse substrates. The oxygen-metabolizing ability of P450s is due to the presence in the active site of iron in the form of protoporphyrin IX (heme iron). In the P450 catalytic cycle, the enzyme binds to its substrated and the heme iron is reduced from a valency of +3 to +2 by an electron transferred from NADPH via another flavoprotein called NADPH-P450 oxidoreductase. Then O_2 binds to the heme and is reduced by another electron. A series of reactions occur that result in splitting of O_2, production of H_2O and oxidation of the substrate. A phenomenon called uncoupling can occur with certain P450 substrates in which H_2O_2 is produced. This uncoupling of electron transfer results in a higher NADPH consumption/product ratio and the possibility of detrimental consequences of O^-_2. Cytochrome b_5 can sometimes participate in donating the second electron with certain P450 forms and substrates (Figure 6). Mitochrondrial P450s have been the same catalytic cycle except that they use the flavoprotein adrenodoxin reductase and the iron sulpher protein adrenodoxin for electron transfer. The steroidogenic P450s have rigid substrate oxidation specificities while the xenobiotic-metabolizing P450s have a loose active site able to accommodate different chemical structures. Thus, a single P450 can metabolize a large number of structurally-diverse chemicals. In addition, a single P450 form can sometimes oxidize a single substrate at different positions.

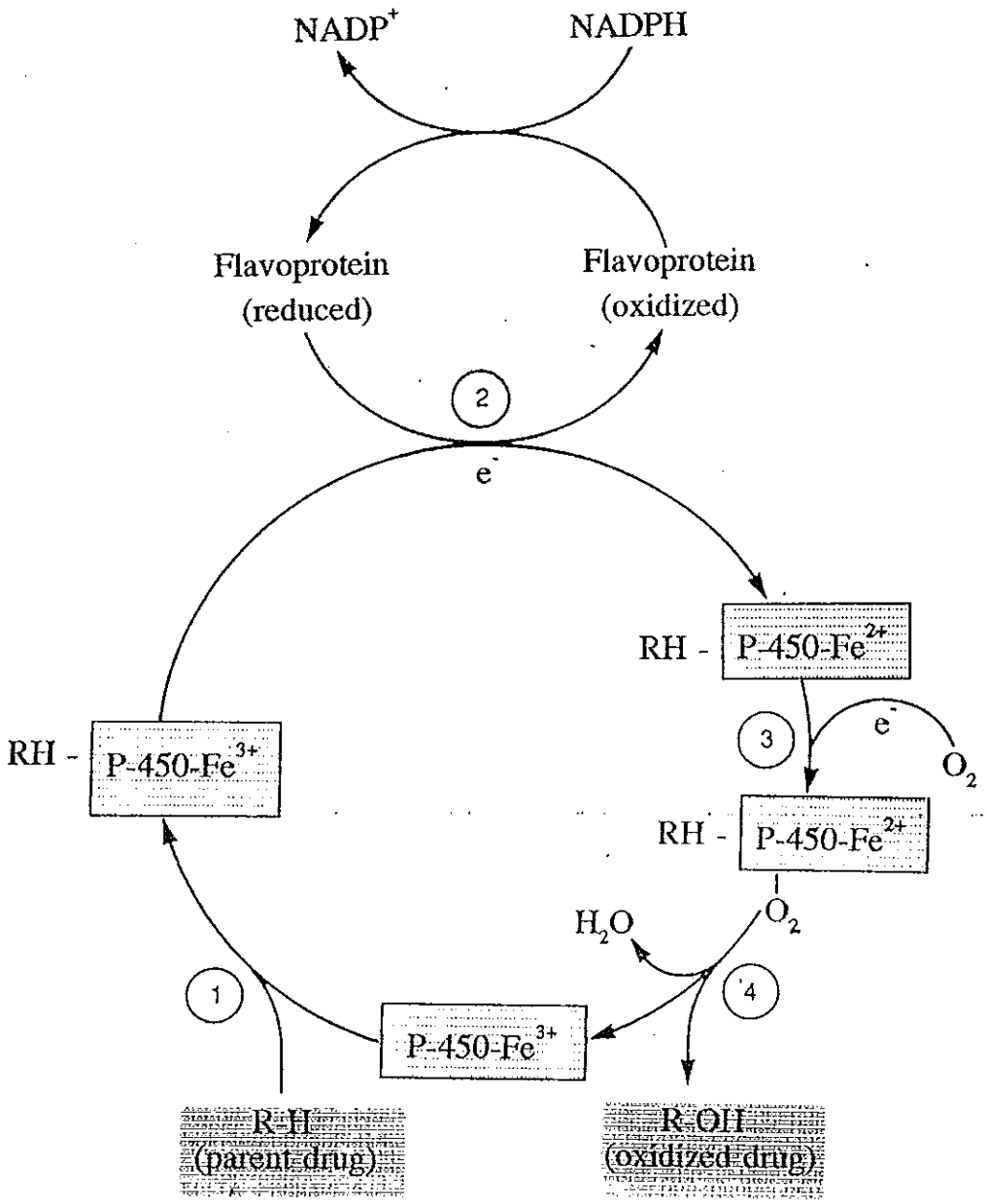

Figure 6 Cytochrome P-450 cycle in drug oxidations

Source : Gilman, A.G. and Goodman, L.S., 1991 : 16.

Cytochrome P-450s have been classified based on primary amino acid sequence similarities. With few exceptions, all P450s within a single family display less than 40% sequence similarity with P450s in any other family. All P450s within a subfamily are always greater than 55% similar to each other.

P450s are named with the root CYP (cytochrome P-450) followed by an Arabic number designating the family, a capital letter denoting the subfamily and another Arabic number designating the individual P450 number. For example, CYP3A4 is the number four P450 in subfamily A of family 3.

Presently, the P450 superfamily consists of 12 families in mamals. Four families, CYP11, CYP17, CYP19 and CYP21 having one or two P450s each, encode the classic steroidogenic enzymes. Two families with single P450s, CYP7 and CYP27, are involved in bile acid synthesis from cholesterol. The CYP5 family encodes the thromboxane synthase and the CYP24 family encodes a 1,25-dihydroxy vitamin D_3 24-hydroxylase. A single subfamily designated CYP4A, containing several P450s, encode fatty acid hydroxylases which are capable of oxidizing natural and synthetic fatty acids and drugs having long carbon chains. The CYP4A family also contains CYP4B1 and CYP4C1 that have no detectable fatty acid hydroxylase activity. The main xenobiotic-metabolizing P450s fall within the CYP1, CYP2 and CYP3 families. The CYP1 family contains 2 subfamilies, CYP1A and CYP1B. The CYP2 family has 7 subfamilies in

mammals. The CYP3 family consists of 2 subfamilies, one of which contains a single P450 CYP3B1 expressed in olfactory epithelia.

Levels of each human P450s family were studied by Shimada (1994). The results showed that about 70% of liver P450s could be accounted for by CYP1A2, 2A6, 2B6, 2C, 2D6, 2E1 and 3A. CYP3A (about 30% of total P450s) and CYP2C (about 20%) were found to be the major forms. Considerable levels of CYP1A2 (about 13%) and CYP2E1 (about 7%) could be determined, where as the CYP2A6 (about 4%), 2D6 (about 2%) and 2B6 (<1%) were the minor P450 forms.

Table 2 Mammalian P450 families and subfamilies

Substrates	Isozymes of cytochromeP-450s
Xenobiotics	CYP1A, CYP1B, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, CYP3A, CYP3B, CYP4B, CYP4C
Cholesteral	
- Bile acid	CYP7, CYP27
- Steroidogenic	CYP11, CYP17, CYP19, CYP21
Fatty acid	CYP4A, CYP5

2. P450s Regulation

P450s expression is partly regulated by enzyme inducers and inhibitors. There are several distinct classes of mammalian P450 inducers that activated transcription of one or more P450 genes. Some P450s are

controlled by another mechanism such as ethanol increased CYP2E1 by substrated-induced protein stabilization. Most P450s inducers can stimulate the activities of certain subsets of phase II and other drug-metabolizing enzymes (Gonzalez and Nebert, 1990).

In the P450s inhibition, inhibitor processes are classified in 4 groups (Tasta and Jenner, 1981).

A. Directly Acting Reversible Inhibitors.

The examples of these inhibitors are gases, alcohol, isocyanides, etc.

B. Indirectly Acting Reversible Inhibitors (Inhibitors Acting Through Metabolic Intermediates Forming Complexes with P450s).

The prodrugs of these inhibitors are metabolized to reactive metabolic intermediates by P450s. Metabolic intermediates have a strong affinity for the heme iron and form reversible complexes with enzyme and to inhibit the activity of that enzyme. The examples of this group are methylenedioxybenzene derivatives, SKF-525A, hydrazine, etc.

C. Irreversible Inhibitors (Inhibitors Acting by Destruction of P450s).

Irreversible inhibitors are a mixed group of compounds having in common only the ability to trigger and/or to catalyze the destruction of P450s. These inhibitors include the chemicals which are directly acting irreversible inhibitors and the chemicals which require metabolism by P450s to form highly reactive intermediates which bind covalently to the enzyme (indirectly acting irreversible inhibitors). The examples for these inhibitors are carbon tetrachloride (CCl_4), chloroform, thiopental, etc.

D. Inhibitors Alterating the Synthesis and/or Degradation of P450s.

These inhibitors decrease levels of P450s either by inhibition on some step(s) in P450s biosynthesis, or by enhancing the activity of degradative enzymes. The examples of these inhibitors are metal ions, amantadine, etc.

Table 3 Major drugs metabolized by the cytochrome P-450 enzymes including their inducers and inhibitors (after Spinler, et al. 1995 : 92)

P450 Enzymes	Substrate	Inducer	Inhibitor
CYP1A2	Caffeine, imipramine, phenacetin	Nicotine, omeprazole	-
CYP2B6	Cyclophosphamide, ifosfamide	Phenobarbital, phenytoin	-
CYP2C8	Benzphetamine, diazepam, diclofenac	-	-
CYP2C9	Hexobarbital, ibuprofen, imipramine, tobultamide, warfarin	-	-
CYP2C18	Omeprazole, oxicam drugs, proguanil, propranolol, retinoic acid, (S)-tetrahydrocannabinol, (S)-mephenytoin	-	Cimetidine
CYP2D6	Antiarrhythmic agents, antihypertensives, antipsychotics, β-blockers, monoamine oxidase inhibitors, morphine and morphine derivatives, tricyclic antidepressants	-	Cimetidine, dextropropoxyphene, quinidine
CYP2E1	Acetaminophen, alcohol, chlorzoxazone	Ethanol	
CYP3A3	Benzphetamine, erythromycin, cyclosporine, vinca alkaloids	Phenobarbital, phenytoin, rifampin	Imidazole derivatives (metronidazole, ketoconazole, clotrimazole, miconazole)
CYP3A4	Benzphetamine, cocaine, cyclosporine, erythromycin, imipramine, lidocaine, nifedipine, quinidine, vinca alkaloids	Phenobarbital, phenytoin, rifampin	Cimetidine, erythromycin, imidazole derivatives
CYP3A5, CYP3A7	Lovastatin, midazolam, quinidine, 17-α-ethynylestrodiol, nifedipine, terfenidine, triazolam, vinca alkaloids	Phenobarbital, phenytoin, rifampin	Imidazole derivatives
CYP4A11	Leukotriene receptor antagonists	Clofibrate	

CHAPTER 3

MATERIALS AND METHODS

Chemicals and Reagents

The Standard acetaminophen (Lot No. 85F-0269) was purchased from Sigma Chemical Co., St. Louis, U.S.A. Acetaminophen (500 mg/tablet, Lot No. F810095), erythromycin stearate (Erythrocin ®, 250 mg/filmtab tablet Lot No. 07026 AF 21) and cimetidine (Tagamet ®, 400 mg/tablet Lot No. 261004) were obtained from The Government Pharmaceutical Organization, Bangkok, Thailand ; Abbott Laboratories, North Chichaco, U.S.A ; and OLIC (Thailand) Ltd., respectively. Acetonotrile (HPLC grade), 85% phosphoric acid (analytical grade), trichloroacetic acid (TCA) and potassium hydroxide were purchased from J.T. Baker Inc.; Merck ; Fluka ; and Riedel-de Haen Ab., respectively. Water was purified for HPLC by the Milli Q Water Purification System (Millipore, Bedford, M.A, U.S,A)

Instrumentation

The HPLC system consisted of a Jasco PU-980 pump, a Rheodyne injector with a 20 µl sample loop and a Jasco UV 975 detector. Detection was made with the variable-wavelength UV detector set at 254 nm and peak area was measured with a Jasco 807-IT integrator. A Jasco recorder

attenuation was set at 32 mV.F.S. and chart speed was 2 mm/min. Separation was achieved on a reversed-phase μ-Bondapak C_{18} column (30 cm x 3.9 mm I.D., particle size 10 μm). A guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

Methods

1. Subjects

Seven Thai male volunteers aged 21-33 yr, weighing 57-68 kg were enrolled in this study. None were smokers. The subjects were considered to be healthy as determination by medical history, physical examination, and essential laboratory tests (complete blood count, renal and liver function tests). All subjects were asked to avoid from taking other drugs which were not include in this study, alcoholic beaverages, and coffee at least 1 week prior to and during the study. They gave written informed consent to the study which was approved by the Ethics Committee, Faculty of Science , Prince of Songkla University, Hat Yai, Thailand.

2. Protocol

Pharmacokinetics of acetaminophen were studied on three separate occasions at least 2 weeks apart in all subjects as the following sequences : (a) acetaminophen administration alone, (b) erythromycin, 250 mg orally 4

times daily, was given 7 days before acetaminophen administration, and (c) erythromycin, 250 mg orally 4 times daily and cimetidine, 400 mg orally twice daily, were given 7 days before acetaminophen administration.

Blood collection

After an overnight fast, each subject received 1000 mg of acetaminophen (500 mg/tablet, 2 tablets) with 150 ml tap water. No food was allowed at least 2 hours after drug ingestion. An intravenous canula, kept patent with 0.5 ml of a dilute heparin solution (100 units/ml) after each sample, was inserted into a forearm vein. Venous blood samples (5 ml) were collected in heparinized tubes before and 10, 20, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6 and 8 hours after an oral single dose acetaminophen administration. Blood samples were centrifuged at 3000 rpm for 15 min and plasma was separated and stored at -20 °C for subsequent analysis. Acetaminophen is stable in plasma at this temperature for a period of years (Lau and Critchley, qouted in Adriaenssen, 1994 ; 1564).

3. Sample Analysis

The plasma acetaminophen concentrations were measured by a high performance liquid chromatographic (HPLC) method (Miners, et al., 1983).

3.1 Mobile Phase

The mobile phase was 5 % acetonitrile in 95 % 20 mM orthophosphoric acid and adjusted to pH 3.0 with 20 % potassium hydroxide.

The mobile phase was freshly prepared in each day and was filtered through 0.45 micropore filtered paper (Nyron 66), then degassed by sonification for 30 minutes before using. The flow rate was 2 ml/min. All analysis were performed at room temperature (about 25 ± 1 °C)

3.2 Stock Standard Solution

Standard solution of acetaminophen was prepared by dissolving 25 mg standard acetaminophen in 50 ml distilled water. At this concentration, acetaminophen was well dissolved. The stock solution was stored at 4 °C and divided in to the aliquot to prepare the working standard acetaminophen in solution (deionized water) and plasma (Appendix-1), which were used to prepare the calibration curves day by day.

3.3 Calibration Curve

The calibration curve which is the standard curve for the plasma acetaminophen concentration calculation should be linear on at least 3 occasions and coefficient of variation (CV = SD/mean normalised peak area ratio × 100) of <10% could be achieved (both intra-day and inter-day).

In this study, the calibration curves were prepared by adding a standard acetaminophen solution to blank human plasma so that the final concentrations of acetaminophen in plasma were 1, 2.5, 5.0, 10.0, 20.0 and 30.0 μg/ml. The calibration curve for acetaminophen peak area was linear in the range 1-30 μg/ml and passed through the origin (Figure 11 , r = 0.999). The CV were 0.52-3.16% (Table 4 and 5).

3.3.1 Recovery

Potential loss of acetaminophen during the 10% trichloroacetic acid precipitation was determined by comparing the peak area of acetaminophen extracted from plasma sample in the range of 1-30 μg/ml with that of an equal concentration of standard acetaminophen prepared in distilled water. The percent recovery was calculated by

$$\frac{\text{peak area of standard acetaminophen in plasma}}{\text{peak area of standard acetaminophen in distilled water}} \times 100$$

and they were over 94 % (Table 6).

3.3.2 Precision and Variability

To determine intra-day precision and variability, The standard acetaminophen was spiked in blank plasma at low, medium and high concentrations and 10 replicates of each were carried out on one day. All should be of ± 10% of spiked value and the CV at each concentration should be of <10%.

To determine inter-day precision and variability, the standard acetaminophen was spiked in blank plasma at low, medium and high concentrations and each concentration was carried out on 10 different days. Accuracy should be of ± 10% of spiked value and the CV at each concentration should be of <10%.

The precision of the entire working dose range was determined by replicate analyses of blank human plasma (n =10) spiked with six different concentrations (1, 2.5, 5.0, 10.0, 20.0 and 30.0 μg/ml) for inter-assay variance and three different concentrations (1, 5 and 20 μg/ml) for intra-assay variance. To determine the intra-assay variance, ten replicate assays of each concentration were carried out in the same day. The inter-assay variance was determined by assaying the peak area of spiked blank plasma on ten days. Coefficients of variation (CV) were calculated from these values. They were 0.52-3.16% (Table 4 and 5).

3.4 Sample Preparation

A 200 μl of plasma sample or working standard was mixed with an equal volume of 10 % trichloroacetic acid in a 1.5 ml polypropylene microcentrifuge tube and centrifuged at 14,000 rpm for 5 minutes to precipitate proteins. A 100 μl of the clear supernatant (5 times of the volume of the injected loop) was injected onto the column. The chromatographic condition used for assay were good to separate acetaminophen from other endogenous substances in plasma, and both erythromycin and cimetidine did not interfere with these analyses (Figure 7-10). This method is repeated to have good reproducibility (Miners, et al., 1983). The lower limit of detection for acetaminophen in this study was 0.25 μg/ml.

4. Data Analysis

4.1 Pharmacokinetic Calculations

The following parameters were estimated from the individual plasma acetaminophen concentration-time curve. The pharmacokinetics behavior of acetaminophen is represented by mathematical functions derived from a one-compartment model in which the distribution of drug between blood and tissues is instantaneous and the elimination of the drug is a first-order process (Mitchell, et al.,1983).

The maximum plasma acetaminophen concentration (C_{max}) and time to reach C_{max} (t_{max}) were determined by visual inspection of the semi-logarithm concentration-time curve.

The absorption rate constant (Ka). The value for Ka was obtained by using the method of residuals which calculated from the following step

A. Plot the drug concentration versus time on semi-logarithmic graph paper with the concentration values on the logarithmic axis.

B. Back extrapolate the log linear portion of the decline phase. Let C_1 denote the plasma concentration along this extrapolated line.

C. Subtract the observed plasma concentration (C_2) from the corresponding extrapolate value at each time point.

D. Plot the residuals (C_1-C_2) against time on the same semi-logarithmic graph paper.

The absorption rate constant was obtained from multiplied the slope of the straight line fitted to residuals by 2.303 yields the value for Ka.

The absorption half-life $[t_{1/2}$ (abs)] was obtained from dividing Ka by 0.693

The elimination rate constant (Ke) was determined by linear least squares regression analysis with plasma acetaminophen concentration-time curve data points from 2 to 8 hr after dosing on semi-logarithmic graph paper.

The elimination half-life ($t_{1/2}$) was calculated as 0.693/Ke.

The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule and extrapolation to infinity.

The apparent oral clearance (Cl/f) was calculated as dose/(AUCxbody weights).

The apparent volume of distribution (Vd/f) was calculated as Cl/f divided by Ke.

4.2 Statistical analysis.

All results are expressed as mean ± S.D. Difference in acetaminophen pharmacokinetic parameters among control and both treatments were tested for statistical significance by analysis of variance (ANOVA) with $P < 0.05$ taken as the minimum level of significance. Duncan's multiple range test was used to test for significant differences between means.

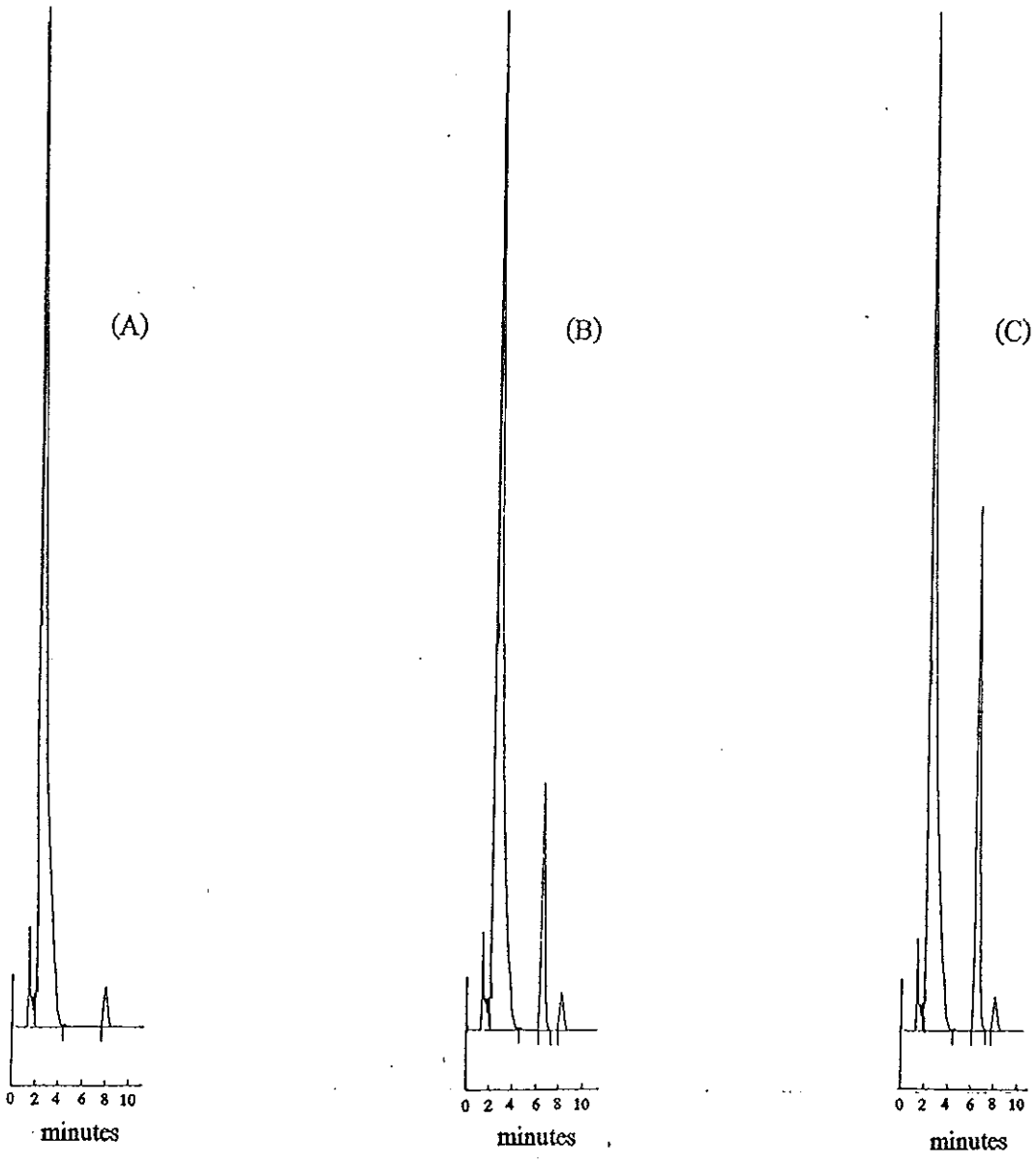

Figure 7 Chromatograms of 20 µl human plasma sample. Key : (A) blank; (B) spiked with standard acetaminophen, 10 µg/ml; (C) spiked with standard acetaminophen, 20 µg/ml. The mobile phase was 5% (V/V) acetonitrile in 95% phosphate buffer pH 3.0 at a flow rate of 2.0 ml/min. Chart speed was 2 mm/min. Attenuation was 32 mV F.S.

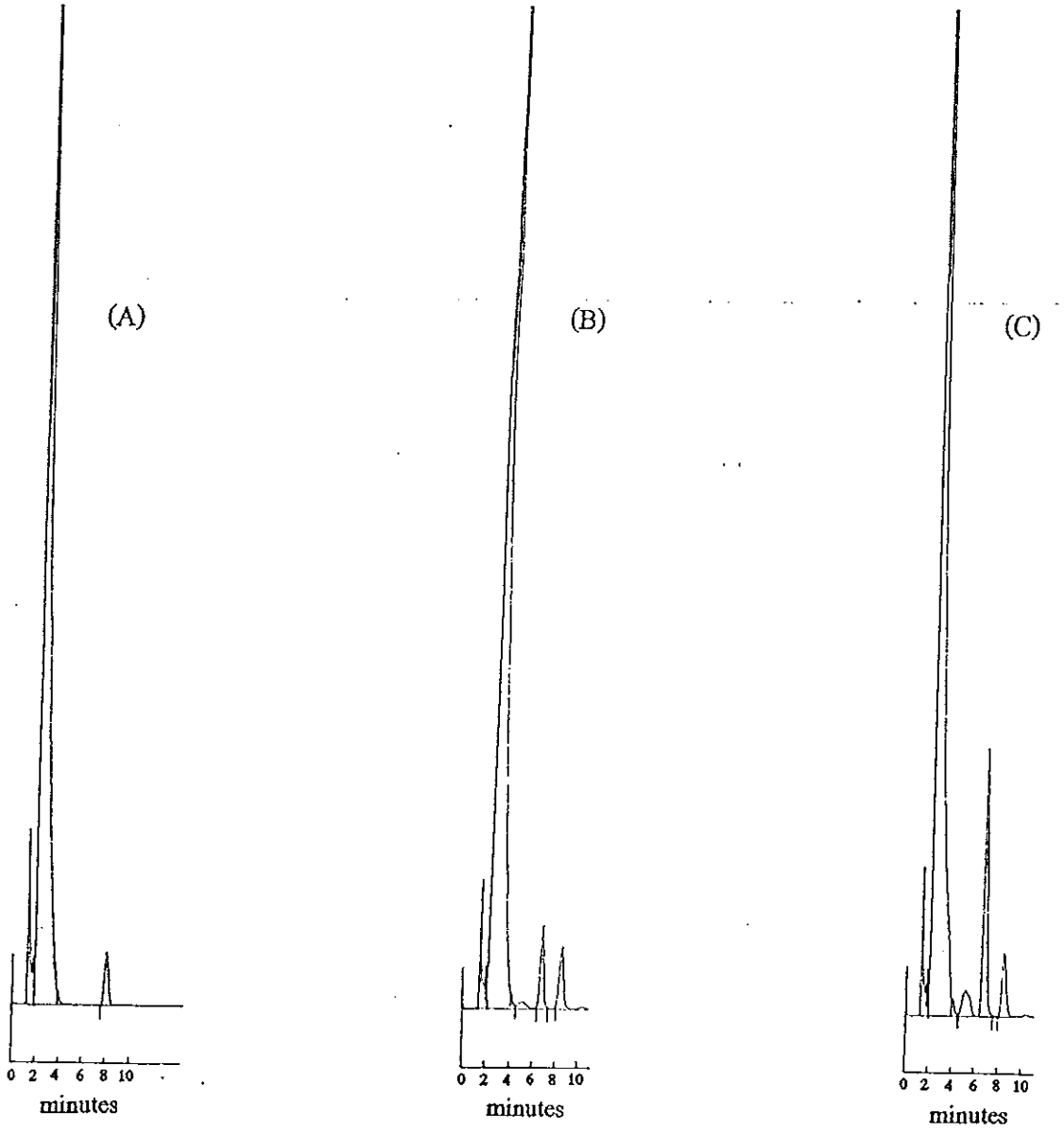

Figure 8 Chromatograms of 20 µl human plasma sample. Key : (A) blank; (B) and (C) plasma obtained from a subject without pretreatment, 10 and 20 min after 1000 mg of acetaminophen was given, respectively. The mobile phase was 5% (V/V) acetonitrile in 95% phosphate buffer pH 3.0 at a flow rate of 2.0 ml/min. Chart speed was 2 mm/min. Attenuation was 32 mV F.S.

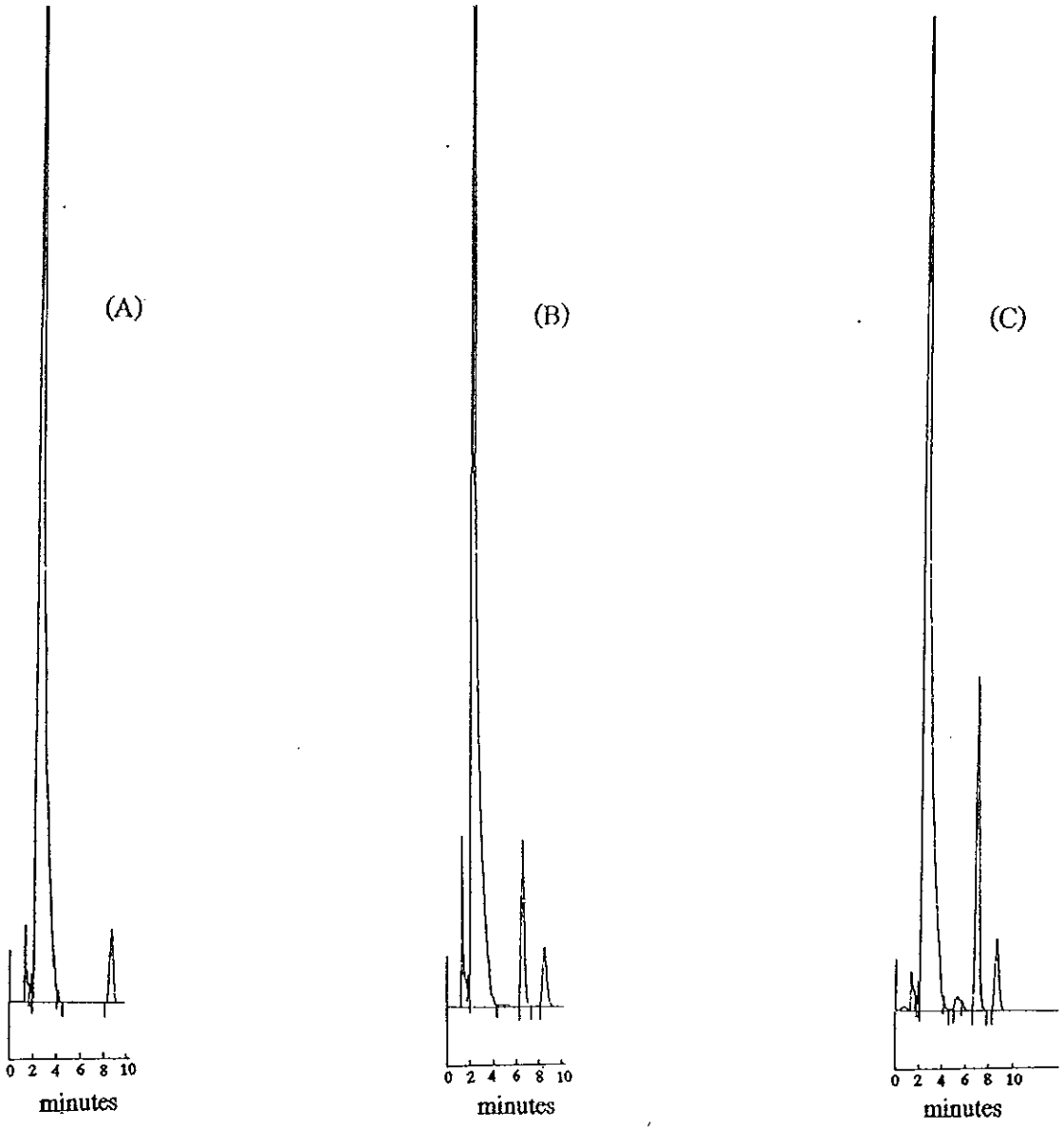

Figure 9 Chromatograms of 20 µl human plasma sample. Key : (A) blank; (B) and (C) plasma obtained from a subject pretreatment with erythromycin, 10 and 20 min after 1000 mg of acetaminophen was given, respectively. The mobile phase was 5% (V/V) acetonitrile in 95% phosphate buffer pH 3.0 at a flow rate of 2.0 ml/min. Chart speed was 2 mm/min. Attenuation was 32 mV F.S.

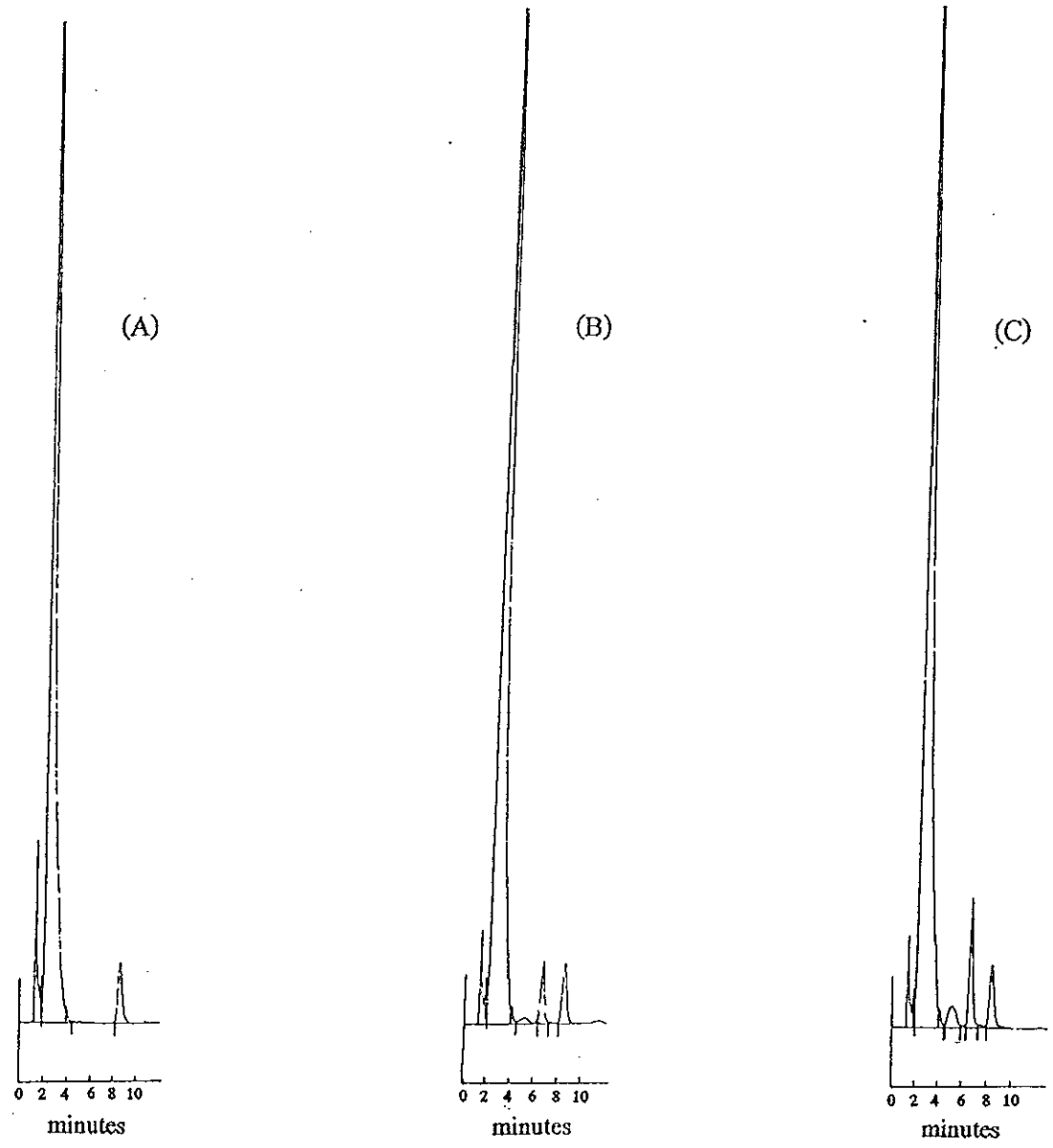

Figure 10 Chromatograms of 20 μl human plasma sample. Key : (A) blank; (B) and (C) plasma obtained from a subject pretreatment with erythromycin and cimetidine, 10 and 20 min after 1000 mg of acetaminophen was given, respectively. The mobile phase was 5% (V/V) acetonitrile in 95% phosphate buffer pH 3.0 at a flow rate of 2.0 ml/min. Chart speed was 2 mm/min. Attenuation was 32 mV F.S.

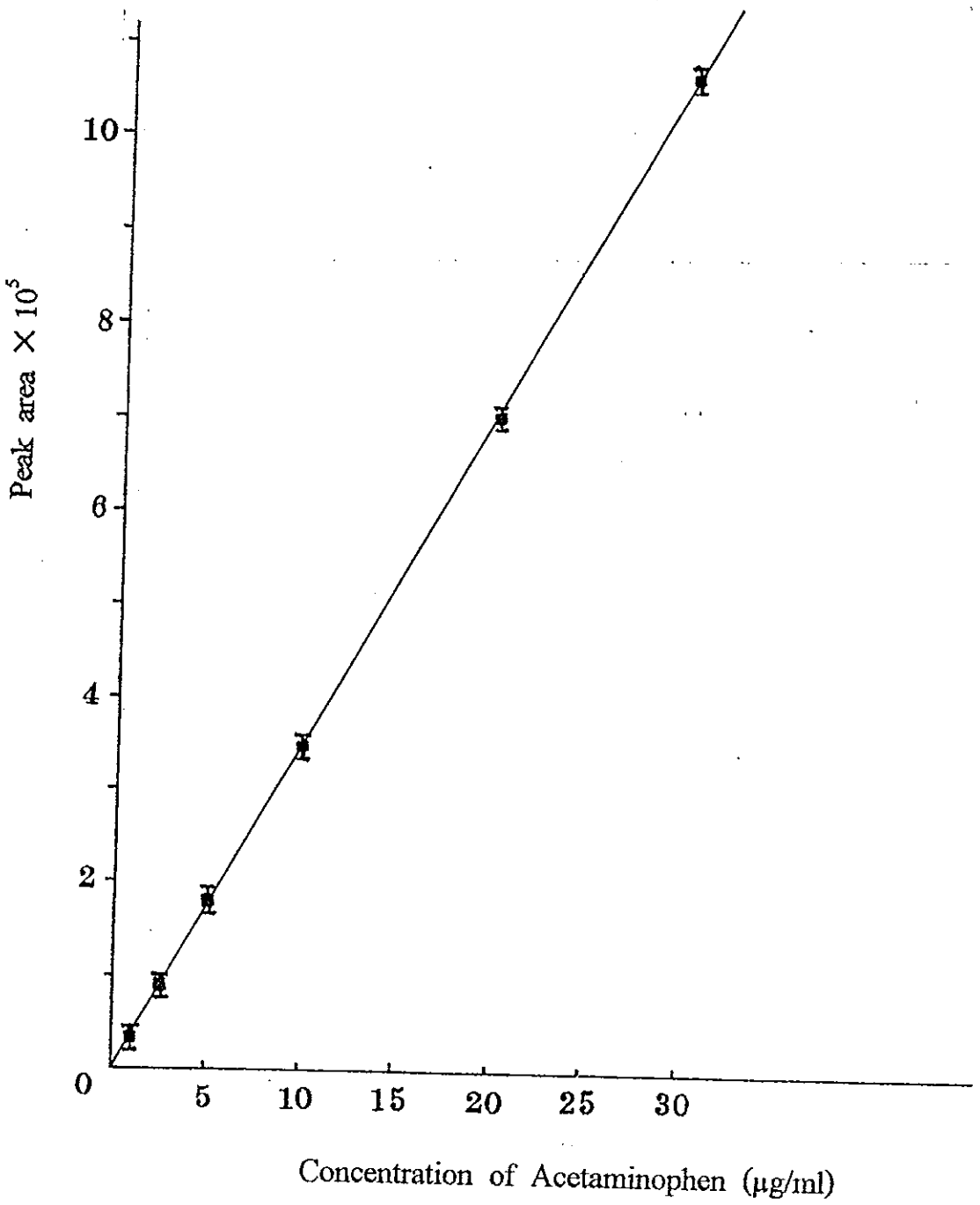

Figure 11 Mean calibration curve of standard acetaminophen in plasma, correlation coefficient (r) =0.999

Table 4 Calibration Curve of Acetaminophen[a] (Inter-assay variance)

Concentration (μg/ml)	Mean peak area ± S.D. (n=10)	CV (%)[b]
1.0	34550.35 ± 1090.33	3.16
2.5	88299.5 ± 1500.25	1.7
5.0	178290.32 ± 2614.17	1.47
10.0	349109.57 ± 5915.58	1.69
20.0	706764.53 ± 11565.67	1.64
30.0	1069067.2 ± 15364.68	1.44

[a] Various concentrations of standard acetaminophen were added to drug-free plasma sample prior to precipitation as described in the text

[b] Standard deviation divided by mean, expressed in percent

Table 5 The intra-assay variance of three different acetaminophen concentrations in plasma[a]

Concentration (μg/ml)	Mean peak area ± S.D. (n=10)	CV (%)[b]
1.0	33926 ± 912.54)	2.69
5.0	182221.6 ± 1887.48	1.04
20.0	699230 ± 3622.46	0.52

[a] Various concentrations of standard acetaminophen were added to drug-free plasma sample prior to precipitation as described in the text

[b] Standard deviation divided by mean, expressed in percent

Table 6 Relative recovery of standard acetaminophen in plasma

Concentration (μg/ml)	Peak area in H_2O[a] (Mean ± S.D.) (n = 10)	Peak area in plasma[b] (Mean ± S.D.) (n = 10)	% Recovery[c]
1.0	36630.33 ± 1004.93	34550.35 ± 1090.33	94.3
2.5	93644.25 ± 2877.07	88299.5 ± 1500.25	94.3
5.0	189493.14 ± 4058.05	178290.32 ± 2614.17	94.09
10.0	369039 ± 7059.23	349109.57 ± 5915.58	94.6
20.0	736660.89 ± 14007.31	706764.53 ± 15364.68	95.9
30.0	1091502.4 ± 9277.75	1069067.2 ± 15364.68	97.9

[a] Various concentrations of standard acetaminophen were add to water and followed by trichloroacetic acid same as in plasma

[b] Various concentrations of standard acetaminophen were add to drug-free plasma sample prior to precipitation by trichloroacetic acid

[c] Mean peak area in plasma divided by mean peak area in H_2O, expressed in percent

CHAPTER 4

RESULTS

The assay validation of our experimental method showed that the standard curve was linear in the acetaminophen concentration range of 1-30 μg/ml. The correlation coefficient (r) and the coefficient of variation (CV) were 0.999 and 0.52-3.16%, respectively. The recovery of acetaminophen in plasma was over 94%. The chromatograms showed that a peak of acetaminophen was well seperated from the other peaks in plasma. Neither peak of erythromycin nor cimetidine interfered with this system.

Seven adult male healthy Thai volunteers who were non-smoker and avoided taking alcohol and unregistered drugs in this study, were enrolled and completed in this study. Two subjects reported gastrointestinal discomfort during on erythromycin pretreatment, and one subject reported slightly dizziness during erythromycin-cimetidine pretreatment. However, they were well tolerated to all drugs through the study. The mean plasma acetaminophen concentration versus time profiles and standard deviation at each point of time are illustrated in Figure 12, and the pharmacokinetic parameters of acetaminophen are shown in Table 7-9. There were no significant differences ($P > 0.05$) among acetaminophen pharmacokinetic parameters obtained from non-pretreated (acetaminophen alone), erythromycin

pretreated, and erythromycin-cimetidine pretreated subjects as listed in Table 10. The values (mean ± S.D.) for C_{max}, T_{max}, $t_{1/2}$, Vd/f, Cl/f, and AUC in subjects receiving acetaminophen alone were 18.91 ± 6.98 μg/ml, 0.76 ± 0.39 hr, 2.18 ± 0.28 hr, 0.97 ± 0.15 l/kg, 5.23 ± 0.58 ml/min/kg and 53.34 ± 4.55 mg/l.hr, respectively; in erythromycin pretreated subjects they were 19.01 ±5.59 μg/ml, 0.56 ± 0.19 hr, 2.11 ± 0.15 hr, 1.00 ± 0.08 l/kg, 5.50 ± 0.62 ml/min/kg and 50.77 ± 2.68 mg/l.hr, respectively; and in the erythromycin-cimetidine pretreated subjects they were 15.36 ± 4.10 μg/ml, 0.84 ± 0.41 hr, 2.08 ± 0.14 hr, 1.03 ± 0.08 l/kg, 5.74 ± 0.58 ml/min/kg and 47.71 ± 4.66 mg/l.hr, respectively. The plasma acetaminophen concentration-time profile selected from one subject, showed that after a short absorptive phase, the plasma concentration declined monoexponentially and was fitted to a one-compartment open model (Figure 14). The summary data (Table 11) are compared to other published data of the pharmacokinetics of acetaminophen in human volunteers, and Table 12 shown the pharmacokinetics of acetaminophen after pretreatment with cimetidine. In the present study, the pharmacokinetic parameters (C_{max}, t_{max}, Vd/f, AUC, and Cl/f) of acetaminophen in subjects receiving acetaminophen alone and erythromycin-cimetidine pretreated subjects are similar to those reports.

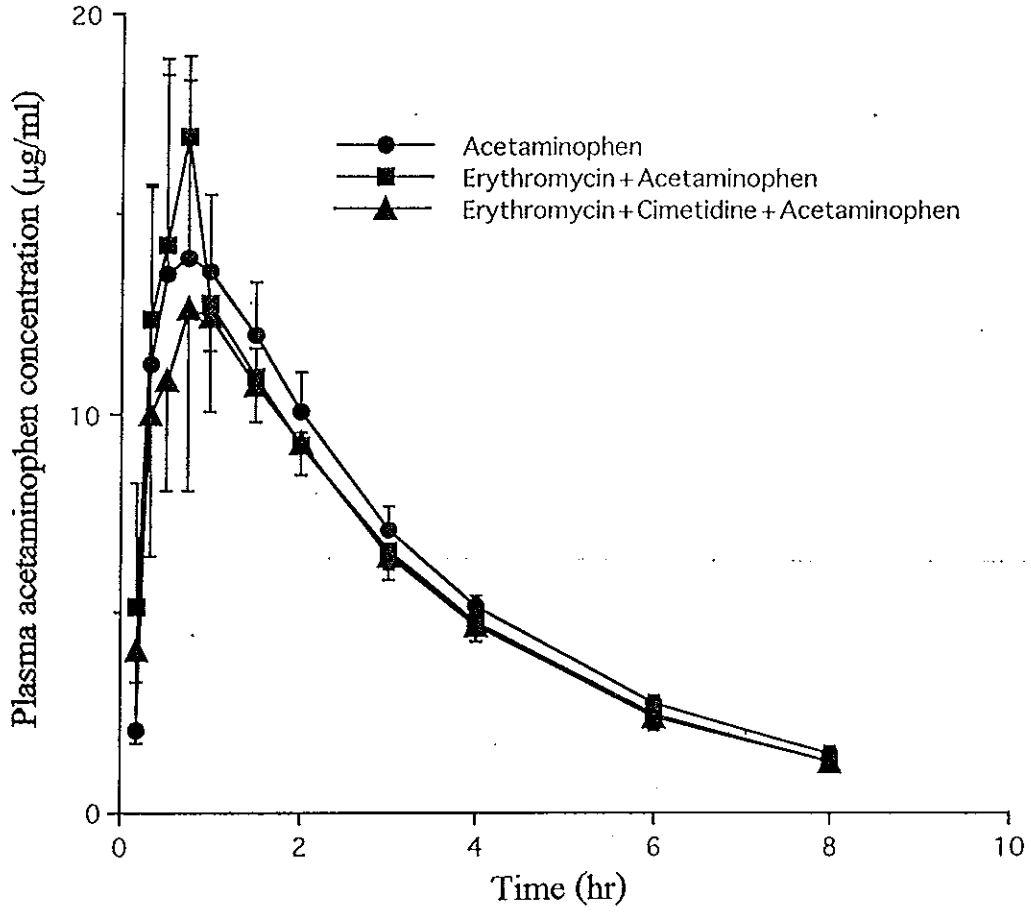

Figure 12 Mean plasma acetaminophen concentrations after acetaminophen administration alone (—●—), after erythromycin pretreatment (—■—), and after erythromycin-cimetidine pretreatment (—▲—) in 7 normal volunteers.

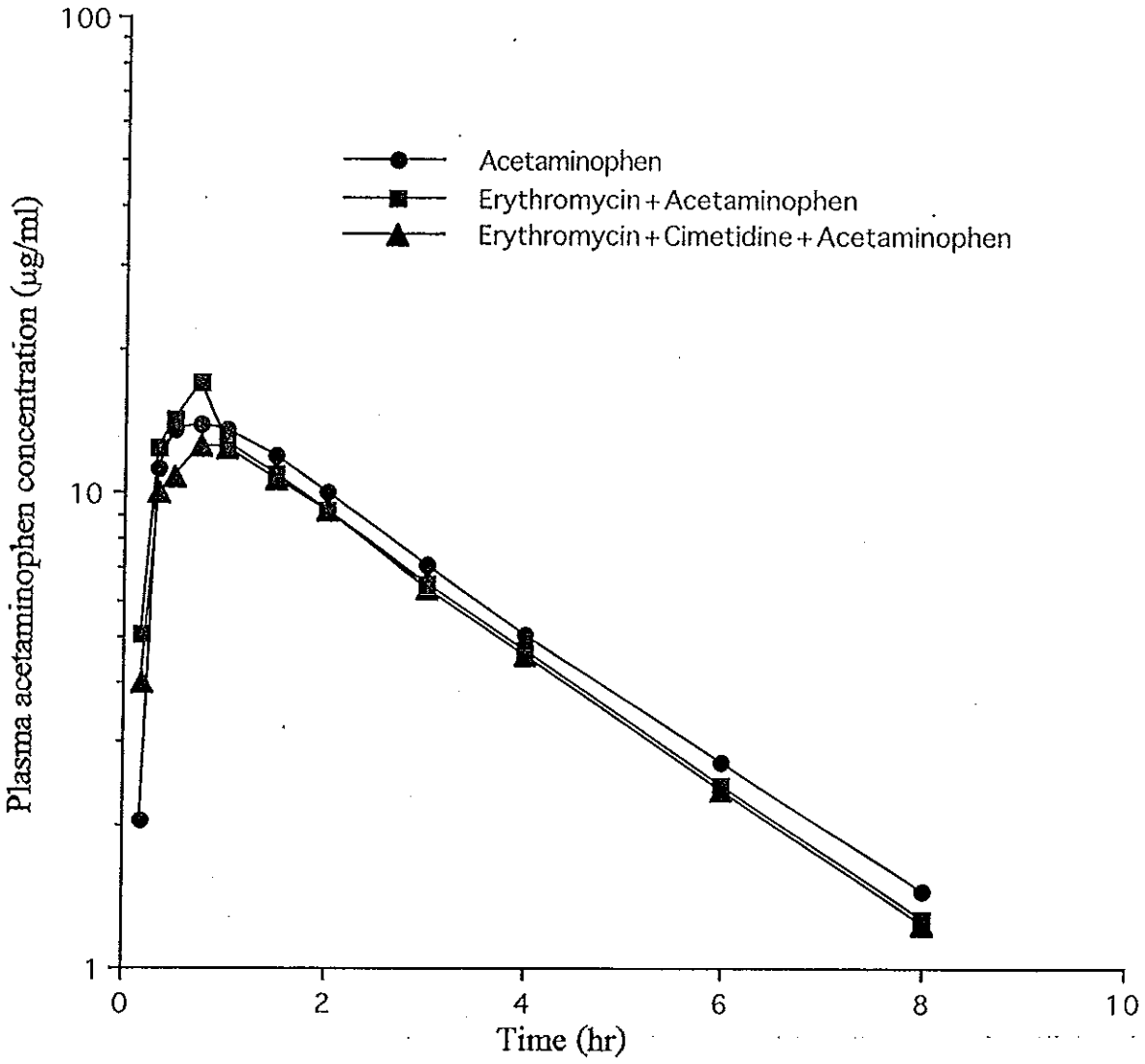

Figure 13 Semi-logarithmic mean plasma acetaminophen concentrations after acetaminophen administration alone (—●—), after erythromycin pretreatment (—■—), and after erythromycin-cimetidine pretreatment (—▲—) in 7 normal volunteers.

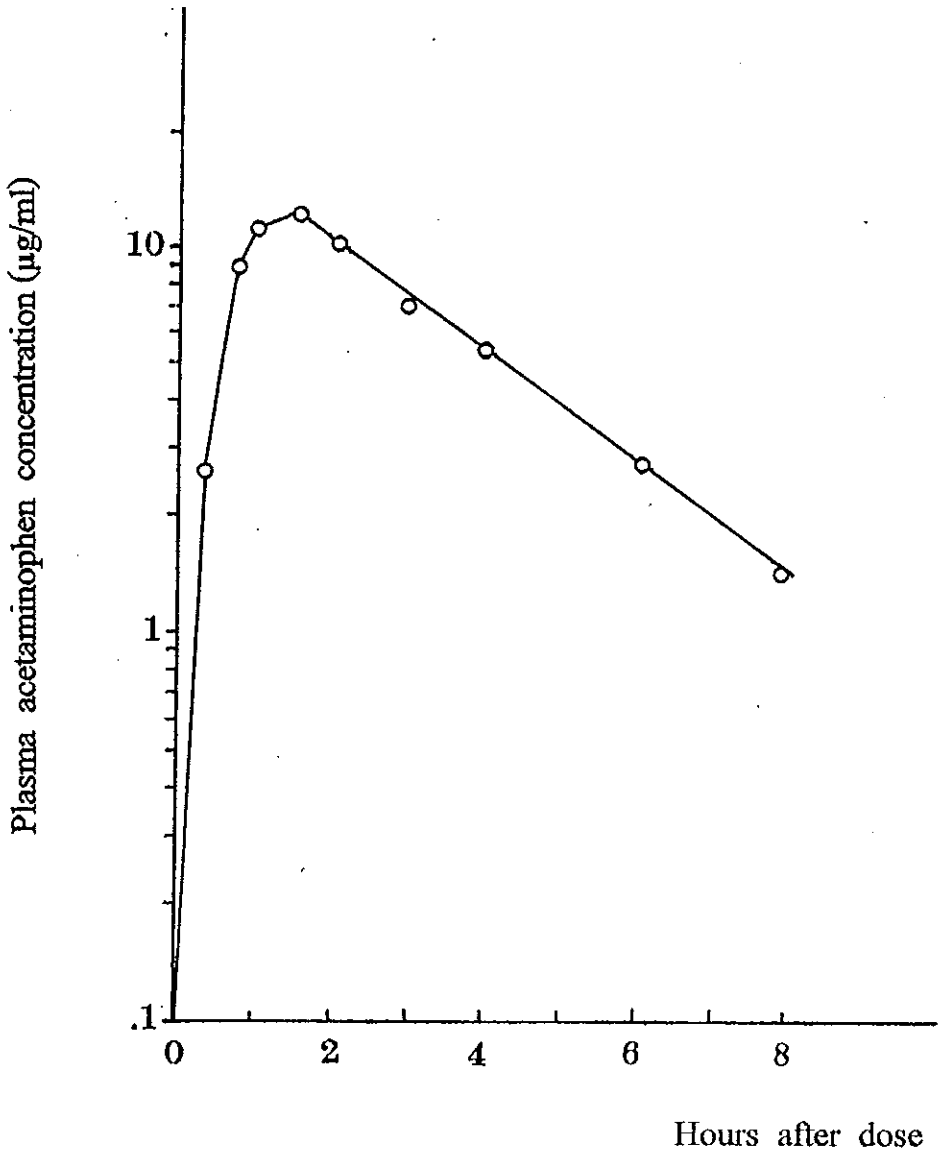

Figure 14 Semi-logarithmic plasma acetaminophen concentration -time profile following the oral administration of a single 1000 mg dose of acetaminophen to the subject pretreated with erythromycin (Subject number 2).

Table 7 Pharmacokinetics of acetaminophen in subjects receiving acetaminophen alone

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$ (abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (μg/ml)	Vd/f (l/kg)	Cl/f (ml/min/kg)	Corr[a]
1	33	60.5	49.23	6.653	0.386	0.10	1.80	0.33	33.25	0.87	5.60	0.999
2	30	65	48.37	3.465	0.331	0.20	2.09	1.50	12.40	0.96	5.30	0.999
3	32	68	56.67	3.465	0.295	0.20	2.35	0.75	16.35	0.88	4.33	0.998
4	21	62	49.03	3.465	0.264	0.20	2.63	0.50	15.00	1.25	5.48	0.995
5	33	61	60.09	4.060	0.332	0.17	2.09	0.50	19.60	0.82	4.55	0.999
6	25	57	53.96	1.440	0.296	0.48	2.34	1.00	14.75	1.10	5.42	0.999
7	27	57	56.01	1.733	0.359	0.40	1.93	0.75	21.00	0.92	5.94	0.999
x ± S.D.	28.7 ± 4.6	61.5 ± 4.0	53.34 ± 4.55	3.47 ± 1.71	0.323 ± 0.04	0.25 ± 0.14	2.18 ± 0.28	0.76 ± 0.39	18.91 ± 6.98	0.97 ± 0.15	5.23 ± 0.58	

[a]correlation coefficient of elimination phase

Table 8 Pharmacokinetics of acetaminophen in erythromycin pretreated subjects

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$ (abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (μg/ml)	Vd/f (l/kg)	Cl/f (ml/min/kg)	Corr[a]
1	33	60.5	45.87	0.945	0.374	0.73	1.85	0.75	29.50	0.96	6.01	0.994
2	30	65	52.85	3.465	0.300	0.20	2.31	0.50	20.50	0.97	4.85	0.999
3	32	68	51.64	2.870	0.328	0.24	2.11	0.75	12.75	0.87	4.75	0.9989
4	21	62	49.49	3.200	0.318	0.22	2.15	0.33	17.80	1.02	5.43	0.999
5	33	61	53.00	6.930	0.319	0.10	2.17	0.33	16.00	0.97	5.16	0.998
6	25	57	49.39	3.620	0.319	0.19	2.17	0.75	14.75	1.11	5.92	0.999
7	27	57	53.13	3.465	0.349	0.20	1.99	0.50	21.75	1.10	6.37	0.997
x ± S.D.	28.7 ± 4.6	61.5 ± 4.0	50.77 ± 2.68	3.50 ± 1.77	0.326 ± 0.017	0.27 ± 0.21	2.11 ± 0.15	0.56 ± 0.19	19.01 ± 5.59	1.00 ± 0.08	5.50 ± 0.62	

[a]correlation coefficient of elimination phase

Table 9 Pharmacokinetics of acetaminophen in erythromycin-cimetidine pretreated subjects

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$ (abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (μg/ml)	Vd/f (l/kg)	Cl/f (ml/min/kg)	Corr[a]
1	33	60.5	46.37	2.00	0.367	0.35	1.89	0.42	20.00	0.97	5.94	0.997
2	30	65	41.52	1.39	0.338	0.50	2.05	1.00	12.20	1.10	6.18	0.998
3	32	68	51.75	1.54	0.301	0.45	2.30	0.75	14.75	0.94	4.74	0.999
4	21	62	48.27	1.83	0.314	0.38	2.21	0.33	16.80	1.06	5.57	0.999
5	33	61	44.65	2.41	0.335	0.29	2.07	1.50	11.30	1.10	6.12	0.999
6	25	57	45.90	2.00	0.344	0.35	2.01	1.15	11.30	1.11	6.37	0.997
7	27	57	55.52	2.77	0.347	0.25	2.00	0.75	21.20	0.91	5.27	0.999
x ± S.D.	28.7 ± 1.7	61.5 ± 0.57	47.71 ± 4.66	1.99 ± 0.48	0.339 ± 0.022	0.37 ± 0.09	2.08 ± 0.14	0.84 ± 0.41	15.36 ± 4.10	1.03 ± 0.08	5.74 ± 0.58	

[a]correlation coefficient of elimination phase

Table 10 Pharmacokinetic parameters of acetaminophen in subjects receiving acetaminophen alone (A), pretreatment with erythromycin (B), and erythromycin-cimetidine (C) (mean ± S.D)

Treatment	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$ (abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (μg/ml)	Vd/f (l/kg)	Cl/f (ml/min/kg)
A (n=7)	28.7 ±4.6	61.5 ±4.0	53.34 ±4.55	3.47 ±1.71	0.323 ±0.04	0.25 ±0.14	2.18 ±0.28	0.76 ±0.39	18.91 ±6.98	0.97 ±0.15	5.23 ±0.58
B (n=7)	28.7 ±4.6	61.5 ±4.0	50.77 ±2.68	3.50 ±1.77	0.326 ±0.017	0.27 ±0.21	2.11 ±0.15	0.56 ±0.19	19.01 ±5.59	1.00 ±0.08	5.50 ±0.62
C (n=7)	28.7 ±1.7	61.5 ±0.57	47.71 ±4.66	1.99 ±0.48	0.339 ±0.022	0.37 ±0.09	2.08 ±0.14	0.84 ±0.41	15.36 ±4.10	1.03 ±0.08	5.74 ±0.58

Table 11 Acetaminophen pharmacokinetic data are compared to other published data

Data	Sources				
	El-Azab 1996	Ismail 1995	Kamali 1993	Divoll 1981	Present study[a]
Subjects	8 healthy male	8 Thai patients with uncomplicated malaria	5 healthy male and 5 healthy female	8 healthy male	7 healthy male
Age (yr)	13.7±3.1	19-41	20-29	24-37	21-35
Dose (mg)	1000	1000	1500	650	1000
Route	oral	oral	oral	intravenous	oral
C_{max} (μg/ml)	17.1 ± 3.9	14.5 ± 5.8	18.2 ± 1.9	-	18.9 ± 2.7
T_{max} (hr)	1.4 ± 0.67	1	1 ± 0.16	-	0.76 ± 0.2
$t_{1/2}$ (hr)	2.4 ± 0.31	3.7 ± 1.1	2.12 ± 0.15	-	2.18 ± 0.11
Vd/f (l/kg)	-	1.2 ± 0.7	1 ± 0.05	1.09	0.97 ± 0.06
Cl/f (ml/min/kg)	-	3.9 ± 1.3	6.23 ± 0.51	5.07	5.23 ± 0.22

[a]Calculation of pharmacokinetic data based on subjects received acetaminophen alone

Table 12 Acetaminophen pharmacokinetics in subjects after pretreatment with erythromycin-cimetidine are compared to published data

Data	Sources			
	Chen and Lee 1985	Miners 1984	Abernethy 1983	Present study
Subjects	4 healthy male	12 healthy male	11 volunteers	7 healthy male
Age (yr)	22	25.3 ± 2.2	21-40	21-35
Dose of acetaminophen (mg)	750	1000	650	1000
Acetaminophen administration	oral	oral	intravenous	oral
Pretreatment	Cimetidine[a] Cimetidine[b]	Cimetidine[c]	Cimetidine[d]	Cimetidine plus Erythromycin
$AUC_{0-\alpha}$ (mg/l.hr)	41 ± 5.33 47.6 ± 9.45	-	-	47.71 ± 1.76
$t_{1/2}$ (hr)	2.65 ± 0.24 2.9 ± 0.6	2.9 ± 0.09	2.66 ± 0.13	2.08 ± 0.05
Vd/f (l/kg)	- -	-	1.08 ± 0.07	1.03 ± 0.03
Cl/f (ml/min/kg)	4.76 ± 0.45 4.16 ± 0.67	5.6 ± 0.19	4.81 ± 0.38	5.74 ± 0.22

[a]Acetaminophen plus single-dose cimetidine

[b]Acetaminophen following one-week pretreated with cimetidine

[c]Acetaminophen following one-week pretreated with cimetidine

[d]Acetaminophen following pretreated with cimetidine 6 hourly for 2 doses

CHAPTER 5

DISCUSSION

As acetaminophen is the drug that has been widely used for relieving pain and fever, while erythromycin and cimetidine are also commonly used for infections and peptic ulcer, respectively. Both erythromycin and cimetidine are cytochrome P-450 inhibitors. Thus it is likely that acetaminophen will be prescribed concomitantly with erythromycin and cimetidine. So drug interaction among these drugs may occur. The minor metabolic pathway of acetaminophen (about 10% of therapeutic dose) is metabolized by cytochrome P-450 which is the microsomal oxidative pathways. Raucy, et al. (1989) reported that CYP2E1, and 1A2 are responsible for the oxidative metabolism of acetaminophen in human liver microsome. Thummel, et al. (1993) studied in human liver microsomes and found that CYP3A4 also play a role in this pathway. In the present study, we investigated the effect of erythromycin (specific inhibitor of CYP3A4) administration alone or co-administration with cimetidine (specific inhibitor of CYP3 family) on the pharmacokinetics of acetaminophen in seven healthy Thai male volunteers.

The semilogarithmic plots of the plasma acetaminophen concentration-time curve (Figure 14) show that the data are well described by a one compartment model with first-order kinetics for both absorption and elimination. The pharmacokinetic parameters such as the absorption

rate constant (Ka), the maximal concentration (C_{max}), the time to reach the maximal concentration (T_{max}), the apparent volume of distribution (Vd/f), the apparent oral clearance (Cl/f), the elimination half-life ($t_{1/2}$) and the elimination rate constant (Ke) of the subjects receiving erythromycin, and erythromycin-cimetidine pretreatment were not significantly different ($P > 0.05$) from those receiving acetaminophen alone. These findings suggest that erythromycin administration alone or in combination with cimetidine did not affect the pharmacokinetics of acetaminophen (Table 10).

Individual variation in plasma concentrations 1 hr after ingestion of a therapeutic dose of acetaminophen tablets has been observed (Heading, et al., 1973; McGilveray and Mattock, 1972; Prescott, 1974). In the present study, individual variations in the rising portion of blood level during 1 hr after acetaminophen ingestion was also found (Appendix-3). In subjects receiving acetaminophen alone, the maximal plasma concentrations of acetaminophen ranged from 12.4 to 33.25 μg/ml which were not different from the above references (7.4 - 37.0 μg/ml). The absorption rate constants ranged from 1.44 to 6.65 hr^{-1} which were similar to those reported previously in healthy volunteers (0.6 - 14.4 hr^{-1}) (McGilveray and Mattock, 1972; Paisanchanthasiri, 1991). Neither the mean plasma concentrations nor the mean absorption rate constants were not significantly different among the 3 occasions, even though the absorption rate constants of subject number 1 and 5 seem to be different among the 3 occasions. There may be the variation in disintegration time and subsequent dissolution of acetaminophen tablets due to variation in

each tablet formulation. The other factors such as the fasting-state which is considered from acetaminophen concentration-time profiles and the activities during blood collection were similar to the others, could be excluded.

The rate and extent of acetaminophen absorption depend on the gastric emptying time (Clement, et al.,1978). Erythromycin has been shown to increase the gastric emptying rate (Tomomasa, et al.,1986). From these reasons, after receiving erythromycin, the absorption rate constant and the maximal concentration of acetaminophen is likely to be enhanced. However, in the present study, after receiving erythromycin pretreatment, the mean of the absorption rate constant and the maximal concentration of acetaminophen did not alter from receiving acetaminophen alone (Table 10). The possible explanation is that the subjects were all fasted over night. In this condition the gastric emptying time is usually rapid. So the effect of erythromycin may be hidden. In contrast, cimetidine was reported to have no effect on the gastric emptying time (Powell and Donn,1984). So the effect of erythromycin-cimetidine pretreatment on the absorption rate constant and the maximal concentration of acetaminophen should likely to be the same as the effect of erythromycin pretreatment. But in the present study, after receiving erythromycin-cimetidine pretreatment, the mean of the absorption rate constant and the maximal concentration of acetaminophen seem to be lower than the values after receiving acetaminophen alone. The reason is unclear. However, Charbon, et al. (1980) reported that cimetidine reduced the increasing blood flow from

histamine in the left gastric arteries in dogs. This effect of cimetidine may reduced the absorption of acetaminophen.

In 1989, Raucy, et al. studied in human liver microsomes and reported that CYP1A2 and 2E1 are responsible in oxidative metabolism of acetaminophen. Studying in vivo, there are many reports which showed about drug interaction between acetaminophen and other drugs. For example, propranolol which is an inhibitor of CYP1A2 (Masubuchi, et al., 1994; Tassaneeyakul, et al., 1993), has been shown that it significantly decreased the clearance of the glucuronide conjugation (27 ± 6%) and glutathione-derived conjugation (16 ± 3%) of acetaminophen by Baraka, et al. (1990). Isoniazid which is the inhibitor of CYP2E1 (Schmalix, et al., 1995), has been demonstrated that it markedly inhibited the clearance of the oxidative pathway of acetaminophen (69.7%) and decreased the acetaminophen clearance (15.2%) by Epstein, et al. (1991). Caffeine which is an inducer of CYP1A2 (Chen, et al.,1996), decreased the plasma acetaminophen levels and enhanced the elimination of acetaminophen after simultaneous administration of both drugs as shown by Rainska-Giezek (1995). Zimmerman and Maddrey (1995) showed that one reason of the death of the regular users of alcohol after ingestion of acetaminophen with therapeutic intent is the induction of CYP2E1 by ethanol. These studies in vitro and in vivo showed that CYP1A2 and 2E1 are responsible in oxidative metabolism of acetaminophen.

By studying of drug interaction between acetaminophen and an enzyme inducer, phenytoin which induce many isozymes of cytochrome

P450 such as CYP2B6, 3A3, 3A4 and 3A5 (Spinler, et al., 1995), has been shown to increase the clearance of the glucuronide conjugation and glutathione-derived conjugation of acetaminophen by Miners, et al. (1984). This report may support the result of studying in vitro which was shown by Thummel, et al. (1993), that CYP3A4 is partly responsible in oxidative metabolism of acetaminophen. However, our results showed that erythromycin and cimetidine did not alter the pharmacokinetics of acetaminophen. Even though the dose of erythromycin stearate (250 mg qid × 7 days) and cimetidine (400 mg bid × 7 days) used in this study has been shown to be sufficient to inhibit hepatic drug metabolizing enzymes of many drugs (in literature review). Although there have been many reports shown that cimetidine did not has the statistically significant effect on acetaminophen pharmacokinetics in humans (Abernethy, et al.,1983; Miners, 1984 and Chen and Lee, 1985). However, in vitro study in human liver microsomes, using high dose of cimetidine (5 to 10 times of the therapeutic effect concentration), approximately 80% of the formation of reactive metabolites of acetaminophen was inhibited (Slattery, et al.,1989).

Cimetidine has been shown to spare the glucuronidation pathway of lorazepam and oxazepam (Patwardhan, et al.,1980), 7-hydroxy-4-methycoumarin (Irshaid and Abu-Khalaf, 1992) and 3-azido-3-deoxythymidine or AZT (Rajaonarison, et al.,1991). There are no reports which support the interference of sulphation by cimetidine. In case of erythromycin, there are no reports of drug interaction between erythromycin and other drugs which are metabolized via glucuronic acid

and sulphate conjugations either in clinical practice or in controlled study. In our study, although we did not determine glucuronic acid and sulphate conjugates of acetaminophen, erythromycin and cimetidine seem unlikely to have effect on them. On the other hand, the activities of various isozymes of cytochrome P-450 in the oxidative metabolism of acetaminophen in human liver microsome have been shown that CYP1A2 is of 40% and CYP2E1 is of 50% (Raucy, et al.,1989). Thummel, et al. (1993) recently reported that the activity of CYP3A4 is of 10% in this pathway. Our results demonstrated that CYP3A4 inhibitors, erythromycin and cimetidine, produced no significant effect on the pharmacokinetics of acetaminophen. Therefore, it is likely that the inhibition of CYP3A4 would have less effect on the oxidative metabolism of acetaminophen. The hypothesis that CYP3A4 is responsible in the oxidative metabolism of acetaminophen in vivo, is still unclear. Although the inhibition of the less activity of enzyme may not show the significance in the pharmacokinetic alteration, the induction of that enzyme may increase its amount and subsequent activity. So that the alteration in pharmacokinetics of the target drug may be occured. The another way to support the above hypothesis, is to measure the quantitative alteration of the acetaminophen metabolites after receiving the specific inducer of CYP3A such as rifampicin (Pichard, et al.,1990). Madhusudanarao, et al. (1988) reported that clearance of acetaminophen was increased in pulmonary tuberculosis patients on the short course chemotherapy in which rifampicin was

included. However, we could not find any controlled study concerning with the effect of rifampicin on pharmacokinetics of acetaminophen.

In summary, this study demonstrated that erythromycin given alone or in combination with cimetidine did not affect the pharmacokinetics of acetaminophen administered at the therapeutic dose. Therefore, co-administration of erythromycin, cimetidine and acetaminophen in therapeutic dosages in clinical uses would unlikely to produce any pharmacokinetic interactions.

APPENDIX-1

Preparation of standard acetaminophen in plasma blank for calibration curve.

Stock A = Acetaminophen 500 mg/l

Stock B = Acetaminophen 100 mg/l

Stock C = Acetaminophen 25 mg/l

1.0 μg/ml = 20 μl of 25 mg/l + 480 μl of plasma or deionized water

2.5 μg/ml = 50 μl of 25 mg/l + 450 μl of plasma or deionized water

5.0 μg/ml = 100 μl of 25 mg/l + 400 μl of plasma or deionized water

10.0 μg/ml = 50 μl of 100 mg/l + 450 μl of plasma or deionized water

20.0 μg/ml = 100 μl of 100 mg/l + 400 μl of plasma or deionized water

30.0 μg/ml = 150 μl of 100 mg/l + 350 μl of plasma or deionized water

APPENDIX-2

Protein precipitation

1. Take 200 µl of sample or standard solution
2. Add 200 µl of freshly prepared 10 % trichloroacetic acid, leave on ice for 5 min
3. Centrifuge at 14,000 rpm for 5 min

APPENDIX-3

Plasma concentration of acetaminophen at 0 - 8 hr in subjects receiving acetaminophen alone

Subject No.	Concentrations (μg/ml) at Time (hr)											
	0	0.17	0.33	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0
11	0	1.75	33.25	21.75	15.40	12.55	10.75	8.85	5.70	3.90	1.85	0.85
21	0	0.70	2.65	4.35	9.00	11.30	12.40	10.35	7.15	5.45	2.75	1.40
31	0	3.20	10.00	15.50	16.35	14.20	11.40	10.10	7.25	5.35	2.80	1.75
41	0	2.60	9.25	15.00	11.0	11.00	9.80	8.25	6.20	4.80	3.20	1.60
51	0	3.30	14.00	19.60	15.70	15.20	14.00	11.00	7.75	5.30	2.80	1.50
61	0	0.30	1.80	6.20	9.00	14.75	12.40	10.60	7.75	5.70	3.10	1.80
71	0	2.60	6.90	12.25	21.00	16.00	13.13	11.25	7.70	5.20	2.60	1.30
x ± SD	0	2.06 ± 0.45	11.12 ± 4.02	13.52 ± 2.44	13.92 ± 1.68	13.57 ± 0.74	11.98 ± 0.54	10.06 ± 0.42	7.07 ± 0.31	5.10 ± 0.22	2.73 ± 0.17	1.46 ± 0.12

APPENDIX-4

Plasma concentration of acetaminophen at 0 - 8 hr in erythromycin-pretreated subjects

Subject No.	Concentrations (μg/ml) at Time (hr)											
	0	0.17	0.33	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0
12	0	1.25	3.25	4.00	29.25	14.90	10.80	9.65	5.50	4.05	1.75	1.00
22	0	3.80	9.90	20.50	17.35	12.85	10.10	9.30	6.50	4.85	2.70	1.50
32	0	5.50	11.60	11.25	12.75	12.25	12.20	9.50	6.85	4.75	2.65	1.30
42	0	6.00	17.80	14.25	13.25	11.25	11.25	8.75	6.20	4.50	2.35	1.30
52	0	12.30	16.0	14.30	14.00	12.00	10.60	9.10	6.55	5.10	2.80	1.30
62	0	3.60	10.40	12.25	14.75	11.30	10.25	8.75	6.25	4.75	2.60	1.25
72	0	3.20	17.50	22.75	17.25	14.75	11.20	9.30	7.50	5.13	2.25	1.25
x	0	5.09	12.35	14.19	16.94	12.76	10.91	9.19	6.48	4.73	2.44	1.27
± SD		± 1.34	± 1.97	± 2.24	± 2.16	± 0.57	± 0.27	± 0.13	± 0.23	± 0.14	± 0.14	± 0.06

APPENDIX-5

Plasma concentration of acetaminophen at 0 - 8 hr in erythromycin-cimetidine pretreated subjects

Subject No.	Concentrations (μg/ml) at Time (hr)											
	0	0.17	0.33	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0
13	0	4.40	12.50	19.00	14.70	12.20	10.20	9.20	5.70	3.90	1.85	1.00
23	0	8.70	8.76	7.00	7.20	12.20	9.35	8.20	5.50	4.20	1.90	1.10
33	0	2.60	4.95	7.75	14.75	13.50	11.13	9.80	6.90	5.20	2.80	1.60
43	0	3.90	16.80	10.75	12.00	13.60	9.70	8.28	6.20	4.80	2.30	1.30
53	0	1.60	7.75	7.75	9.20	8.20	11.30	9.30	6.60	4.60	2.60	1.20
63	0	4.20	9.00	8.50	9.50	11.30	11.30	9.50	6.30	4.50	2.55	1.13
73	0	2.75	10.25	14.85	21.20	16.00	12.00	10.25	7.25	5.20	2.70	1.25
x	0	4.02	10.00	10.80	12.65	12.43	10.71	9.21	6.35	4.63	2.39	1.23
± SD		± 0.87	± 1.43	± 1.70	± 1.78	± 0.91	± 0.37	± 0.29	± 0.24	± 0.18	± 0.14	± 0.07

APPENDIX-6

ที่ ทม 1209/ 113๐

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90110

หนังสือรับรองการศึกษาวิจัย

การศึกษาวิจัยเรื่อง : "ผลของการบริหารยาอีริโธมัยซินและไซเมทิดินร่วมกันต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนในอาสาสมัครที่มีสุขภาพปกติ"

ผู้วิจัย : นางดวงแข รักไทย
นักศึกษาปริญญาโท สาขาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณา และเห็นชอบจากคณะกรรมการจริยธรรม ซึ่งเป็นคณะกรรมการพิจารณาโครงการวิจัย ตลอดจนติดตามผลในส่วนของการทดลองที่กระทำต่ออาสาสมัคร ของคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 25 ธันวาคม 2539

..... ประธานคณะกรรมการ
(รศ.เพริศพิชญ์ คณาธารณา)
รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา

..... กรรมการ
(นายแพทย์สมหมาย ปลอดสมบูรณ์)
ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์

.....กรรมการ
(รศ.ถาวร เกียรติทับทิว)
ภาควิชารัฐประศาสนศาสตร์ คณะวิทยาการจัดการ

.....กรรมการ
(นายแพทยวิบูลย์ ฤทธิทิศ)
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

.....กรรมการ
(แพทย์หญิงสุวิณา รัตนชัยวงศ์)
ภาควิชาชีวเวชศาสตร์ คณะแพทยศาสตร์

.....กรรมการ
(ผศ.วุฒิพร พรหมขุนทอง)
ภาควิชาวาริชศาสตร์ คณะทรัพยากรธรรมชาติ

ใบยินยอม

Consent form

1. ชื่อโครงการ : ผลของการบริหารยาอีริโธมัยซินและไซเมทิดีนร่วมกันต่อเภสัชจลน์ศาสตร์ของ ของยาอะเซตามิโนเฟนในอาสาสมัครที่มีสุขภาพปกติ
2. ข้าพเจ้า (นาย, นาง, นางสาว).....นามสกุล.....อายุ.....ปี ยินยอมเข้ารับการศึกษาเกี่ยวกับ " ผลของการบริหารยาอีริโธมัยซินและไซเมทิดีนร่วมกันต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนในอาสาสมัครที่มีสุขภาพปกติ"
3. วัตถุประสงค์ของการศึกษา
   1). ศึกษาเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนชนิดรับประทานในขนาด 1000 มก. ครั้งเดียว ในอาสาสมัครคนไทยที่มีสุขภาพปกติ
   2). ศึกษาผลกระทบจากการรับประทานยาอีริโธมัยซิน ต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟน ในอาสาสมัครคนไทยที่มีสุขภาพปกติ
   3). ศึกษาผลกระทบจากการรับประทานยาอีริโธมัยซินและไซเมทิดีนร่วมกัน ต่อเภสัชจลนศาสตร์ของยาอะเซตามิโนเฟนในอาสาสมัครคนไทยที่มีสุขภาพปกติ
   4). สามารถใช้เป็นข้อมูลให้แพทย์ทราบถึง ปฏิกิริยาระหว่างยา (drug interaction) ทั้ง 3 ชนิดนี้
4. วิธีการศึกษา
   4.1 อาสาสมัครที่เข้าร่วมโครงการต้องเป็นผู้ที่มีสุขภาพสมบูรณ์และแข็งแรงดี
   4.2 ใช้อาสาสมัครเพศชาย อายุระหว่าง 20-35 ปี
   4.3 อาสาสมัครทุกคนต้องไม่ได้รับยาชนิดอื่นมาก่อนที่จะเริ่มทำการทดลอง เป็นเวลาอย่างน้อย 1 เดือน ยกเว้นวิตามินหรือยาที่แพทย์ในโครงการเป็นผู้สั่งให้
   4.4 อาสาสมัครทุกคนต้องไม่สูบบุหรี่หรือดื่มสุราในระหว่างที่ทำการทดลอง
   4.5 ก่อนเริ่มทำการทดลองอาสาสมัครทุกคนจะได้รับการเจาะเลือด เพื่อตรวจความปกติ/ผิดปกติของเม็ดเลือด และค่าชีวเคมีของเลือด ที่โรงพยาบาลสงขลานครินทร์
   4.6 ก่อนเริ่มทำการทดลอง ให้อาสาสมัครอดอาหารมาก่อนอย่างน้อย 8 ชั่วโมง ในการทดลองครั้งที่ 1 อาสาสมัครทุกคนจะได้รับประทานยาอะเซตามิโนเฟนขนาด 1000 มก. (500 มก. 2 เม็ด) หลังจากได้ยาแล้วประมาณ 2 ชั่วโมงจึงจะยินยอมให้อาสาสมัครรับประทานอาหารได้ ส่วนในการทดลองครั้งที่ 2 และ 3 จะแบ่งอาสาสมัครออกเป็น 2 กลุ่มโดยการจับฉลาก กลุ่มที่ 1 จะได้รับยาอีริโธมัยซิน ขนาด 250 มก. วันละ 4 ครั้ง ก่อนอาหาร และก่อนนอน รับประทานติดต่อกันเป็นเวลา 7 วัน ในการทดลองครั้งที่ 2 และจะได้รับอีริโธมัยซินขนาด 250 มก. วันละ 4 ครั้ง ก่อนอาหาร และก่อนนอน ร่วมกับยาไซเมทิดีน ขนาด 800 มก. (400 มก. 2 เม็ด) ก่อนนอน รับประทานติดต่อกัน

เป็นเวลา 7 วัน ในการทดลองครั้งที่ 3 ส่วนกลุ่มที่ 2 จะได้รับยาสลับกับกลุ่มแรก กล่าวคือ จะได้รับยาอีริโธมัยซินและไซเมทิดีนในการทดลองครั้งที่ 2 และจะได้รับเฉพาะยาอีริโธมัยซินในการทดลองครั้งที่ 3 โดยขนาด เวลา และระยะเวลาการรับประทานยาเป็นเช่นเดียวกับกลุ่มแรก ในการทดลองครั้งที่ 2 และ 3 เมื่อรับประทานยาจนครบ 7 วันแล้ว ก็จะทำการทดลองเหมือนการทดลองครั้งที่ 1

4.7 ในขณะทำการทดลองแต่ละครั้ง จะเจาะเลือดครั้งละ 5 มล. ในช่วงเวลา 0-8 ชั่วโมง โดยเจาะเลือดก่อนรับประทานยาอะเซตามิโนเฟน และเมื่อเวลา 10, 20, 30, 45, 60, 90, 120, 180, 240, 360 และ 480 นาที หลังได้รับยา การเจาะเลือดนั้นจะทำการเจาะเพียงครั้งเดียวแล้วคาสาย catheter ไว้ เพื่อดูดเอาตัวอย่างเลือดที่เวลาต่างๆกัน นำเอาเลือดที่ได้ปั่นแยกเอาพลาสมาทันที และเก็บไว้ที่อุณหภูมิ -20° C เพื่อทำการวิเคราะห์ต่อไป

5. ผลข้างเคียงของการใช้ยา

1. ยาอะเซตามิโนเฟนหรือยาพาราเซตามอล เป็นยาลดไข้แก้ปวดที่ใช้ได้ผลดีและนิยมใช้กันมากในปัจจุบัน ขนาดที่ใช้ในการรักษา 500-1000 มก. จะมีผลข้างเคียงของยาน้อยมาก แต่พิษของยาจะเกิดขึ้นถ้าได้รับเกินขนาด (รับประทานครั้งหนึ่งมากกว่า 10 กรัม) โดยเกิดภาวะตับวาย
2. ยาอีริโธมัยซิน เป็นยาปฏิชีวนะที่นิยมใช้ในผู้ที่แพ้เพนนิซิลิน ผลข้างเคียงของยานี้ที่ให้โดยการรับประทาน คือ เกิดการระคายเคืองทางเดินอาหาร อาจเกิดปฏิกิริยาการแพ้ยา เช่น ผื่น ลมพิษ ดีซ่าน
3. ยาไซเมทิดีน เป็นยาที่ใช้รักษาแผลในกระเพาะอาหารและลำไส้เล็กส่วนต้น ผลข้างเคียงคือ อาจมีอาการท้องเดินเล็กน้อย มีปฏิกิริยาการแพ้ยาในบางราย ในรายที่ได้รับยานี้ติดต่อกันเป็นเวลานานๆ(มากกว่า 1 เดือน) อาจมีหน้าอกโต (gynecomastia) ในผู้ชาย ปวดศรีษะ มึนงง ยับยั้งการทำงานของเอ็นไซม์ที่ตับ

6. ความรับผิดชอบต่ออาสาสมัครที่ร่วมโครงการ

หากอาสาสมัครผู้เข้าร่วมโครงการทดลอง เกิดอาการผิดปกติทั้งทางร่างกายและจิตใจอันเป็นผลสืบเนื่องมาจากการทดลองนี้ ไม่ว่าจะด้วยสาเหตุใดก็ตาม ผู้ทำการทดลองต้องรับผิดชอบในการรักษาพยาบาลอาสาสมัครจนกว่าจะหายเป็นปกติ

7. โอกาสในการซักถาม : หากข้าพเจ้ามีข้อสงสัยเกี่ยวกับการศึกษาครั้งนี้ ข้าพเจ้ามีสิทธิในการซักถามได้ทุกขั้นตอนทั้งก่อนและระหว่างทำการทดลอง และหากข้าพเจ้าไม่พอใจในผลของการกระทำในการศึกษาวิจัย ข้าพเจ้ามีสิทธิในการถอนตัวจากโครงการได้ทุกเมื่อ

8. คำยินยอมเข้าร่วมโครงการ : ข้าพเจ้าได้อ่าน และเข้าใจถึงวัตถุประสงค์ของการศึกษาครั้งนี้เป็นอย่างดี และยินดีให้ความร่วมมืออย่างดีที่สุด

9. ค่าตอบแทน : อาสาสมัครทุกคนจะได้รับค่าตอบแทนในการเข้าร่วมโครงการขั้นตอนละ 1000 บาท ไม่ว่าจะร่วมโครงการจนเสร็จสิ้นหรือไม่ก็ตาม

หมายเหตุ : อาสาสมัครทุกคนต้องให้ความร่วมมือเป็นอย่างดี โดยเฉพาะอย่างยิ่งต้องไม่สูบบุหรี่ ไม่ดื่มสุรา หรือรับประทานยาอื่นใดโดยมิได้แจ้งให้คณะผู้ทำการวิจัยทราบ ถ้าอาสาสมัครท่านใดไม่ทำตามระเบียบที่ตกลงไว้ คณะผู้ทำการทดลองสามารถถอนท่านออกจากการทดลองได้ทันที

.....	.....
(ลายเซ็นอาสาสมัคร)	วัน เดือน ปี
.....	.....
(ลายเซ็นพยาน)	วัน เดือน ปี
.....	.....
(ลายเซ็นแพทย์)	วัน เดือน ปี

แบบบันทึกประวัติและการตรวจร่างกาย
ของอาสาสมัครไทย

เลขที่.....

วันที่.....

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1. ประวัติส่วนตัว

ชื่อ.....นามสกุล.....

อายุ.....ปี เพศ ( ) ชาย ( ) หญิง

น้ำหนัก.....กก. ส่วนสูง.....ซม.

อาชีพ.....ที่อยู่.....

2. ประวัติการเจ็บป่วย

2.1 <u>ประวัติการเจ็บป่วยในปัจจุบัน</u>

(1).....

(2).....

(3).....

2.2 <u>ประวัติการเจ็บป่วยในอดีต</u>

( ) เคยนอนพักรักษาตัวในโรงพยาบาล ระบุชื่อโรค.....

( ) เคยได้รับการผ่าตัด ระบุชื่อโรค.....

( ) เคยเป็นโรคภูมิแพ้

( ) เคยแพ้ยา ระบุชื่อยาและอาการ.....

( ) เคยมีอาการตัวเหลือง ตาเหลือง เมื่อปี.....

3. ประวัติการเจ็บป่วยในครอบครัว

3.1 <u>ประวัติโรคกรรมพันธุ์</u> มีญาติป่วยเป็นโรค

( ) โรคภูมิแพ้

( ) โรคเบาหวาน

( ) โรคลมบ้าหมู

( ) โรคเลือด.....

3.2 <u>โรคติดเชื้อ</u>

( ) วัณโรค

( ) ตับอักเสบ

( ) อื่นๆ

4. ประวัติและอุปนิสัยส่วนตัว

บุหรี่ ( ) ไม่สูบ ( ) สูบ : จำนวน.....มวน/วัน

สุรา ( ) ไม่ดื่ม ( ) ดื่ม : จำนวน.....แก้ว/วัน

ยาที่ใช้/รับประทานเป็นประจำ ระบุชื่อยา.....

5. การตรวจร่างกาย

GA : .....

Vital Sign : BT.....°C PR...../min

RR...../min BP.....mmHg

Skin : .....

Heart : .....

Lung : .....

Abdomen : .....

Extremities : .....

Neuroexamination :

Conciousness : ( ) poor ( ) fair ( ) good

Pupils : diameter.....mm

RTL.....

Reflex : .....

.....

Muscle Power : .....

สรุปการตรวจร่างกาย

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ

แพทย์ผู้ตรวจร่างกาย.....

6. การตรวจทางห้องปฏิบัติการ

6.1 CBC ผล.....

6.2 LFT ผล.....

7. สรุปผลของการตรวจร่างกาย

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ ระบุ.....

ผู้บันทึก.....

BIBLIOGRAPHY

Textbooks

Ginsburg, C.M. 1980. "Macrolide : Erythromycin", In Antimicrobial Therapy, pp. 84-134. Kajan, B.M., eds. Philadelphia : W.B. Saunder Company.

Heading, R.C.; Logan, R.F.A.; McLoughlin, G.P.; Lidgard, G. and Forrest, J.A.H. 1977. "Effect of Cimetidine on Gastric Emptying", In Cimetidine : Proceedings of the Second International Symposium on Histamine H_2-Receptor Antagonists, pp. 145-154. Burland, W.L. and Alison-Simkins, M. eds. Amsterdam : Excerpta Medica.

Kucers, A. and Bennett, M.N. 1979. The Use of Antibiotics. pp. 496-506. 3rd ed. London : Heinemann Medical Books LTD.

Plaa, G.L. 1980. "Toxic Responses of the Liver", In Casarett and Doull's Toxicology : The Basis Science of Poisons, p. 221. Doull, J.; Klaassen, C.D. and Amdur, M.O.,eds. 2nd ed. New York : Macmillan Publishing.

Sande, M.A. and Mandell, G.L. 1991. "Antimicrobial Agents : Erythromycin", In Goodman and Gilman's the Pharmacological Basis of Therapeutics, pp. 1130-1134. Gilman, A.G. and Goodman, L.S., eds. New York : Macmillan Publishing.

Journals

Abernethy, D.R.; Greenblatt, D.J.; Divoll, M.; Ameer, B. and Shader, R.I. 1983. "Differential Effect of Cimetidine on Drug Oxidation (Antipyrine and Diazepam) vs. Conjugation (Acetaminophen and Lorazepam) : Prevention of Acetaminophen Toxicity by Cimetidine", Journal of Pharmacology and Experimental Therpeutics. 224 (1983), 508-513.

Al-obaidy, S.S.; McKiernan, P.J.; Li Wan Po, A.; Glasgow, J.F.T. and Collier, P.S. 1996. "Metabolism of Paracetamol in Children with Chronic Liver Disease", European Journal of Clinical Pharmacology. 50 (1996), 69-76.

Alam, S.N.; Roberts, R.J. and Fischer, L.J. 1977. "Age-Related Differences in Salicylamide and Acetaminophen Conjugation in Man", Journal of Pediatrics. 90 (1977), 130-135.

Ambrose, P.J. and Harralson, A.F. 1981. "Lack of Effect of Cimetidine on Theophylline Clearance", Drug Intelligence and Clinical Pharmacy. 15 (1981), 389-390.

Bachmann, K.; Schwartz, J.I.; Forney, R.; Frogameni, A. and Jauregui, L.E. 1984. "The Effect of Erythromycin on the Disposition Kinetics of Warfarin", Pharmacology. 28 (1984), 171-176.

Baraka, O.Z.; Ford, J.M.; Truman, C.A. and Roberts, C.J.C. 1990. "Effect of Propranolol on Paracetamol Metabolism in Man", British Journal of Clinical Pharmacology. 29 (1990), 261-264.

Bartle, W.R. 1980. "Possible Warfarin-Erythromycin Interaction", Archives Internal Medicine. 140 (1980), 985-987.

Bartle, W.R.; Walker, S.E. and Shapero, T. 1982. "Effect of Cimetidine on Phenytoin Metabolism", Clinical Pharmacology and Therapeutics. 31 (1982), 1202.

Beaulac-Baillargeon, L. and Rocheleau, S. 1994. "Paracetamol Pharmacokinetics During the First Trimester of Human Pregnancy", European Journal of Clinical Pharmacology. 46 (1994), 451-454.

Benson, G.D. 1983. "Acetaminophen in Chronic Liver Disease", Clinical Pharmacology and Therapeutics. 33 (1983), 95-101.

Bolme, P.; Eriksson, M.; Habte, D. and Paalzow, L. 1982. "Paracetamol to Children with Different Nutrition Status", Clinical Pharmacology and Therapeutics. 31 (1982), 205.

Bonkowsky, H.L.; Mudge, G.H. and MeMurtry, R.J. 1978. "Chronic Hepatic Inflammation and Fibrosis Due to Low Doses of Paracetamol", Lancet. 1 (1978), 1016-1018.

Breen, K.J.; Bury, R.; Desmond, P.V. Mashford, M.L.; Morphett,B.; Westwood, B. and Shaw, R.G. 1982. "Effect of Cimetidine and Ranitidine on Hepatic Drug Metabolism", Clinical Pharmacology and Therapeutics. 31 (1982), 272-300.

Carranco, E.; Kareus, J.; Co, S.; Peak, V. and Al-Rajich, S. 1985. "Carbamazepine Toxicity Induced by Concurrent Erythromycin Therapy", Archives of Neurology. 42 (1985), 187-188.

Charbon, G.A.; Brouwers, H.A.A. and Sala, A. 1980. "Histamine H_1 and H_2-Receptors in the Gastrointestinal Circulation", Naunyn-Schmiedeberg's Archives of Pharmacology. 312 (1980), 123-129.

Chen, L.; Bondoc, F.Y.; Lee, M.J.; Hussin, A.H.; Thomas, P.E. and Yang, C.S. 1996. "Caffeine Induces Cytochrome P4501A2 : Induction of CYP1A2 by Tea in Rats", Drug Metabolism and Disposition. 24 (1996), 529-533.

Chen, M.M. and Lee, C.S. 1985. "Cimetidine-Acetaminophen Interaction in Humans", Journal of Clinical Pharmacology. 25 (1985), 227-229.

Clements, J.A.; Heading, R.C.; Nimmo, W.S. and Prescott, L.F. 1978. "Kinetics of Acetaminophen Absorption and Gastric Emptying in Man", Clinical Pharmacology and Therapeutics. 24 (1978), 420-431.

Clissold, S.P. 1986. "Paracetamol and Phenacetin", Drugs. 32 (1986), 46-59.

Cox, S.R.; Kroboth, P.D.; Anderson, P.H. and Smith, R.B. 1986. "Mechanism for the Interaction Between Triazolam and Cimetidine", Biopharmaceutics & Drug Disposition. 7 (1986), 567-575.

Danan, G.; Descatoire, V. and Pessayre, D. 1981. "Self-Induction by Erythromycin of Its Own Transformation into a Metabolite Forming an Inactive Complex with Reduced Cytochrome P-450", Journal of Pharmacology and Experimental Therapeutics. 218 (1981), 509-514.

Daneshmend, T.K. and Roberts, C.J.C. 1981. "Cimetidine and Bioavailability of Labetalol", Lancet. 1 (1981), 565.

DeLaforge, M.; Jaouen, M. and Mansuy, D. 1983. "Dual Effect of Macrolide Antibiotics on Rat Liver Cytochrome P-450. Induction and Formation of Metabolite-Complex. A Structure-Activity Relationship", Biochemical Pharmacology. 32 (1983), 2309-2318.

Desmond, P.V.; Shaw, R.G.; Bury, R.W.; Mashford, M.L. and Breen, K.J. 1981. "Cimetidine Impairs the Clearance of an Orally Administered High Clearance Drug, Chlormethiazole", Gastroenterology. 80 (1981), 1330.

Divoll, M.; Abernethy, D.R.; Ameer, B. and Greenblatt, D.J. 1982. "Acetaminophen Kinetics in the Elderly", Clinical Pharmacology and Therapeutics. 31 (1982), 151-156.

Donovan, M.A.; Heagerty, A.M.; Patel, L.; Castleden, M. and Pohl, J.E.F. 1981. "Cimetidine and Bioavailibility of Propranolol", Lancet. 1 (1981), 164.

D' Souza, M.A.; Pollock,S.H. and Solomon, H.M. 1988. "Cyclosporine-Cimetidine Interaction", The American Society for Pharmacology and Experimental Therapeutics. 16 (1988), 57-59.

El-Azab, G.; Youssef, M.K.; Higashi, Y.; Murakami, T. and Yata, N. 1996. "Acetaminophen Plasma Level After Oral Administration in Liver Cirrhotic Patients Suffering from Schistosomal Infection", International Journal of Clinical Pharmacology and Therapeutics. 34 (1996), 299-303.

Epstein, M.M.; Nelson, S.D.; Slattery, J.T.; Kalhorn, T.F.; Wal, R.A. and Wright, J.M. 1991. "Inhibition of the Metabolism of Paracetamol by Isoniazid", British Journal of Clinical Pharmacology. 31 (1991), 139-142.

Freeman, D.J.; Grant, D.R. and Carruthers, S.G. 1991. "The Cyclosporine-Erythromycin Interaction : Impaired First Pass Metabolism in Pig", British Journal of Pharmacology. 103 (1991), 1709-1712.

Galinsky, R.E. and Levy, G. 1984. "Absorption and Metabolism of Acetaminophen Shortly Before Parturition", Drug Intelligence Clinical Pharmacology. 18 (1984), 977-979.

Gazzard, B.G.; Ford-Hutchinson, A.W.; Smith, M.J.H. and William, R. 1973. "The Binding of Paracetamol to Plasma Proteins of Man and Pig", Journal of Pharmacy and Pharmacology. 25 (1973), 964-967.

Gonzalez, F.G. 1989. "The Molecular Biology of Cytochrome P450s", Pharmacological Reviews. 40 (1989), 244-276.

Gonzalez, F.G. and Idle, J.R. 1994. "Pharmacokinetic Phenotyping and Genotyping", Clinical Pharmacokinetics. 26 (1994), 59-70.

Gubbins, P.O. and Bertch, K.E. 1991. "Drug Absorption in Gastrointestinal Disease and Surgery. Clinical Pharmacokinetic and Therapeutic Implications', Clinical Pharmacokinetics. 21 (1991), 431-447.

Gugler, R.; Brand, M. and Somogyi, A. 1981. "Impaired Cimetidine Absorption Due to Antacids and Metoclopramide", European Journal of Clinical Pharmacology. 20 (1981), 225-228.

Hajek, K.K.; Cook, N.I. and Novak, R.F. 1982. "Mechanism of Inhibition of Microsomal Drug Metabolism by Imidazole", Journal of Pharmacology and Experimental Therapeutics. 223 (1982), 97-104.

Heading, R.C.; Nimmo, J.; Prescott, L.F. and Tothill, P. 1973 "The Dependence of Paracetamol Absorption on the Rate of Gastric Emtying", Clinical Pharmacology. 47 (1973), 415-421.

Hemsworth, T.C. and Renton, K.W. 1981. "Depression of Theophylline Metabolism and Elimination by Troleandomycin and Erythromycin", Biochemical Pharmacology. 30 (1981), 1299-1304.

Hetzel, D.; Birkett, D. and Miners, J. 1979. "Cimetidine Interaction with Warfarin", Lancet. 2 (1979), 639.

Holt, S.; Heading, R.C.; Carter, D.C.; Prescott, L.F. and Tothill, P. 1979a. "Effect of Gel Fibre on Gastric Emptying and Absorption of Glucose and Paracetamol", Lancet. 1 (1979), 636-639.

Holt, S.; Heading, R.C.; McLoughlin, G.P. and Prescott, L.F. 1979b. "Paracetamol Absorption in Coeliac Disease and Crohn's Disease", Gut. 20 (1979), A449.

Husserl, F.E. 1983. "Erythromycin-Warfarin Interaction", Archives Internal Medicine. 143 (1983), 1831-1836.

Irshaid, Y. and Abu-Khalaf, M. 1992. "Lack of Effect of Certain Histamine H_2-receptor Blockers on the Glucuronidation of 7-Hydroxy-4-Methylcoumarin by Human Liver Microsomes", Pharmacology and Toxicology. 71 (1992), 294-296.

Ismail, S.; Na Bangchang, K.; Karbwang, J.; Back, D.J. and Edwards, G. 1995. "Paracetamol Disposition in Thai Patients During and After Treatment of Falciparum Malaria", European Journal of Clinical Pharmacology. 48 (1995), 65-69.

Jaffe, J.M.; Colaizzi, J.L. and Barry, H. 1971. "Effect of Dietary Component on GI Absorption of Acetaminophen Tablets in Man", Journal of Pharmaceutical Science. 60 (1971), 1646-1650.

Jaster, P.J.; Abbas, D. and Wichita, K.S. 1986. "Erythromycin-Carbamazepine Interaction", Neurology. 36 (1986), 594-595.

Jensen, S.C. and Gugler, R. 1985. "Cimetidine Interaction with Liver Microsomes In Vitro and In Vivo : Involvement of an Activated Complex with Cytochrome P-450", Biochemical Pharmacology. 34 (1985), 2141-2146.

Joel, S.P.; Clark, P.I.; Heap, L.; Webster, L.; Robbins, S.; Craft, H. and Slevin, M.L. 1995. "Pharmacological Attempts to Improve the Bioavailability of Oral Etoposide", Cancer Chemotherapy and Pharmacology. 37 (1995), 125-133.

Johnson, G.K. and Tolman,K.G. 1977. "Chronic Liver Disease and Acetaminophen", Annals of Internal Medicine. 87 (1977), 302-304.

Jollow, D.J.; Mitchell, J.R.; Potter, W.Z.; Davis, D.C.; Gillette,J.R. and Brodie, B.B. 1973. "Acetaminophen-Induced Hepatic Necrosis II. Role of Covalent Binding in Vivo", Journal of Pharmacology and Experimental Therapeutics. 187 (1973), 195-202.

Jonkman, J.G. and Hendeles, L. 1983. "The Theophylline-Erythromycin Interaction", Chest. 84 (1983), 309-310.

Josting, D.; Winne, D. and Bock, K.W. 1976. "Glucuronidation of Paracetamol, Morphine and 1-Naphthol in the Rat Intestinal Loop", Biochemical Pharmacology. 25 (1976), 613-616.

Kamali, F, 1993. "The Effect of Probenecid on Paracetamol Metabolism and Pharmacokinetics", European Journal of Clinical Pharmacology. 45 (1993), 551-553.

Kelly, J.F.; Tang-Liu, D.D.S.; Lazo, S.; Upton, R.A. and Williams, R.L. 1982. "The Effect of Cimetidine on Theophylline Metabolism in the Elderly", Clinical Pharmacology and Therapeutics. 31 (1982), 238-239.

Kirch, W.; Kohler, H.; Spahn, H. and Mutschuler, E. 1981. "Interaction of Cimetidine with Metoprolol, Propranolol or Atenolol", Lancet. 2 (1981), 531-532.

Klotz, U.; Anttila, V.J. and Reimann, I. 1979. "Cimetidine-Diazepam Interaction", Lancet. 2 (1979), 699.

Knodell, R.G.; Holtzman, J.L.; Crankshaw, D.L.; Steele, N.M. and Stanley, L.N. 1982. "Drug Metabolism by Rat and Human Hepatic Microsomes in Response to Interaction with H_2-Receptor Antagonists", Gastroenterology. 82 (1982), 84-88.

Kochak, G.M.; Rakhit, A.; Thompson, T.N. and Hurley, M.E. 1988. "Pentopril-Cimetidine Interaction Caused by a Reduction in Hepatic Blood Flow", Journal of Clinical Pharmacology. 28 (1988), 222-227.

Lalonde, R.L.; Koob, R.A.; McLean, W.M. and Balsys, A.J. 1982. "Influence of Cimetidine on Theophylline Pharmacokinetics at Steady State", Clinical Pharmacology and Therapeutics. 31 (1982), 241-242.

LaForce, C.F.; Miller, M.F. and Chai, H. 1981. Effect of Erythromycin on Theophylline Clearance in Asthmatic Children", The Journal of Pedriatrics. 99 (1981), 153-156.

LaForce, C.F.; Szefler, S.J.; Miller, M.F.; Ebling, W. and Brenner, M. 1983. "Inhibition of Methylprednisolone Elimination in the Presence of Erythromycin Therapy", Journal of Allergy and Clinical Immunology. 72 (1983), 34-39.

Larrey, D,; Brentano, F.C.; Breil, P.; Vitaux, J.; Theodore, D.; Babany, G. and Pessayre, D. 1983a. "Effect of erythromycin on Hepatic Drug-Metabolizing Enzymes in Human", Biochemical Pharmacology. 32 (1983), 1063-1068.

Larrey, D.; Tinel, M. and Pessayre, D. 1983b. "Formation of Inactive Cytochrome P-450 Fe(II)-Metabolite Complexs with Several Erythromycin Derivatives But not with Josamycin and Medicamycin in Rats", Biochemical Pharmacology. 32 (1983), 1487-1493.

Lau, G.S.N. and Critchley, J.A.J.H. 1994. "The Estimation of Paracetamol and Its Major Metabolites in Both Plasma and Urine by a Single High Performance Liquid Chromatography Assay", Journal of Pharmaceutical & Biomedical Analysis. 12 (1994),1563-1572. quoting Adriaenssens, P.J. 1980. "Paracetamol Metabolism in Man", PhD Thesis. University of Edinburgh.

Levy, G.; Khanna, N.N.; Soda, D.M.; Tsuzuki, O. and Stern, L. 1975. "Pharmacokinetics of Acetaminophen in the Human Neonate ; Formation of Acetaminophen Glucuronide and Sulfate in Relation to Plasma Bilirubin Concentration and d-Glucaric Acid Excretion", Pediatrics. 55 (1975), 818-825.

Ludden, T.M. 1985. "Pharmacokinetic Interactions of the Macrolide Antibiotics", Clinical Pharmacokinetics. 10 (1985), 63-67.

Maddux, M.S.; Leeds ,N.H.; Organek, H.W.; Hasegawa, G.r. and Bauman, J.L. 1982. "The Effect of Erythromycin on Theophylline Pharmacokinetics at Steady State", Chest. 81 (1982), 563-565.

Madhusudanarao, K.; Bapna, J.s.; Adithan, C.; Ray, K.; Venkatadri, N.; Kamatchi, G.L.; Seetharaman, M.L. and Bahadur, P. 1988. "Effect of Short Course Chemotherapy on Salivary Paracetamol Elimination", Clinical Experimental Pharmacology and Physiology. 15 (1988), 639-643.

Marshall, P.J.; Kulmacz, R.J. and Lands, W.E.M. 1987. "Constraints on Prostaglandin Biosynthesis in Tissues", The Journal of Biological Chemistry. 262 (1987), 3510-3517.

Masubuchi, Y.; Hosokawa, S.; Horie, T.; Suzuki, T. Ohmori, S.; Kitada, M. and Narimatsu, S. 1994. "Cytochrome P450 Isozymes Involved in Propranolol Metabolism in Human Liver Microsomes. The Role of CYP2D6 as Ring-Hydroxylase and CYP1A2 as N-Desisopropylase", Drug Metabolism and Disposition. 22 (1994), 909-915.

May, D.C.; Jarboe, C.H.; VanBakel, A.B. and William, W.M. 1982. "Effect of Cimetidine on Caffeine Disposition in Smokers and Nonsmokers", Clinical Pharmacology and Therapeutics. 31 (1982), 656-661.

McGilveray, I.J. and Mattock, G.L. 1972. "Some Factors Affecting the Absorption of Paracetamol", Journal of Pharmacy and Pharmacology. 24 (1972), 615-619.

Miller, R.P.; Robert, R.J. and Fischer, L.J. 1976. "Acetaminophen Elimination Kinetics in Neonates, Children and Adults", Clinical Pharmacology and Therapeutics. 19 (1976), 284-294.

Miners, J.O.; Attwood, J. and Birkett, D.J. 1983. "Influence of Sex and Oral Contraceptive Steroid on Paracetamol Metabolism", British Journal of Clinical Pharmacology. 16 (1983), 503-509.

Miners, J.O.; Attwood, J. and Birkett, D.J. 1984. Determinants of Acetaminophen Metabolism : Effect of Inducers and Inhibitors of Drug Metabolism on Acetaminophen's Metabolic Pathway", Clinical Pharmacology and Therapeutics. 35 (1984), 480-486.

Mitchell, M.C.; Hanew, T.; Meredith, C.G. and Schenker, S. 1983. "Effect of Oral Contraceptive Steroids on Acetaminophen Metabolism and Elimination", Clinical Pharmacology and Therapeutics. 34 (1983), 48-53.

Mitchell, J.R.; Jollow, D.J.; Potter, W.Z.; Davis, O.C.; Gillette, J.R. and Brodie, B.B. 1973a. "Acetaminophen-Induced Hepatic Necrosis I. Role of Drug Metabolism", Journal of Pharmacology and Experimental Therapeutics. 187 (1973), 185-194.

Mitchell, J.R.; Jollow, D.J.; Potter, W.Z.; Davis, O.C.; Gillette, J.R. and Brodie, B.B. 1973b. "Acetaminophen-Induced Hepatic Necrosis IV. Protective Role of Glutathione", Journal of Pharmacology and Experimental Therapeutics. 187 (1973), 211-217.

Mitchell, M.C.; Schenker, S, Avant, G.R. and Speeg, K.V. 1981. "Cimetidine Protects Against Acetaminophen Hepatotoxicity in Rats", Gastroenterology. 81 (1981), 1052-1060.

Moldeus, P. 1978. "Paracetamol Metabolism and Toxicity in Isolated Hepatocytes from Rat and Mouse", Biochemical Pharmacology. 1 (1978), 2859-2863.

Moldeus, P.; Andersson, B. Norling, A. and Ormstad, K. 1980. "Effect of Chronic Ethanol Administration on Drug Metabolism in Isolated Hepatocytes with Emphasis on Paracetamol Activation", Biochemical Pharmacology. 10 (1980), 67-74.

Mucklow, J.C.; Fraser, H.S.; Bulpitt, C.J.; Kahn, C.; Mould, G. and Dollery, C.T. 1980. "Environmental Factors Effecting Paracetamol Metabolism in London Factory and Office Workers", British Journal of Clinical Pharmacology. 10 (1980), 67-74.

Muirhead, M.R.; Somogyi, A.A.; Rolan, P.E. and Bochner, F. 1986. "Effect of Cimetidine on Renal and Hepatic Drug Elimination : Studies with Triamterene", Clinical Pharmacology and Therapeutics. 40 (1986), 400-407.

Nimmo, W.S. and Prescott, L.F. 1978. The Influence of Posture on Paracetamol Absorption", British Journal of Clinical Pharmacology. 5 (1978), 348-349.

Nimmo, W.S.; Wilson, J. and Prescott, L.F. 1975. "Narcotic Analgesics and Delayed Gastric Emptying During Labour", Lancet. 1 (1975), 890-893.

Paisanchanthasiri, C. 1991. "Pharmacokinetics of Acetaminophen in Children with Thalassemia Diseases", Master of Science Thesis in Biology Sciences, Prince of Songkla University. (Unpublished)

Patwardhan, R.V.; Johnson, R.; Sinclair, A.; Schenker, S. and Speeg, K.V. 1981. "Lack of Tolerance and Rapid Recovery of Cimetidine Inhibited Chlordiazepoxide (Librium) Elimination in Man", Gastroenterology. 81 (1981), 547-551.

Patwardhan, R.V.; Yarborough, G.W.; Desmond, P.V.; John, R.F.; Schenker, S. and Speeg, K.V. 1980. "Cimetidine Spares the Glucuronidation of Lorazepam and Oxazepam", Gastroenterology. 79 (1980), 912-916.

Pelkonen, O. and Puurunen, J. 1980. "The Effect of Cimetidine on In Vitro and In Vivo Microsomal Drug Metabolism in the Rat", Biochemical Pharmacology. 29 (1980), 3075-3080.

Pessayre, D.; Tinel, M.; Larrey, D.; Cobert, B.; Funck-Brentano, C. and Babany, G. 1983. "Inactive of Cytochrome P-450 by a Troleandomycin Metabolite. Protective Role of Glutathione", Journal of Pharmacology and Experimental Therapeutics. 224 (1983), 685-691.

Peterson, F.T.; Knodell, R.G.; Lindemann, N.J. and Steele, N.M. 1983. "Prevention of Acetaminophen and Cocaine Hepatotoxicity in Mice by Cimetidine Treatment", Gastroenterology. 85 (1983), 122-129.

Peterson R.G. and Rumack, B.H. 1978. "Pharmacokinetics of Acetaminophen in Children", Pediatrics. 62 (1978), 877-879.

Pichard, L.; Fabre, I.; Fabre, G.; Domergue, J.; Saint-Aubert, B.; Mourad, G. and Maurel. 1990. "Cyclosporine A Drug Interactions. Screening for Inducers and Inhibitors of Cytochrome P-450 (Cyclosporine A Oxidase) in Primary Cultures of Human Hepatocytes and in Liver Microsomes", Drug Metabolism and Disposition. 18 (1990), 595-606.

Powell, J.R. and Donn, K.H. 1984. "Histamine H_2-Antagonist Drug Interactions in Perspective : Mechanistic Concepts and Clinical Implication", The American Journal of Medicine. 77 (1984), 57-84.

Prescott, L.F. 1974. "Gastrointestinal Absorption of Drugs", Medical Clinics of North America. 58 (1974), 907-916.

Rainska - Giezek, T. 1995. "Influence of Caffeine on Toxicity and Pharmacokinetics of Paracetamol", Annal Academic Medical Statin. 41 (1995), 69-85.

Rajaonarison, J.F.; Lacarelle, B.; De-Sousa, G.; Catalin, J. and Rahmani, R. 1991. "In Vitro Glucuronidation of 3-Azido-3-Deoxythymidine by Human Liver. Role of UDP-Glucuronosyltransferase 2 Form", Drug Metabolism and Disposition. 19 (1991), 809-815.

Raucy, J.L.; Lasker, J.M.; Lieber, C.S. and Black, M. 1989. "Acetaminophen Activation by Human Liver Cytochromes P450IIE1 and P450IA2", Archives of Biochemistry and Biophysics. 271 (1989), 270-283.

Rawlins, M.D.; Henderson, D.B. and Hijab, A.R. 1977. "Pharmacokinetics of Paracetamol (Acetaminophen) After Intravenous and Oral Administration", European Journal of Pharmacology. 11 (1977), 283-286.

Reilly. P.B.; Carrington, L.E. and Winzor, D.J. 1983. "The Interaction of Cimetidine with Rat Liver Microsomes", Biochemical Pharmacology. 32 (1983), 831-835.

Reisz, G.; Pingleton, S.K.; Melethil, S. and Ryan, P.B. 1983. "The Effect of Erythromycin on Theophylline Pharmacokinetics in Chronic Bronchitis", American Review of Respiratory Diseases. 127 (1983), 581-584.

Renton K.W.; Gray, J.D. and Hung, O.R. 1981. "Depression of Theophylline Elimination by Erythromycin", Clinical Pharmacology and Therapeutics. 30 (1981), 422-426.

Richer, C.; Mathieu, M.; Bah, H.; Thuillex, C.; Duroux, P. and Giudicelli, J.F. 1982. "Theophylline Kinetics and Ventilatory Flow in Bronchial Asthma and Chronic Airflow Obstruction. Influence of Erythromycin", Clinical Pharmacology and Therapeutics. 31 (1982), 579-586.

Richter, A. and Smith, S.E. 1974. "Bioavailability of Different Preperations of Paracetamol", British Journal of Clinical Pharmacology. 1 (1974), 495-498.

Roberts, R.K.; Grice, J.; Wood, L.; Petroff, V. and McGuffie, C. 1981. "Cimetidine Impairs the Elimination of Theophylline and Antipyrine", Gastroenterology. 81 (1981), 19-21.

Rollinshoff, W.; Sticken, R. and Paumgartner, G. 1981. "Inhibition of Drug Metabolism by Cimetidine in Man. Dependence on Pretreatment Microsomal Liver Function", Gastroenterology. 80 (1981), 1347.

Schmalix, W.A.; Barrenscheen, M.; Landsiedel, R.; Janzowski, C.; Eisenbrand, G.; Gonzalez, F.; Eliasson, E.; Ingleman-Sundberg, M.; Perchermier, M. and Greim, H. 1995. "Stable Expression of Human Cytochrome P450 2E1 in V79 Chinese Hamster cells", European Journal of Pharmacology. 293 (1995), 123-131.

Serlin, M.J.; Challiner, M.; Park, B.K.; Turcan P.A. and Breckenridge, A.M. 1980. "Cimetidine Potentiates the Anticoagulant Effect of Warfarin by Inhibition of Drug Metabolism", Biochemical Pharmacology. 29 (1980), 1971-1972.

Serlin M.J.; Sibieon, R.G.; Mossman, S.; Breckenridge, A.M.; Williams, J.R.B.; Atwood, J.L. and Willoughby, J.M.T. 1979. "Cimetidine : Interaction with Oral Anticoagulants in Man", Lancet. 2 (1979), 317-319.

Shimada, T.; Yamazaki, H.; Mimuru, M.; Invi, Y. and Guengerich, F.P. 1994. "Interindividual Variations in Human Liver Cytochrome P-450 Enzymes Involved in the Oxidation of Drugs, Carcinogens and Toxic Chemicals : Studies with Liver Microsomes of 30 Japanese and Caucasians", Journal of Pharmacology and Experimental Therapeutics. 270 (1994), 414-423.

Shively, C.A. and Vessell, E.S. 1975. "Temporal Variation Acetaminophen and Phenacetin Half-Life in Man", Clinical Pharmacology and Therapeutics. 18 (1975), 413-424.

Sketris, I.S. and Belitsky, P. 1986. "Erythromycin-Cyclosporine Interaction", Drug Intelligence and Clinical Pharmacy. 20 (1986), 504-505.

Slattery, J.T.; McRorie, T.I.; Reynolds,R.; Kalhorn, T.F.; Kharasch, E.D. and Eddy, A.C. 1989. "Lack of Effect of Cimetidine on Acetaminophen Disposition in Humans", Clinical Pharmacology and Therapeutics. 46 (1989), 591-597.

Somogyi, A. and Gugler, R. 1983. "Clinical Pharmacokinetics of Cimetidine", Clinical Pharmacokinetics. 8 (1983), 463-495.

Speeg, K.V.; Patwardhan, R.V.; Avant, G.R.; Mitchell, M.C. and Schenker, S. 1982. "Inhibition of Microsomal Drug Metabolism by Histamine H_2-Receptor Antagonists Studied In Vivo and In Vitro in Rodents", Gastroenterology. 82 (1982), 89-96.

Spinler, S.A.; Cheng, J.W.M.; Kindwall, K.E. and Charland, S.L. 1995. "Possible Inhibition of Hepatic Metabolism of Quinidine by Erythromycin", Clinical Pharmacology and Therapeutics. 57 (1995), 89-94.

Staiger, H.C.; Zimmermann, R.; Schmidt, S.; De Vries, J.; Walter, E.; Josephi, E. and Weber, E. 1982. "Influence of Cimetidine on the Pharmacodynamics of Phenprocoumon", Clinical Pharmacology and Therapeutics. 31 (1982), 233.

Tassaneeyakul, W.; Birkett, D.J.; Veronese, M.E.; McManus, M.E.; Turkey, R.H.; Quattrochi, L.C.; Gelboin, H.V. and Miners, J.O. 1993. "Specificity of Substrate and Inhibitor Probes for Human Cytochrome P450 1A1 and 1A2", Journal of Pharmacology and Experimental Therapeutics. 265 (1993), 401-407.

Telerman-Toppet, N.; Duret, M.E. and Coers, C. 1981. "Cimetidine Interaction with Carbamazepine", Annals of Internal Medicine. 94 (1981), 544.

Testa, B. and Jenner, P. 1981. "Inhibition of Cytochrome P-450s and Their Mechanism of Action", Drug Metabolism Review. 12 (1981), 1-117.

Thummel, K.E.; Lee, C.E.; Kunze, K.L.; Nelson, S.D. and Slattery, J.T. 1993. "Oxidation of Acetaminophen to N-Acetyl-p-aminobenzo quinoneimine by Human CYP3A4", Biochemical Pharmacology. 45 (1993), 1563-1569.

Tomomasa, T.; Arai, H.; Wakabayashi, K. and Itoh, Z. 1986. "Erythromycin induces Migrating Motor Complex in Human Gastrointestinal Tract", Digestive Diseases and Science. 31 (1986), 157-161.

Veng Pederson, P. and Miller, R. 1980. "Pharmacokinetics and Bioavailability of Cimetidine in Man", Jounal of Pharmaceutical Science. 69 (1980), 394-398.

Ware, A.J.; Upchurch, K.S.; Eigenbrodt, E.H. and Norman, D.A. 1978. "Acetaminophen and the Liver", Annals of Internal Medicine. 88 (1978), 267-268.

Wojcicki, J.; Gawronska-Schlarz, B.; Kazimierezyk, J.; Baskzewiez, Z. and Raezynski, A. 1979. "Comparative Pharmacokinetics of Paracetamol in Men and Women Considering Follicular and Luteal Phases", Arzneimmittel-Forschung. 29 (1979), 350-352.

Wong, Y.Y.; Ludden, T.M. and Bell, R.D. 1983. "Effect of Erythromycin on Carbamazepine Kinetics", Clinical Pharmacology. 33 (1983), 460-464.

Wroblewski, B.A.; Singer; W.D. and Whyte, J. 1986. "Carbamazepine-Erythromycin Interaction, Case Studies and Clinical Significance", JAMA. 255 (1986), 1165-1167.

Zarowitz, B.J.; Szefler, S.J. and Lasezkay, G.M. 1981. "Effect of Erythromycin Base on Theophylline Kinetics", Clinical Pharmacology and Therapeutics. 29 (1981), 601-605.

Zimmerman, H.J. and Maddrey, W.C. 1995. "Acetaminophen (Paracetamol) Hepatotoxicity with Regular Intake of Alcohol : Analysis of Instances of Therapeutic Misadventure", Hepatology. 22 (1995), 767-773.

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