First-Pass Effect of cis-3,4-Dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl)-benzamide (U-54494) in Rats—A Model with Multiple Cannulas for Investigation of Gastrointestinal and Hepatic Metabolism

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A multiple cannulated rat model was utilized to investigate the relative contribution of the gut and liver as sites of first-pass metabolism of orally administered U-54494A, an anticonvulsant drug candidate. Each rat received a dose of U-54494A by oral, intraportal, and intravenous routes on three separate occasions. Intraportal and intravenous doses were administered through chronic cannulas surgically implanted in the portal vein and superior vena cava, respectively. Blood samples were collected over a 6-hr period from the superior vena cava cannula. The mean (n=3) bioavailability of orally dosed U-54494A was 4.5±1.1%, while that dosed intraportally was 19.1±3.0%. The relative contribution of the gut and liver as sites of first-pass extraction and/or metabolism of orally administered drug was 69.9±14.0% and 24.5±12.2%, respectively. Approximately 35 to 40% of the total plasma clearance was attributed to the liver. The plasma concentrations of the four known metabolites of U-54494A were apparently higher for the oral and intraportal routes compared to that after intravenous administration. This investigation confirms that the low oral bioavailability of U-54494A in the rat can be primarily attributed to both extensive intestinal and hepatic first-pass metabolism.

KEY WORDS: multiple administration routes; rat cannulation; metabolism; liver; gut.

INTRODUCTION

As an anticonvulsant drug candidate, cis-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl)-benzamide monohydrochloride (U-54494A) (1) has been investigated for its absorption, disposition, metabolism, and excretion characteristics in animal models as well as in humans. Previous studies indicated that U-54494A was extensively metabolized after oral administration in several species including rat, mouse, dog, monkey, and human. As a result, the oral bioavailability of U-54494A was only 25% in the dog (2) and was even less in rats, mice and monkeys. Four of its metabolites in rat plasma and urine have been identified and designated M-1 (U-83892), M-2 (U-83894), M-3 (U-84042), and M-4 (U-92004) as shown in Fig. 1. The metabolic profile was qualitatively the same between rat, mouse, dog, monkey, and human based on *in vivo* and *in vitro* studies. Since the gastrointestinal absorption of U-54494A has been demonstrated to be 86% in the rat from a mass balance study using [^{14}C]U-54494A, the poor oral availability of this drug appeared to be attributive to the first-pass metabolism and/or extraction in the two principal organs, gut and liver. Many investigators have highlighted various experimental approaches using multiple administration routes to evaluate the sites of first-pass metabolism of drugs in rats and dogs (3–9). However, most of them used either anesthetized rats with intraportal drug administration or conscious rats with intraperitoneal drug administration for investigation of hepatic metabolism (5–8). The experimental approach described in this report involves administration of U-54494A by multiple routes (oral, intraportal, and intravenous) to each conscious individual rat through gavage or two surgically implanted cannulas positioned in the portal vein and superior vena cava, respectively, that are afferent to the potential sites of first-pass metabolism. Thus, the relative contribution of gut and liver as sites of first-pass extraction and/or metabolism of the orally administered U-54494A can be estimated.

MATERIALS AND METHODS

Study Design— The study was conducted using three male Sprague-Dawley rats weighing approximately 300 g. Rats were anesthetized using an injectable cocktail of sterile water:100 mg/ml ketamine:20 mg/ml xylazine:10 mg/ml acepromazine (2.5:1.875:0.475:0.1875, v/v) via subcutaneous route. Under anesthesia, each rat was fitted with cannulas directly implanted in the superior vena cava (SVC) using PE-20 tubing and in the portal vein (PV) using PE 10-Silastic 105 (0.012 in. I.D.) tubing (10–12). These vascular cannulas were exteriorized at the nape of the neck and kept flushed with isotonic saline once a day for three days after the surgery and once a week thereafter during the study period to maintain patency. Intramuscular doses of Combiotic (0.25 ml/kg, Pfizer Inc., New York, NY) were administered postsurgically for three days to prevent infection. The rats were allowed to return to presurgical body weight prior to the initiation of the study. Considering the potential of limited patency time of the portal vein cannulas, in the first dosing period, all rats received U-54494A administration intraportally (ipv) by rapid injection over one minute via the PV cannula. In the second and third dosing periods, each rat received U-54494A either orally (po) by gavage, or intravenously (iv) by rapid injection over one minute via the SVC cannula in a crossover design. The vascular cannulas were flushed with isotonic saline after each injection to insure complete drug delivery. A 7-day washout period was employed between doses. Rats were fasted approximately 16 hours prior to and 6 hours post each administration. Considering the low oral bioavailability and the assay sensitivity, the dose level selected for oral administration was 20 mg/kg and for intraportal and intravenous administration was 5 mg/kg. Based on previous studies, a proportional increase in plasma concentrations when the dose increased from 5 to 20 mg/kg was assumed. A water solution formulation containing

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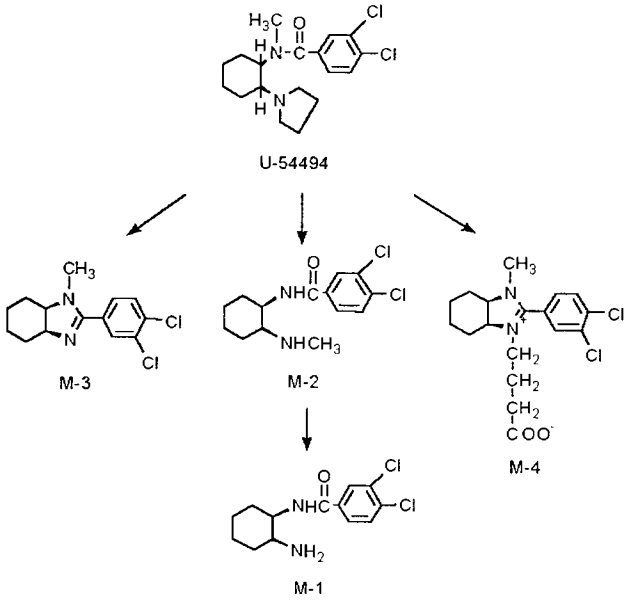

Fig. 1. Metabolic scheme of U-54494A in the rat.

3 mg/ml U-54494, the free base of U-54494A, was used for oral, intraportal, and intravenous administration. Blood samples (300 μl) were collected prior to dosing and at several time points up to 6 hr after dosing through the SVC cannula for each administration route. Blood samples were placed on ice after sampling and plasma was harvested within one hour of sampling. Plasma samples were stored at −20°C until analysis.

Analytical Method— Plasma samples were analyzed for the concentrations of U-54494 and the four known metabolites using an HPLC method as described in a manuscript (2). The extraction method was modified to obtain better assay sensitivity and extraction recovery since only 100 μl of plasma sample was available for extraction in this study. Briefly, 100 μl of plasma sample was mixed with 200 μl of blank rat plasma, 50 μl of internal standard solution and 300 μl of acetonitrile-water (3:7, v/v) before loading on the solid phase extraction (SPE) column (phenyl, 100 mg/ml, Varian, Harbor City, CA). The loaded sample was washed with 100 μl of methanol-water (3:7, v/v) followed by 1 ml of water. The compounds of interest were then eluted with 500 μl of methanol-acetone (3:2, v/v) into a 2 ml autosampler vial. The eluate was vacuum aspirated in the manifold to near dryness, and the residue was reconstituted with 200 μl of methanol-water (3:7, v/v) for HPLC analysis. The HPLC system parameters and the quantitation method used for the analysis remained unchanged as described in the manuscript (2). This modified method had been previously validated with regard to precision, accuracy, specificity, sensitivity and linearity. Drug concentrations are reported in free base equivalents.

Data Analysis— The pharmacokinetic profiles for the parent drug and four known metabolites formed after each administration route of U-54494A to the rat were evaluated with non-compartmental pharmacokinetic techniques (13). The observed maximum plasma concentrations (C_{max}) and the time at which they occurred (T_{max}) were obtained from the concentration-time data. The elimination rate constant, k, was calculated using least squares linear regression analysis on the terminal portion of the log plasma concentration-time profile. The elimination half-life $t_{1/2}$ was calculated by dividing ln 2 by k. The area under the plasma concentration-time curve from time zero up to the time of the last sample with quantifiable analyte concentration (AUC_t) was calculated using the linear trapezoidal rule, and from time zero to infinity (AUC) using the sum of AUC_t and the residual area (the plasma concentration of the last time point divided by k). The percent bioavailability for the oral (F_{po}) and intraportal (F_{ipv}) administration was determined as follows:

$$F_{po} = \frac{AUC_{po} \cdot DOSE_{iv}}{AUC_{iv} \cdot DOSE_{po}} \times 100\% \quad and$$

$$F_{ipv} = \frac{AUC_{ipv} \cdot DOSE_{iv}}{AUC_{iv} \cdot DOSE_{po}} \times 100\%$$

where $DOSE_{po}$ and AUC_{po}, $DOSE_{ipv}$ and AUC_{ipv}, and $DOSE_{iv}$ and AUC_{iv} are the doses and AUCs when the drug was administered orally, intraportally, and intravenously, respectively. The total plasma clearance (Cl_p) after intravenous administration was calculated using the following equation:

$$Cl_p = \frac{DOSE_{iv}}{AUC_{iv}}$$

and thus apparent oral and intraportal clearances were:

$$Cl_p/F_{po} = \frac{DOSE_{po}}{AUC_{po}} \quad and$$

$$Cl_p/F_{ipv} = \frac{DOSE_{ipv}}{AUC_{ipv}}$$

The hepatic clearance, Cl_H, was estimated as follows:

$$Cl_H = Q_H \cdot E_{liver}$$

where Q_H is the reported value of hepatic plasma flow rates for rats (1.86 to 2.06 l/hr/kg, (14–16), and E_{liver} is the liver extraction ratio for U-54494 in rats and was calculated according to the relationships described by Morrison *et al.* (3) and Cassidy and Houston (6):

$$E_{liver}(\%) = \left[1 - \frac{AUC_{ipv} \times DOSE_{iv}}{AUC_{iv} \times DOSE_{ipv}}\right] \times 100$$

and similarly, the gut extraction ratio (E_{gut}) was determined as

$$E_{gut}(\%) = \left[1 - \frac{AUC_{po} \times DOSE_{ipv}}{AUC_{ipv} \times DOSE_{po} \times A_{po}}\right] \times 100$$

where A_{po} is the fraction of dose absorbed after an oral administration of U-54494A to rats. The relative contribution of the gut and liver as sites of first-pass extraction and/or

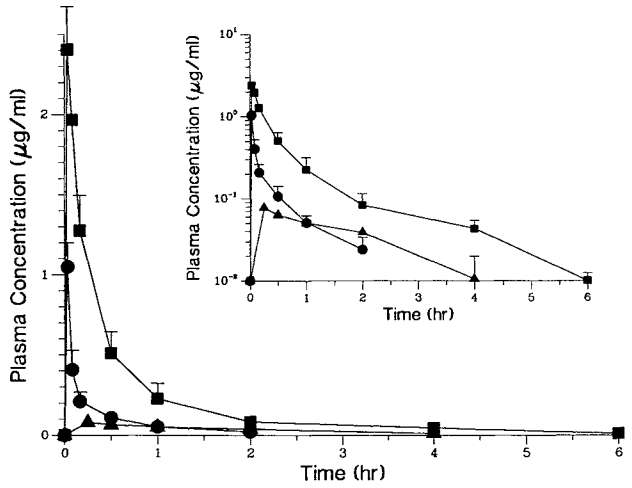

Fig. 2. Mean (n=3) plasma concentration-time profiles of U-54494 following intravenous (-■-), intraportal (-●-), and oral (-▲-) administration to rats. A semi-log plot is also shown in this figure.

metabolism of the orally administered U-54494A were estimated as follows:

$$E_{gut\ first\ pass}\ (\%) = E_{gut}\ (\%)\ and$$

$$E_{liver\ first\ pass}\ (\%) = [100 - E_{gut\ first\ pass}\ (\%)] \times E_{liver}$$

RESULTS AND DISCUSSION

It approximately took 30–40 minutes to complete the implantation of SVC and PV cannulas for each rat. The three rats tolerated the surgery well and returned to their pre-surgical body weight within one week. No abnormal clinical signs were observed in the rats treated by any of the three administration routes. The cannulas for each rat kept patent during the study period (one month). Our experience to date showed that more than 90% of SVC cannulas and 70% of the PV cannulas were patent up to 4 weeks after implantation, which allows us to complete a study with multiple routes of drug administration.

Bioavailability and Pharmacokinetics of U-54494— The mean plasma concentration-time profiles of U-54494 in rats with standard deviation (SD) bars following iv, ipv, and po administration are shown in Fig. 2. Selected pharmacokinetic parameters of the parent compound for each rat are listed in Table I, along with their mean and SD values for the three rats. Plasma as compared to concentrations of U-54494 varied according to the route of administration; the intravenous route provided the greatest AUC, intraportal the next, and oral the lowest. The bioavailabilities of U-54494 by the oral and intraportal routes were determined as 4.49±1.14% and 19.12±2.99%, respectively (Table I). The lower bioavailability and higher plasma clearance for the oral route compared to the intraportal routes and for the intraportal route compared to the intravenous route clearly indicated the first-pass effect of gut and liver on the U-54494 bioavailability in rats. The estimated mean hepatic drug clearance ranged from 1.65 to 2.04 l/hr/kg which only accounted for 35 to 40% of the total plasma clearance (4.25±0.32 l/hr/kg).

Plasma Concentrations of The Four Known Metabolites— The mean plasma concentration-time data of the four known metabolites, M-1, M-2, M-3, and M-4, formed after the three routes of administration of U-54494A to rats are graphically presented in Fig. 3. Calculated pharmacokinetic parameters, C_{max}, T_{max}, AUC, and $t_{1/2}$ for the four metabolites are listed in Table II. The four identified metabolites were observed after either of the administration routes. Rapid biotransformation of U-54494 to M-2, M-3, and M-4 following the intraportal administration was observed as indicated by the short T_{max} of 0.10–0.14 hr. The formation of M-1 was slowest with a T_{max} of 4.50±1.00 hr after ipv dose, consistent with the metabolic scheme that M-1 was formed by further metabolism of M-2. Owing to the delayed formation of M-1 and the limited time points for blood collection, the values of $t_{1/2}$ and AUC for M-1 could not be calculated. The T_{max} values for the formation of metabolites were much shorter in general for the ipv route compared to the iv route, showing the influence of first-pass hepatic metabolism of U-54494 in rats. The higher plasma concentrations of the four metabolites after po and ipv administration and lower after iv administration also demonstrated the participation of metabolism on the first-pass effect. The AUC of M-4 was approximately 5-fold higher for po and 2-fold higher for ipv, but was 5-fold lower for iv administration when compared to

Table I. Pharmacokinetic Parameters of U-54495 in Rats Following Intravenous, Intraportal, and Oral Administration

Route	Rat #	Dose (mg/kg)	C_{max} (μg/ml)	T_{max} (hr)	AUC (μg · hr/ml)	$t_{1/2}$ (hr)	CL_p/F (l/hr/kg)	F (%)
iv	1	5.19	2.61	0.03	1.33	1.18	3.90	
	2	4.40	2.10	0.03	0.85	1.69	4.54	
	3	5.04	2.49	0.03	1.17	1.03	4.31	
	Mean ± SD	4.88 ± 0.42	2.40 ± 0.27	0.03 ± 0.00	1.17 ± 0.24	1.30 ± 0.34	4.25 ± 0.32	
ipv	1	5.08	1.07	0.03	0.24	0.53	21.17	18.44
	2	5.08	1.18	0.03	0.16	0.77	31.16	16.53
	3	5.29	1.10	0.03	0.28	0.76	19.24	22.39
	Mean ± SD	5.14 ± 0.13	1.12 ± 0.06	0.03 ± 0.00	0.22 ± 0.05	0.69 ± 0.14	23.86 ± 6.40	19.12 ± 2.99
po	1	20.10	0.06	0.25	0.20	2.56	99.50	3.92
	2	19.68	0.08	0.25	0.25	2.06	78.10	5.81
	3	20.10	0.09	0.25	0.18	1.57	114.86	3.75
	Mean ± SD	19.96 ± 0.24	0.08 ± 0.02	0.25 ± 0.00	0.21 ± 0.04	2.06 ± 0.50	97.49 ± 18.46	4.49 ± 1.14

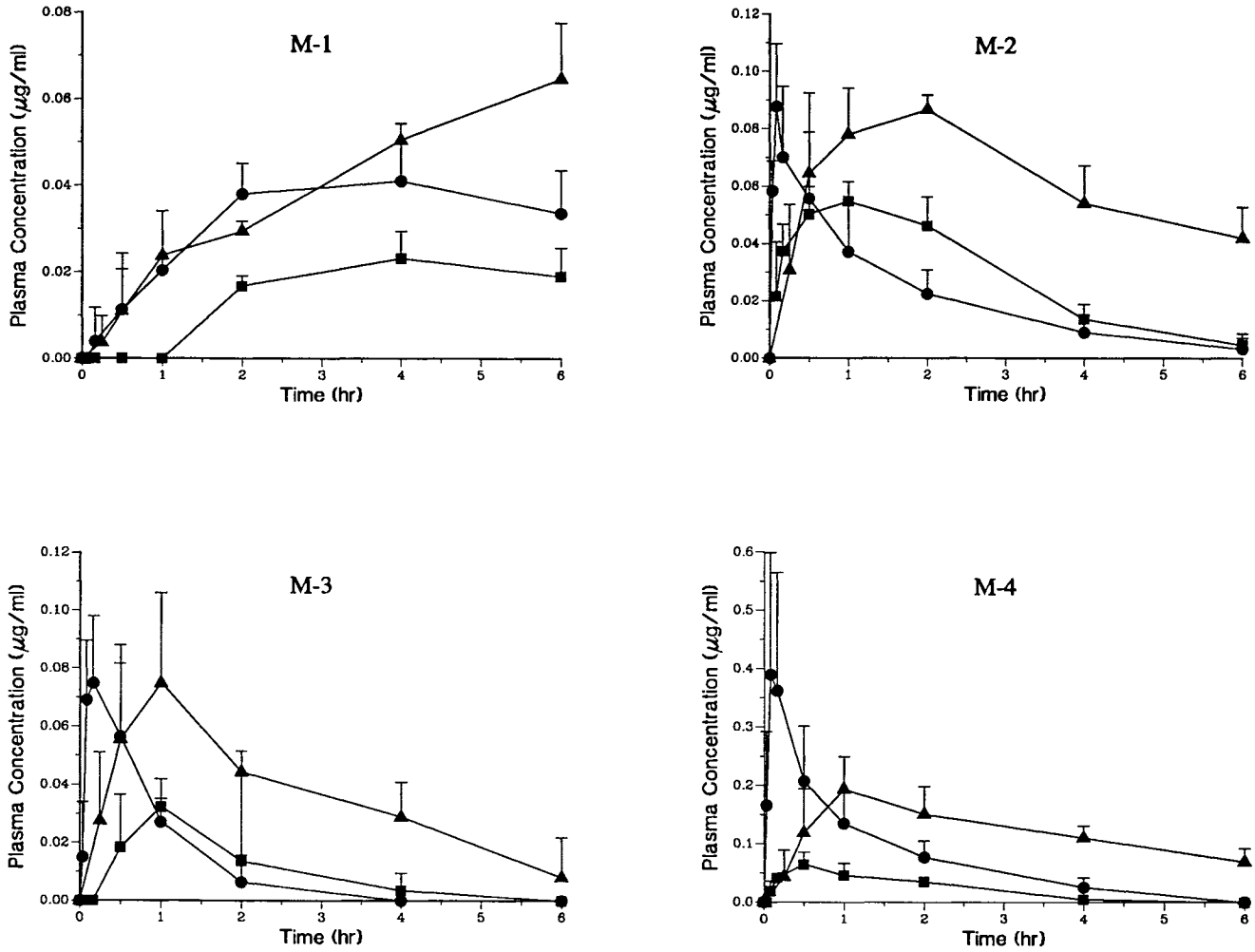

Fig. 3. Mean (n = 3) plasma concentration-time profiles of the known metabolites following intravenous (–■–), intraportal (–●–), and oral (–▲–) administration of U-54494A to rats.

Table II. Pharmacokinetic Parameters of Known Metabolites Following Intravenous, Intraportal, and Oral Administration of U-54494 to Rats

Route	U-54494 Dose (mg/kg)	Compound	C_{max} (μg/ml)	T_{max} (hr)	AUC (μg · hr/ml)	$t_{1/2}$ (hr)
iv	4.88 ± 0.42	M-1	0.03 ± 0.01	4.70 ± 1.16	0.10 ± 0.01[a]	—[b]
		M-2	0.06 ± 0.01	1.17 ± 0.76	0.11 ± 0.01	1.33 ± 0.16
		M-3	0.04 ± 0.03	1.34 ± 1.02	0.06 ± 0.04[a]	—
		M-4	0.07 ± 0.03	0.50 ± 0.00	0.18 ± 0.06	1.85 ± 0.46
ipv	5.14 ± 0.13	M-1	0.04 ± 0.01	4.50 ± 1.00	0.19 ± 0.04[a]	—
		M-2	0.09 ± 0.02	0.10 ± 0.04	0.14 ± 0.05	1.71 ± 0.41
		M-3	0.08 ± 0.02	0.14 ± 0.04	0.08 ± 0.03	0.69 ± 0.22
		M-4	0.41 ± 0.24	0.12 ± 0.05	0.50 ± 0.21	1.66 ± 0.46
po	19.95 ± 0.24	M-1	0.06 ± 1.14	6.00 ± 0.00	0.22 ± 0.01[a]	—
		M-2	0.09 ± 0.01	1.17 ± 0.76	0.63 ± 0.16	4.54 ± 0.31
		M-3	0.08 ± 0.04	1.00 ± 0.00	0.29 ± 0.11	2.38 ± 0.91
		M-4	0.18 ± 0.05	1.00 ± 0.00	1.10 ± 0.27	3.91 ± 0.87

[a] Data presented are AUC_t.
[b] Not calculated.

Table III. Relative Contribution of Gut and Liver as First-Pass Metabolism Sites for U-54494 in Rats Following an Oral Administration

Rat ID	E_{gut} (%)	E_{liver} (%)	$E_{gut\ first\text{-}pass}$ (%)	$E_{liver\ first\text{-}pass}$ (%)	$E_{gut\ +\ liver\ first\text{-}pass}$ (%)
1	75.3	81.6	75.3	20.2	95.5
2	54.0	83.5	54.0	38.4	92.4
3	80.5	77.6	80.5	15.1	95.6
Mean ± SD	69.9 ± 14.0	80.9 ± 3.0	69.9 ± 14.0	24.5 ± 12.2	94.5 ± 1.8

those for the parent compound, further suggesting the participation of first-pass metabolism in the sites of gut and liver. Most likely, the primary metabolic pathway entails enzyme mediated formation of an intermediate (oxidation product) either in gut or in liver. This intermediate was further dehydrated to form M-4. The type of isoforms responsible for U-54494 metabolism remained unknown. Future studies using intestinal and liver microsomes to investigate the mechanism of U-54494 metabolism were planned.

Extraction Ratios and Relative Contribution of Gut and Liver as First-Pass Metabolism Sites— Since U-54494A was nearly completely absorbed, direct evidence of the first-pass effect of U-54494 has been demonstrated by the differences in dose normalized AUCs after oral, intraportal, and intravenous administration in this study. Drugs which exhibit a first-pass effect are generally characterized by extensive metabolism from the gut and liver during their passage through these organs. The calculated extraction ratio of U-54494 by gut and liver were 69.9±14.0% and 80.9±3.0%, respectively, assuming that 86% of the U-54494 dose was absorbed in the rat after oral administration (Table III). Thus, about 70% of the absorbed U-54494 after an oral administration was assumed to be extracted and/or metabolized in the gut. Similarly, approximately 81% of intraportally dosed U-54494 appeared to be extracted and/or metabolized in the liver before it reached the central venous circulation. The relative contribution as first-pass effect sites for orally administered U-54494 was 69.9±14.0% in gut and 24.5±12.2% in liver (Table III). The importance of metabolic activity in gut wall has recently become apparent, as reported by many investigators such as Conway *et al.* (7) who indicated that an intraperitoneal dose of terbutaline in rats provided a 6- to 7-fold increase in availability compared with the oral route. Ilett *et al.* (9) reported that an extensive first-pass conjugation of isoprenaline in dog intestine was observed. Morrison *et al.* (3) also found that the gut was the major site of hydrolysis (87%), whereas the liver (<10%) and lungs (<2%) were minor sites, of orally administered zofenopril calcium in dogs. These findings are supported by the observations from our study that the contribution of gut was almost triple of that of the liver after the oral administration of U-54494A to the rats.

CONCLUSIONS

Low bioavailability, 4.5 and 19.1%, was observed when U-54494A was administered orally and intraportally, respectively, to rats. About 35 to 40% of total plasma clearance appeared to occur in the liver. With respect to first-pass extraction and/or metabolism of orally administered U-54494 in rats, the gut was the major site (69.9%) with the liver following (24.5%). The higher plasma concentrations of the four known metabolites after oral and intraportal administration compared to that after intravenous administration, also supported the explanation that the lack of oral bioavailability of U-54494 in rats can be attributed to the extensive gut and liver first-pass metabolism. Results generated using this animal model are consistent with our previous findings, indicating that the experimental design is feasible and can be used as one of the approaches for investigation of first-pass effect on the drug bioavailability by gastrointestinal and hepatic metabolism. This animal model also offers advantages by providing true pharmacokinetics from freely moving conscious rats when the drug was administered and the blood samples were collected. Furthermore, more accurate results are obtained through administering the drug to the same rat with multiple administration routes, so that the intersubject variability and the animal numbers used for the study can be greatly reduced.

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