Dose-Dependent Pharmacokinetics of MK-417, a Potent Carbonic Anhydrase Inhibitor, in Experimental Polycythemic and Anemic Rats

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MK-417 is a potent carbonic anhydrase inhibitor currently under clinical investigation as a topical ocular hypotensive agent. While present in most of the tissues, carbonic anhydrase predominates in red blood cells. Earlier studies from our laboratory have demonstrated that carbonic anhydrase plays an important role in the elimination kinetics of MK-417 and that the enzyme can be saturated when MK-417 exceeds the stoichiometric concentration of the enzyme. Since carbonic anhydrase is an intracellular enzyme in erythrocytes, conditions which may change the hematocrit can alter the load of MK-417 needed to saturate carbonic anhydrase. It is, therefore, important to determine the effects of anemic and polycythemic states on the pharmacokinetics of MK-417. The anemic state in rats was obtained by replacing whole blood with donor plasma (12–15 ml), while polycythemia was induced by infusion of 12 to 15 ml of whole blood. At low doses (0.05 and 0.1 mg/kg), the pharmacokinetic parameters for MK-417 remained unchanged and there were no significant differences in the pharmacokinetic parameters among the anemic, polycythemic, and normal rats. The total blood clearance and apparent volume of distribution were increased markedly when the dose exceeded 0.2 mg/kg in anemic rats and 0.5 and 1 mg/kg in normal and polycythemic rats, respectively. Clearly, the dose of MK-417 required to saturate the enzyme was different among the three groups of animals. However, the terminal half-life was dose independent and not influenced by hematocrit. At high doses (1 and 2 mg/kg), significant differences in total blood clearance and apparent volume of distribution were observed in the three groups of rats with the following rank: anemic rats > normal rats > polycythemic rats. There was a strong inverse correlation between total blood clearance and hematocrit and between apparent volume of distribution and hematocrit.

KEY WORDS: carbonic anhydrase inhibitor; dose-dependent pharmacokinetics; saturable binding; MK-417.

INTRODUCTION

Carbonic anhydrase, a zinc metalloenzyme, predominates in red blood cells of all mammals, accounting for more than 90% of the enzyme in the body (1). The main function of this enzyme is the regulation of CO_2 and O_2 exchange between blood and both the alveolar air sacs and the peripheral tissues (2,3). Carbonic anhydrase is also found in the secretory cells of the ciliary body in the eyes and plays an essential role in the secretion of aqueous humor (4).

MK-417, *S*(+),[5,6-dihydro-4H-4 isobutylaminothieno (2,3-B)thiopyran-2-sulfonamide-7,7-dioxide] (Fig. 1), is a potent carbonic anhydrase inhibitor currently under clinical investigation as a topical ocular hypotensive agent (5). The drug acts directly on the carbonic anhydrase in the secretory cells of the ciliary body. Earlier studies (6,7) have demonstrated that carbonic anhydrase plays an important role in the elimination kinetics of MK-417 and that the enzyme in the vascular space can be saturated when the dose of MK-417 is sufficiently high to yield blood levels which exceed the concentration of the enzyme in blood.

Since carbonic anhydrase is present mainly in erythrocytes, conditions which may change the hematocrit such as anemia and polycythemia can alter the load of MK-417 needed to saturate carbonic anhydrase and thus alter its pharmacokinetics. The present study compares the pharmacokinetics of MK-417 in normal rats, rats made anemic by replacing whole blood with plasma, and rats made polycythemic by infusing whole blood.

MATERIALS AND METHODS

Materials

MK-417 and an analogue [2-thiophenesulfonamide 5-((3-(((2-methylpropyl)amino)methyl)phenyl)sulfonyl)] used as an internal standard for HPLC assay were synthesized at Merck Sharp & Dohme Research Laboratories (West Point, PA). The radiolabeled MK-417 was prepared with ^{14}C at C-1 of isobutyl amino group. All other reagents were of analytic grade.

Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA), weighing 300–400 g were used in this study. Animals were housed under standard conditions (12-hr light/dark cycle; temperature, 22 ± 0.5°C; humidity, 50 ± 10%) with free access to food and water. All animals had a cannula (PE-50) implanted in the right jugular vein for blood sampling and drug administration. The surgery was performed under light pentobarbital anesthesia (40 mg/kg, i.p.) 1 day before the experiment. During the kinetic study, all animals were housed individually in plastic metabolism cages and were not restrained at any time during the experiments.

The anemic state in rats was obtained by replacing whole blood with donor plasma (12–15 ml), while polycythemia was induced by infusion of 12 to 15 ml of whole blood. These procedures were performed approximately 24 hr before the experiments. The hematocrit was determined periodically during the kinetic study.

To study dose-dependent pharmacokinetics of MK-417, the drug was administered by i.v. injection via the cannula in the right jugular vein at doses of 0.05, 0.1, 0.2, 0.5, 1, and 2 mg/kg. Blood samples were collected in disposable plastic syringes. Prior to collection, the saline in the cannula and a small volume of blood were aspirated into a plastic syringe to be reinjected after the sample was collected. Blood samples (0.3 ml) were taken at 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, and 144 hr following drug administration.

In a separate kinetic study, blood samples were collected more frequently at the early time points in order to measure simultaneously the drug in plasma and erythro-

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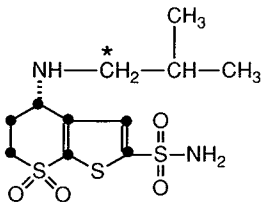

Fig. 1. Chemical structure of MK-417. The position of the radiolabeled ^{14}C is indicated by the asterisk.

cytes. Blood samples were taken at 5, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min following i.v. administration of MK-417 at 2 and 5 mg/kg. Plasma was separated by immediate centrifugation with a microcentrifuge (Brinkmann, Centrifuge 3200). The whole procedure of separation was performed within 1 min. The drug in plasma and whole blood was then analyzed by HPLC and the concentration in erythrocytes was calculated from the differences between plasma and blood concentration using the hematocrit.

Plasma Protein Binding

Binding of MK-417 to plasma protein was determined by the ultrafiltration method. [^{14}C]MK-417 was added to fresh plasma to yield final concentrations of 0.025, 0.25, 3.5, 7.0, and 70 μg/ml. Fresh rat plasma was obtained from anemic, polycythemic, and normal rats. After incubation of plasma samples at 37°C for 5 min, 0.8 ml was immediately transferred to a Centrifree tube (Amicon Co., Danvers, MA) and centrifuged at 1500*g* for 5 min at 37°C. Under these conditions, approximately 150 μl of plasma filtrate was obtained. The unbound fraction of the drug was estimated directly from the ratio of drug concentration in the plasma filtrate to the total drug concentration in the starting plasma. Preliminary experiments revealed negligible binding of the drug to the filtration device.

Analytical Procedures

The concentration of MK-417 in plasma and blood was determined by high-performance liquid chromatography. Whole blood or plasma was buffered with 1 *N* $NaHCO_3$–NaOH (pH 8.9) and placed in a boiling-water bath for 10 min. The heat-treated samples were extracted with 10 ml of toluene/2-propanol/ethyl acetate (49/1/50, v/v/v) and back extracted into 0.4 ml of 0.025 *M* phosphoric acid. The samples were then injected (200 μl) into an HPLC system consisting of a Rainin uptight precolumn packed with partisil ODS (37 μm) and a Whatman partisphere axial compression C-18 5-μm ODS analytical column. The flow rate of the mobile phase, 0.02% H_3PO_4/triethylamine/acetonitrile (50/0.006/50, v/v/v), was 1 ml/min, and the optical density of the column eluate was monitored at 256 nm. There was no interference by endogenous substances. The detection limit of MK-417 was 5 ng/ml in plasma and blood.

Pharmacokinetic Analysis

Total blood clearance was calculated as dose divided by total area under blood concentration–time curve (AUC) following intravenous administration of MK-417. The terminal half-life ($t_{1/2}$) was determined from the slope of the regression line fitted to the log blood concentration–time data of the terminal phase by the method of least squares. The apparent volume of distribution at steady state was estimated by the following equation (8):

$$V_{dss} = \frac{\text{dose i.v.} \times (\text{AUMC})_0^\infty}{[(\text{AUC})_0^\infty]^2} \quad (1)$$

where $(AUMC)_0^\infty$ is the total area under the first moment of the drug concentration curve from zero to infinity. The areas of (AUC) and (AUMC) were estimated by LaGrange numerical integration (program Lagran version 2.1).

Statistical Analysis

Statistical significance was determined by one-way analysis of variance (ANOVA). Pairwise comparisons were two-sided and performed at 0.05 and 0.01 levels of significance using Tukey's multiple-comparison procedure (9).

RESULTS

Figure 2 shows the mean hematocrit values of anemic, polycythemic, and normal rats. Replacing whole blood with plasma caused a substantial decrease in hematocrit (anemic state), while infusion of blood resulted in a significant increase in hematocrit (polycythemic state). The hematocrit remained relatively constant during the 6-day kinetic study. No apparent physical and behavioural changes were observed in these animals.

Following i.v. administration of MK-417 to anemic, polycythemic, and normal rats, drug was not detected in plasma after low doses (0.05 and 0.1 mg/kg) and only up to 1–2 hr after high doses (2 and 5 mg/kg). Figure 3 shows the concentrations of MK-417 in plasma, erythrocytes, and whole blood of normal rats after administration of the high doses. The drug in plasma was eliminated very rapidly, while the decline of the drug in erythrocytes and blood was very slow, with a terminal half-life of approximately 40 hr. Similar results were observed in anemic and polycythemic rats. Comparison of the partial AUC (0 → 2 hr) for plasma and whole blood indicated that the drug was concentrated within erythrocytes in blood and that only a very small portion of the drug was present in plasma (Table I). The whole blood AUC was higher in polycythemic rats than in normal rats, while the anemic rats had a lower AUC. However, there

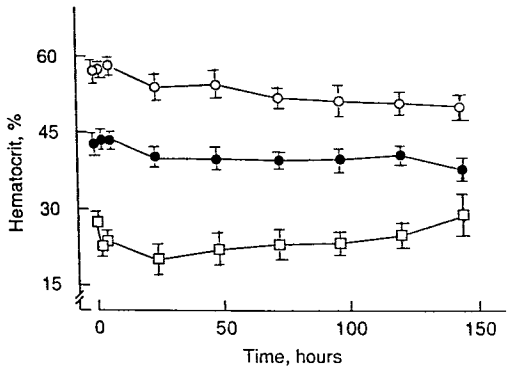

Fig. 2. Mean values of hematocrit of anemic (□), polycythemia (○), and normal rats (●) during 6-day kinetic study. Mean ± SD; n = 26–32.

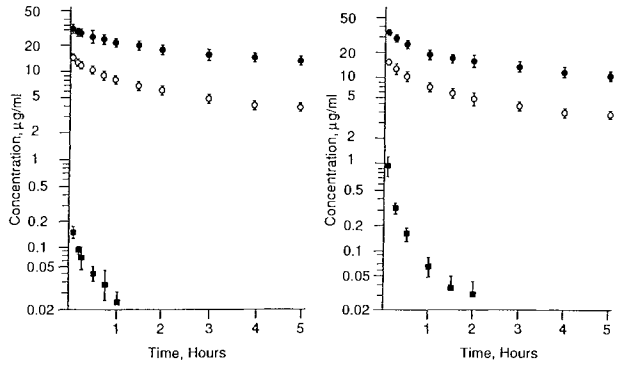

Fig. 3. Mean concentrations of MK-417 in plasma (■), erythrocytes (●), and whole blood (○) of normal rats after i.v. administration of 2 (left) and 5 (right) mg/kg. Mean ± SD; n = 4–6.

were no significant differences in plasma AUC among the three groups of animals. In addition, the plasma AUC for each group of rats at 5 mg/kg was disproportionately higher than that at 2 mg/kg, suggesting saturable binding of the drug to erythrocytes.

The ratio of MK-417 concentration in red blood cells to that in plasma was calculated from plasma and blood concentrations following i.v. administration of 2 and 5 mg/kg dose. The ratio decreased sharply in the polycythemic, normal, and anemic rats as the concentration of drug in blood was higher than 7.0, 4.5, and 3.0 μg/ml, respectively (Fig. 4), suggesting that the concentration of MK-417 required to saturate the enzyme was different among the three groups of animals. It is interesting to note that the ratio was similar among the three groups of rats in the low concentration range (<3.0 μg/ml), while polycythemic rats had a higher ratio than normal and anemic rats in the high concentration range (>5.0 μg/ml).

The mean concentrations of MK-417 in whole blood after i.v. injection of the drug are shown in Figs. 5 and 6. After intravenous administration of 0.05 and 0.1 mg/kg, blood concentrations of MK-417 in anemic, polycythemic, and normal rats were almost identical (Fig. 5). When the dose was increased to 1 and 2 mg/kg i.v. anemic rats cleared MK-417 more rapidly, while drug elimination was relatively slow in polycythemic rate, compared to controls (Fig. 6).

The pharmacokinetic parameters of MK-417 in anemic, polycythemic, and normal rats as a function of dose are summarized in Table II. The total blood clearance and apparent volume of distribution increased markedly in each group of rats, when the dose exceeded 0.2 mg/kg in anemic rats and 0.5 and 1.0 mg/kg in normal and polycythemic rats, respectively. However, the terminal half-life was dose independent and not influenced by hematocrit. At low doses (0.05 and 0.1 mg/kg), there were no significant differences in the kinetic parameters among the three groups of rats. Significant differences in total blood clearance and apparent volume of distribution were observed at 1.0 and 2.0 mg/kg across the three groups of rats with the following rank: anemic rats > normal rats > polycythemic rats. There was a strong inverse correlation between total blood clearance and hematocrit (Fig. 7) and between apparent volume of distribution and hematocrit (Fig. 8).

Binding of MK-417 to plasma protein was low. The unbound fraction was about 0.60 over a wide concentration range of 0.25 to 70 μg/ml. There appeared to be no differences in plasma protein binding of MK-417 among polycythemic, anemic, and normal rats.

DISCUSSION

There are four major observations in this study: (i) MK-417, though poorly bound to plasma proteins, was cleared very slowly in rats; (ii) nonlinear kinetics of MK-417 occurred at different dose levels among anemic, polycythemic, and normal rats; (iii) when MK-417 was administered at low doses (0.05 and 0.1 mg/kg), there were no significant differences in elimination in the three groups of animals; and (iv) following high doses of MK-417 (1 and 2 mg/kg), anemic rats had a greater total blood clearance and apparent volume of distribution than normal and polycythemic rats. Each of these observations may be explained by the strong and saturable binding of the drug to carbonic anhydrase within erythrocytes.

For example, earlier studies in this laboratory have shown that the association constant of MK-417 binding to carbonic anhydrase in rat erythrocytes is $2 \times 10^6\ M^{-1}$ (data to be published),[3] indicating a strong binding of the drug to

[3] J. H. Lin and T. H. Lin. Stereoselective binding of MK-927 to carbonic anhydrase in erythrocytes (submitted for publication).

Table I. AUC of MK-417 in Plasma and Blood from 0 to 2 hr Following i.v. Administration of 2 and 5 mg/kg Doses to Polycythemic, Anemic, and Normal Rats (Mean ± SD; n = 4–6)

	Dose			
	2 mg/kg		5 mg/kg	
Rats	AUC_o^2, plasma (μg · min/ml)	AUC_o^2, blood (μg · min/ml)	AUC_o^2, plasma (μg · min/ml)	AUC_o^2, blood (μg · min/ml)
Anemic	4.82 ± 0.86	360 ± 58	23.6 ± 0.72	512 ± 77
Normal	4.50 ± 1.1	501 ± 86*	19.4 ± 4.3	1058 ± 97*
Polycythemic	4.06 ± 1.2	682 ± 125*	19.5 ± 3.9	1680 ± 183*,**
Analysis of variance	NS	$P < 0.05$	NS	$P < 0.01$

* Significantly different from anemic rats at $P < 0.01$.
** Significantly different from normal rats at $P < 0.01$.

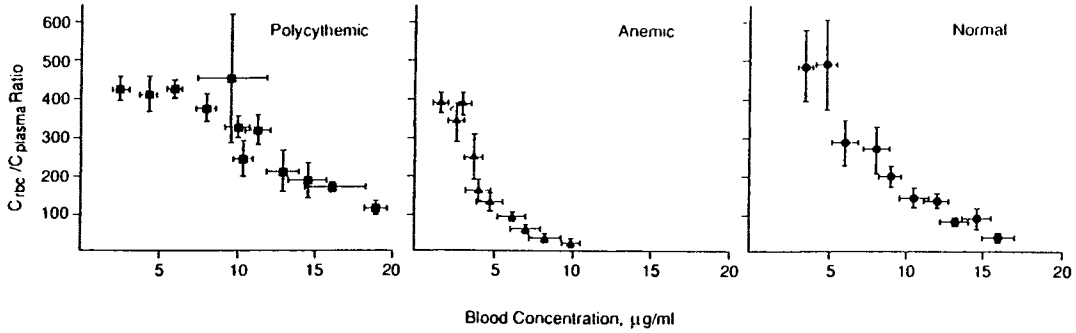

Fig. 4. Blood cell-to-plasma drug concentration ratio of MK-417 as a function of drug concentration in blood. Anemic rats (▲), polycythemic rats (■), and normal rats (●). Mean ± SD; n = 4–6.

carbonic anhydrase. Thus, the slow elimination of MK-417 in rats was most likely due to the strong binding.

The concentration of carbonic anhydrase in rat erythrocytes has been estimated to be about 100 μM in this laboratory.[3] Since more than 90% of carbonic anhydrase in the body is present in erythrocytes (1), and since carbonic anhydrase is an intracellular enzyme, the total amount of carbonic anhydrase in anemic (Ht = 0.25), polycythemic (Ht = 0.60), and normal (Ht = 0.45) rats would be about 1.75, 4.20, and 3.15 μmol/kg, respectively, assuming a blood volume of 70 ml/kg. Doses of 0.05 and 0.1 mg/kg are equivalent to 0.15 and 0.3 μmol/kg, respectively, indicating that even at the lower hematocrit such as anemia there is still sufficient carbonic anhydrase in the red blood cells to bind most of the drug. Thus, MK-417 was confined within the circulating system and the kinetic behavior of MK-417 based on whole blood concentrations was similar at low doses among the three groups of animals (Table II). The low volume of distribution being approximately equal to the blood volume of 70 ml/kg at low doses (Table II) supports the notion that MK-417 was tightly bound to carbonic anhydrase within erythrocytes in blood.

When the dose of MK-417 approaches or exceeds the amount of carbonic anhydrase in erythrocytes, binding of the drug to the enzyme becomes saturated and more unbound drug is available for distribution and elimination. Thus, there was an increase in total blood clearance and apparent volume of distribution when high doses of MK-417 were administered (Table II). In anemic rats, the total amount of the enzyme was much less than that in normal and polycythemic rats, resulting in a greater increase in total blood clearance and apparent volume of distribution (Table II). This explains why there were significant differences in total blood clearance and apparent volume of distribution at high doses in the three groups of animals.

Total blood clearance and apparent volume of distribution of MK-417 significantly increased in each group of rats, when the dose exceeded 0.2 mg/kg (0.6 μmol/kg) in anemic and 0.5 and 1.0 mg/kg (1.5 and 3.0 μmol/kg) in normal and polycythemic rats, respectively (Table II). These results indicated that a lower dose of MK-417 will be required to saturate the enzyme in an anemic animal, while a higher dose will be necessary for polycythemic rats. This is consistent with the notion that anemic rats have less carbonic anhydrase, while polycythemic animals have more enzyme.

Binding of MK-417 to carbonic anhydrase can affect plasma levels of the drug. Since binding of MK-417 to plasma protein was low and independent of concentration, the ratio of drug concentration in erythrocytes to that in plasma reflects the binding to carbonic anhydrase. Thus in anemic, normal, and polycythemic rats a sharp decrease in the ratio of drug in erythrocytes to that in plasma occurred at blood concentrations of 3, 4.5, and 7 μg/ml, respectively (Fig. 4). These data support the concept that the dose of MK-417 required to saturate the binding to carbonic anhydrase in the three groups of rats is dependent on the total amount of the enzyme in blood.

Although increasing doses resulted in increases in both total blood clearance and apparent volume of distribution, the dose appeared to have little effect on the terminal half-

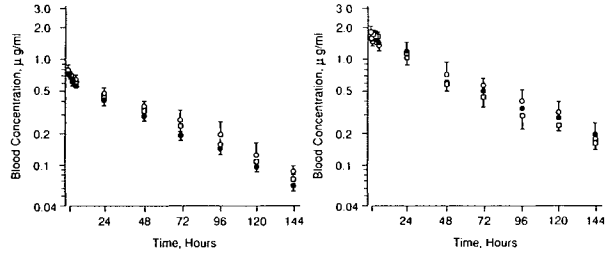

Fig. 5. Mean blood concentration of MK-417 in anemic (□), polycythemic (○), and normal (●) rats following i.v. administration of 0.05 (left) and 0.1 (right) mg/kg. Mean ± SD; n = 4–6.

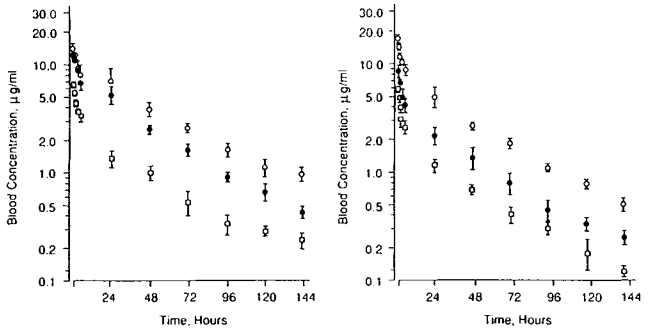

Fig. 6. Mean blood concentration of MK-417 in anemic (□), polycythemic (○), and normal (●) rats following i.v. administration of 1 (left) and 2 (right) mg/kg. Mean ± SD; n = 4–6.

Table II. Pharmacokinetic Parameters of MK-417 in Blood of Anemic, Polycythemia, and Normal Rats After i.v. Administration of Various Doses of the Drug (Mean ± SD, n = 4–7)*

Dose (mg/kg)	Normal rats $t_{1/2}$ (hr)	Normal rats V_{dss} (ml/kg)	Normal rats CL_{total} (ml/hr/kg)	Anemic rats $t_{1/2}$ (hr)
0.05	45.7 ± 3.9	80.8 ± 7.3	1.29 ± 0.09	41.2 ± 3.0
0.1	49.4 ± 9.9	76.7 ± 14.2	1.02 ± 0.19	35.3 ± 2.3
0.2	41.6 ± 3.5	76.0 ± 5.7	1.31 ± 0.08	34.7 ± 9.2
0.5	38.6 ± 5.1	113.0 ± 19.4[a–c]	2.23 ± 0.28[a–c]	35.4 ± 3.5
1	37.2 ± 4.7	147.7 ± 23.2[a–c]	2.74 ± 0.32[a–c]	32.8 ± 6.3
2	38.3 ± 3.2	408 ± 98[a–e]	9.07 ± 1.94[a–e]	33.9 ± 13.0
Analysis of variance	NS	$P < 0.01$	$P < 0.01$	NS

* Superscripts as follows: (a) significantly different from 0.05 mg/kg dose at $p < 0.01$; (b) significantly different from 0.1 mg/kg dose at $P < 0.01$; (c) significantly different from 0.2 mg/kg dose at $P < 0.01$; (d) significantly different from 0.5 mg/kg dose at $P < 0.01$; (e) significantly different from 1 mg/kg dose at $P < 0.01$.

life of MK-417 (Table II). Since a drug's half-life is proportional to the volume of distribution but inversely related to total body clearance, both of which are determined by the binding of the drug to the enzyme, the consequences of binding on this parameter depend on the relative magnitude of changes in total body clearance and volume of distribution (10,11). Therefore, the consistency in the half-life of MK-417 with respect to the dose is the result of quantitatively similar changes in total blood clearance and apparent volume of distribution affected by saturable binding.

For low clearance drugs, binding is an important determinant in drug elimination, and total body clearance is directly proportional to the unbound fraction (10,11). Binding of a drug to protein, and accordingly the unbound fraction, is dependent on the concentration of protein, the number of binding sites, the affinity constant, and the drug level (10,11). Protein binding of most drugs is decreased with hypoalbuminaemia. For example, unbound fraction and total body clearance of several drugs have been reported to be increased in patients with nephrotic syndrome (12,13). Plasma albumin concentration can be as low as 10 g/liter in patients with nephrotic syndrome, while normal subjects have plasma albumin concentrations of at least 35 g/liter (11). Intuitively, a reduction in hematocrit is expected to be associated with a decrease in the blood concentration of carbonic anhydrase and a resultant increase in both the unbound fraction and the total body clearance. Conversely, an increase in hematocrit would be expected to lead to a decrease in both the unbound fraction and the total body clearance. However, this is not the case for carbonic anhydrase, where the enzyme is present mostly in erythrocytes rather than in plasma. In fact, changes in hematocrit had no effect on the elimination kinetics of MK-417 at low doses.

The affinity of MK-417 for carbonic anhydrase is sufficiently high so that the efflux of the drug from the erythrocytes may be very slow. In addition, the erythrocyte cell membrane may provide a significant diffusional barrier between drug and the organs of elimination. Accordingly, reequilibration as the drug in plasma is removed by eliminating processes may not occur within the involved organs. Therefore the drug in erythrocytes is not readily available for elimination and represents another compartment. This means that whole blood can not be considered as a single compartment. In this situation, it would be desirable to analyze both plasma and erythrocyte data in order to gain a better understanding of the effects of hematocrit on the dose-

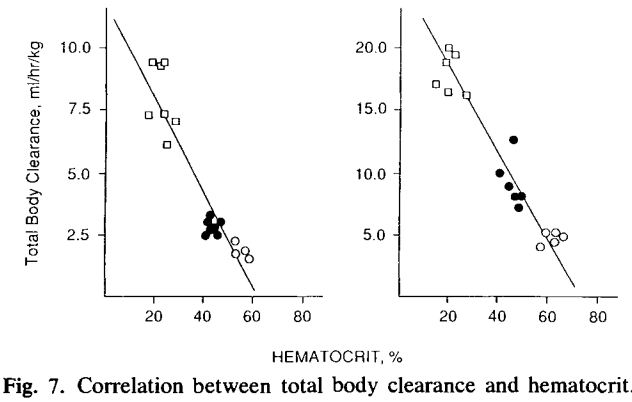

Fig. 7. Correlation between total body clearance and hematocrit. (□) Anemic, (○) polycythemic, and (●) normal rats, respectively. Left, 1 mg/kg; right, 2 mg/kg.

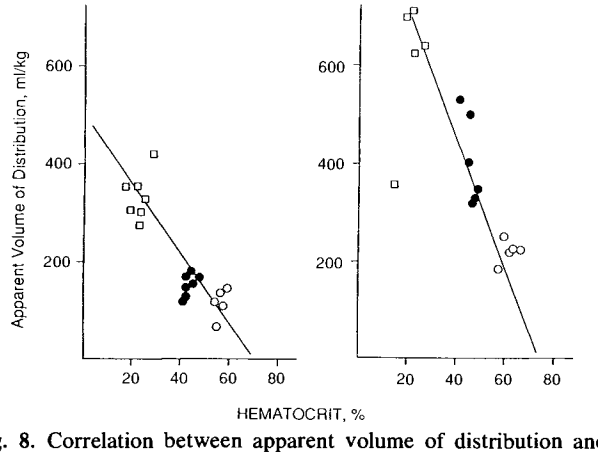

Fig. 8. Correlation between apparent volume of distribution and hematocrit. Symbols as in the legend to Fig. 7. Left, 1 mg/kg; right, 2 mg/kg.

Table II. Continued

Anemic rats		Polycythemia rats		
V_{dss} (ml/kg)	CL_{total} (ml/hr/kg)	$t_{1/2}$ (hr)	V_{dss} (ml/kg)	CL_{total} (ml/hr/kg)
68.4 ± 5.5	1.15 ± 0.08	52.8 ± 7.5	79.4 ± 12.2	1.01 ± 0.16
57.5 ± 4.8	1.08 ± 0.12	54.3 ± 9.0	77.4 ± 6.0	0.980 ± 0.06
105.7 ± 9.9[a,b]	2.06 ± 0.50[a,b]	—	—	—
166.9 ± 20[a–c]	3.63 ± 0.33[a–c]	39.3 ± 6.7	73.0 ± 24.0	1.25 ± 0.12
328.4 ± 46.6[a–d]	8.02 ± 1.4[a–d]	40.9 ± 10.3	111.7 ± 30.7[a,b,d]	1.84 ± 0.25[a,b,d]
831.4 ± 346[a–e]	17.7 ± 2.2[a–c]	40.8 ± 6.2	222.0 ± 28.2[a,b,d,e]	4.37 ± 0.61[a,b,d,e]
$P < 0.01$	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$

dependent kinetics of MK-417. However, because of assay limitations, drug concentrations in plasma could not be detected at time points later than 2 hr after the high doses and kinetic analyses were based only on drug concentration in whole blood.

Nonlinear pharmacokinetics caused by saturable plasma protein binding have been reported for several drugs, such as diflunisal (14,15), naproxen (16,17), and salicylate (18). However, the saturable binding of carbonic anhydrase is a completely different situation. Carbonic anhydrase in erythrocytes serves as a distribution "sink." Therefore, nonlinear kinetics of MK-417 due to saturability in binding to carbonic anhydrase in erythrocytes is a novel finding.

Normally, the circulating blood consists of about 45% blood cells by volume. However, in newborn infants or in polycythemic patients the fraction may be as high as 60%, and in patients with anemia as low as 20% (19). The acute anemia and polycythemia were induced in rats to mimic the clinical situations.

Preliminary data from clinical studies suggest that the topical dose of MK-417 required to lower intraocular pressure is one drop of 1% solution twice a day (5). If each drop is about 30 μl, the dose is approximately 0.02 mg/kg (0.06 μmol/kg), which is much lower than the reported values of carbonic anhydrase (2.5 μmol/kg) in human erythrocytes (20,21). Accordingly, changes in hematocrit would not alter the pharmacokinetics of MK-417 in clinical situation.

In conclusion, this study demonstrates the effects of experimental manipulation of hematocrit on dose-dependent pharmacokinetics of MK-417 in rats. At low doses, there were no significant differences in the pharmacokinetic parameters for MK-417 among anemic, polycythemic, and normal rats. However, significant differences in total blood clearance and apparent volume of distribution were observed at high doses in the three groups of rats. Although the total blood clearance and apparent volume of distribution were increased with increasing dose, the terminal half-life was dose independent and not influenced by hematocrit. The hematocrit effect on the pharmacokinetics of MK-417 is unlikely to be clinically relevant because the therapeutic dose of MK-417 is much lower than the amount of carbonic anhydrase even in anemic patients.

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