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Interspecies Differences and Scaling for the Pharmacokinetics of Xanthine Derivatives

YOSHIMI TSUNEKAWA, TAKAAKI HASEGAWA, MASAYUKI NADAI, KENZO TAKAGI* AND TOSHITAKA NABESHIMA

*Department of Hospital Pharmacy and *Second Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan*

Abstract—Pharmacokinetic characteristics of the new xanthine bronchodilators, enprofylline and l-methyl-3-propylxanthine (MPX), were investigated in mice, rats, guinea-pigs, rabbits and dogs. The possibility of an interspecies pharmacokinetic scale was also evaluated. The concentration of these two drugs in plasma and urine was determined by HPLC. Pharmacokinetic parameters were calculated using model-independent methods. The disappearance curves of the two drugs from plasma varied markedly among animal species. Interspecies differences in the plasma protein binding of each drug were observed for all animals in the study. Differences in the biotransformation of enprofylline and MPX were also confirmed among the various animal species: enprofylline is mainly excreted in an unchanged form in urine while MPX follows a non-renal route of elimination. In all animals, the renal clearance for enprofylline was greater than the glomerular filtration rate, indicating active tubular secretion. Significant allometric relationships were seen between the values of total body clearance and steady state volume of distribution for both total and unbound enprofylline and species body weight, but similar correlations could not be recognized for MPX. Renal clearance of enprofylline was also closely correlated with species body weight, suggesting no interspecies difference with relation to affinity and/or capacity for the active tubular secretion mechanism of enprofylline. Our findings suggest that xanthine derivatives, including enprofylline, are mainly eliminated via the kidney, and an estimate of the basic pharmacokinetics in man can be obtained from data in experimental animals.

Theophylline is one of the most used anti-asthma drugs, but has disadvantages because of its extrapulmonary effects, such as stimulation of the central nervous system (CNS) and heart excitation. The action of alkylxanthines on bronchodilation is thought to be mediated via cAMP phosphodiesterase (PDE) inhibition (Katsuki & Murad 1977; Polson et al 1982) and adenosine antagonism (Fredholm & Persson 1982).

As part of our research on bronchodilators, we were interested in developing compounds with a stronger relaxant effect by making chemical modifications to the xanthine molecule. We recently synthesized various *N*-alkylxanthine derivatives, and have shown that a close relationship exists between their bronchial smooth muscle relaxant effect and cAMP-PDE inhibitory activity, and that a new xanthine derivative, l-methyl-3-propylxanthine (MPX), has a much stronger in-vitro relaxant effect than either theophylline or enprofylline (Apichartpichean et al 1988; Miyamoto et al 1989; Takagi et al 1988; Ogawa et al 1989). We also reported on the positive relationships between the protein binding characteristics of *N*-alkylxanthine derivatives and their hydrophobicity, which are important determinants influencing the pharmacokinetic behaviour of any drug (Hasegawa et al 1991b). In subsequent studies on the structure-pharmacokinetic relationships among these xanthine derivatives in rats, we found that there was a highly significant correlation between the steady-state volume of distribution and unbound drug fraction in plasma due to differences in hydrophobicity (Apichartpichean et al 1991). We investigated the pharmacokinetic characteristics of two drugs in rats and found that enprofylline is almost completely excreted unchanged by an active tubular secretion mechanism (Apichartpichean et al 1991; Nadai et al 1991) and that MPX, despite its resemblance to enprofylline, is almost completely metabolized in the liver (Apichartpichean et al 1988, 1991).

Two detailed studies on animal scaling with theophylline (Gaspari & Bonati 1990) and caffeine (Bonati et al 1984), have been reported: allometric relationships were observed between pharmacokinetic parameters of these two drugs and species body weight. Since an interspecies pharmacokinetic scale would be useful for designing new drugs for man, the present study was designed to evaluate the pharmacokinetic behaviour of enprofylline and MPX in various experimental animals, specifically mice, rats, guinea-pigs, rabbits and dogs, and to determine whether the pharmacokinetics of these two drugs in man could be predicted from the data obtained.

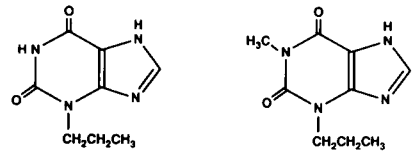

FIG. 1. Chemical structures of enprofylline and l-methyl-3-propylxanthine (MPX).

Correspondence: T. Hasegawa, Department of Hospital Pharmacy, Nagoya University School of Medicine, 65 Tsurunai-cho, Showa-ku, Nagoya 466, Japan.

Materials and Methods

Materials

The *N*-alkylxanthine derivatives enprofylline and MPX (Fig. 1) were synthesized in our laboratory (Apichartpichean et al 1988, 1991; Takagi et al 1988; Hasegawa et al 1990, 1991a). All other chemical reagents were of analytical grade.

Animals

Male ICR mice, male Wistar rats, male Hartley guinea-pigs, JW-NIBS male rabbits and male beagle dogs were utilized in this study.

Protein binding

Drug plasma protein binding in-vitro was studied by equilibrium dialysis using a cellulose membrane (Visking sheet, Sanplatec Corp., Osaka, Japan) with a molecular cut-off at 10000–20000 Da. Plasma solutions containing appropriate concentrations of each drug (< 10 μg mL^{-1}) were prepared from pooled plasma taken from each animal individually and were immediately dialysed against an equal volume of pH 7.4 isotonic phosphate buffer at 37°C for 5 h, by which time equilibrium had been attained. The concentrations of the two drugs on each side of the membrane were determined by HPLC.

Pharmacokinetic studies

The two drugs were administered intravenously at a dose of 2.5 mg kg^{-1}, into the tail vein of mice, right jugular vein of rats and guinea-pigs, marginal ear vein of rabbits or antecubital vein of beagles. Rats and guinea-pigs were first cannulated with polyethylene tubing in the right jugular vein before experimentation under light ether anaesthesia. Blood samples were collected at appropriate intervals after dosing. Blood was taken from the marginal vein of the opposite ear in rabbits, while mice were exsanguinated under light ether anaesthesia. Samples were immediately centrifuged at 11000 rev min^{-1} for 5 min and the resulting plasma samples were stored at −40°C until analysis. Urine samples were also collected over a period of 24 h after dosing in all animals except mice.

HPLC analysis

The concentrations of the two drugs in all species were determined by HPLC (Hasegawa et al 1990). Blank plasma and urine from each animal species were shown to have no interfering peaks at the retention time of either drug.

Pharmacokinetic analysis

The plasma concentration-time data of each drug was analysed using model-independent methods and the non-linear least-squares method program, MULTI (Yamaoka et al 1981). The mean plasma drug concentrations from five mice were used for calculating the pharmacokinetic parameters in mice.

The area under the plasma concentration-time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. Total body clearance (CL_T) was determined by $CL_T = \text{dose}/AUC$. The apparent volume of distribution at steady state (V_{SS}) was calculated by $V_{SS} = CL_T \times MRT$. Mean residence time (MRT) was calculated by $MRT = AUMC/AUC$.

The fraction of drug excreted unchanged in urine (f_e) was calculated by $f_e = A_u/\text{dose}$, where A_u represents the amount of drug excreted in urine. Renal clearance (CL_R) was calculated by $CL_R = CL_T \times f_e$ and non-renal clearance was represented by $CL_T - CL_R$. Total and renal clearance for plasma unbound drug was defined as $CL_{TU} = CL_T/f_u$ and $CL_{RU} = CL_R/f_u$, respectively, with f_u being the average fraction of unbound drug.

Interspecies pharmacokinetic scaling

Pharmacokinetic parameters for enprofylline in man were obtained from the literature (Borga et al 1983). Relationships between pharmacokinetic parameters and the body weights of each animal species were evaluated using allometric relationships (Boxenbaum 1982). Pharmacokinetic parameters were plotted against body weight of the various animal species according to allometric relationships obtained using linear least-squares regression analysis.

Statistical analysis

Results were expressed as mean ± s.e. Regressions were obtained using linear least-squares regression analysis. Statistical significance of the regressions was assessed using Student's *t*-test with $P < 0.05$ indicating a significant difference.

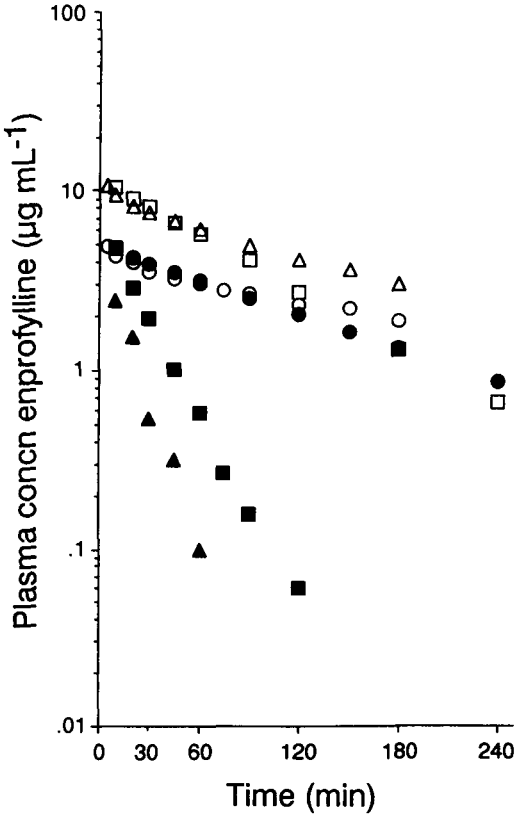

FIG. 2. Semilogarithmic plasma concentration-time profiles following intravenous administration of enprofylline (2·5 mg kg^{-1}) in different animal species: □, rabbit; ○, dog; △, guinea-pig; ■, rat; ●, man; ▲, mouse.

Table 1. Pharmacokinetic parameters of total and unbound enprofylline in different animal species.

Animal species	Body weight (kg)	CL_T (L h^{-1})	CL_{TU} (L h^{-1})	V_{SS} (L)	V_{SSU} (L)	f_u	Urinary recovery (%)	CL_R (L h^{-1})	CL_{RU} (L h^{-1})
Mouse[a]	0·031	0·059	0·081	0·014	0·019	0·730	—[c]	—	—
Rat	0·333	0·288	1·274	0·114	0·504	0·226	86·93	0·251	1·111
Guinea-pig	0·613	0·072	0·986	0·161	2·205	0·073	89·60	0·065	0·890
Rabbit	2·330	0·355	2·934	0·479	3·959	0·121	87·70	0·311	2·570
Dog	13·33	1·907	3·106	8·498	13·84	0·614	58·74	1·120	1·824
Man[b]	76·00	18·92	38·70	38·76	79·26	0·489	89·06	16·75	34·254

Each parameter was expressed as mean (n = 3–4). [a]Data were calculated from the mean plasma concentrations of four animals. [b]Data were cited from the study using healthy volunteers (Borgå et al 1983). [c]Not determined.

Results

Semilogarithmic plots of the mean plasma concentration-time data for enprofylline and MPX after intravenous administration to the various animal species are illustrated in Figs 2, 3, respectively. As shown, a one-compartment model adequately described the plasma concentration-time data for these two drugs in all species except dogs and guinea-pigs. The disappearance of both drugs from plasma was faster in smaller animals than in larger animals. In particular, the plasma disappearance curve of enprofylline in man was similar to those observed in guinea-pigs and dogs.

Pharmacokinetic parameters of the various animal species are summarized in Tables 1, 2. For enprofylline, the values of

Table 2. Pharmacokinetic parameters of total and unbound l-methyl-3-propylxanthine (MPX) in different animal species.

Animal species	Body weight (kg)	CL_T (L h^{-1})	CL_{TU} (L h^{-1})	V_{SS} (L)	V_{SSU} (L)	f_u
Mouse[a]	0·033	0·034	0·056	0·007	0·011	0·611
Rat	0·264	0·051	0·447	0·050	0·439	0·114
Guinea-pig	0·315	0·009	0·563	0·051	3·188	0·016
Rabbit	2·09	0·134	4·79	0·252	9·00	0·028
Dog	13·33	2·44	3·66	8·93	13·37	0·668

Each parameter was expressed as mean (n = 3–4). [a]Data were calculated from the mean plasma concentrations of four animals. Urinary recovery was not detected in the mouse and not determined for the other species.

V_{SS} and CL_T increased as the body weight of each animal increased. The value of CL_T per body weight in mice was higher than in other animal species, but rabbits and dogs were similar in CL_T values per kg body weight. Interspecies differences in the plasma unbound fraction of enprofylline were observed. Urinary excretion of unchanged drug was consistent (~ 90%) amongst all the animal species except dogs where it was approximately 60%, demonstrating extrarenal clearance including biotransformation and elimination in the liver of dogs. In addition, the values of renal clearance of enprofylline were greater than the glomerular filtration rate in all the animals studied (Adolph 1949; Lázníček et al 1990). These findings indicate that the kidney is the major route for elimination in the animal species

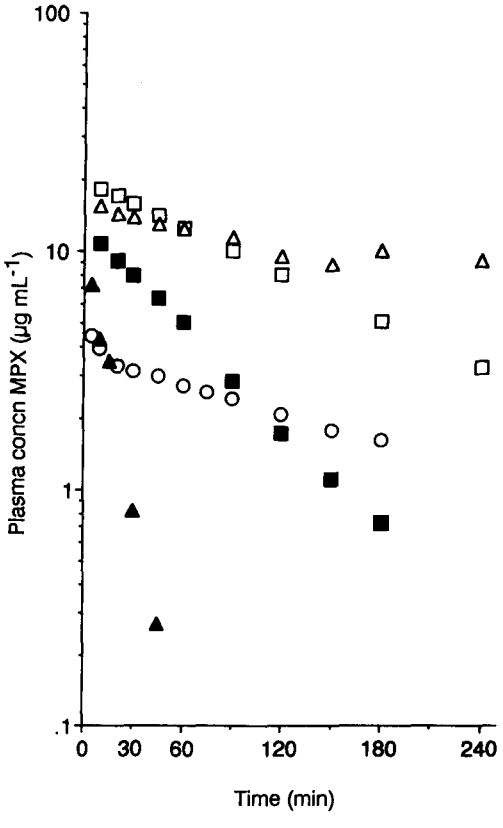

FIG. 3. Semilogarithmic plasma concentration-time profiles following intravenous administration of l-methyl-3-propylxanthine (MPX, 2·5 mg kg^{-1}) in different animal species: □, rabbit; ○, dog; △, guinea-pig; ■, rat; ▲, mouse.

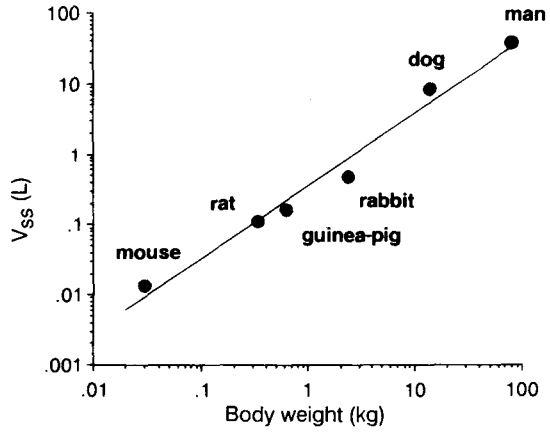

FIG. 4. Allometric relationship between steady state volume of distribution (V_{SS}) and species body weight for enprofylline.

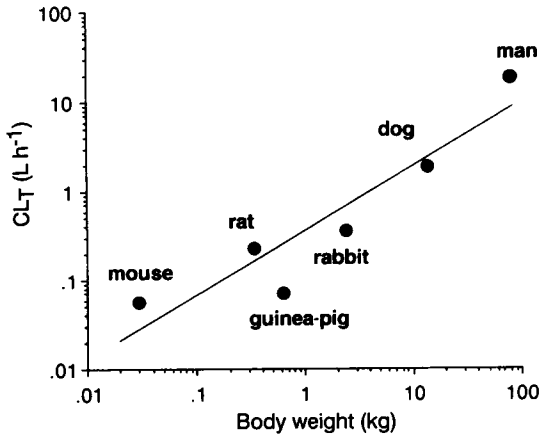

FIG. 5. Allometric relationship between total body clearance (CL_T) and species body weight for enprofylline.

studied and that active tubular secretion plays a major role in the renal excretion mechanism of enprofylline.

The relationships between the volume of distribution in the steady state (V_{SS}), total body clearance (CL_T) and species body weight on log–log plot are illustrated in Figs 4 and 5, respectively; both parameters increased as the body weight of each animal increased. A strong correlation was seen between the steady state volume of distribution (V_{SS}) and species body weight ($r = 0{\cdot}99$) with the allometric equation as follows:

$$V_{SS} = 0{\cdot}365 \times BW^{1{\cdot}048} \quad (1)$$

where BW represents the body weight of the particular animal species. In the same way, a significant linear relationship was also obtained using the logarithmic plot of the total body clearance against the logarithmic value for the species body weight ($r = 0{\cdot}92$) with the equation as follows:

$$CL_T = 0{\cdot}361 \times BW^{0{\cdot}725} \quad (2)$$

It is generally recognized that volume of distribution, total body clearance and other pharmacokinetic parameters which were corrected for plasma protein binding, are useful as an indicator of tissue binding and as a more rational parameter for scaling, respectively. These parameters for unbound drug are summarized in Table 1. These parameters also increased as the body weight of each species increased. Significant relationships were likewise observed between the basic pharmacokinetic parameters, volume of distribution (V_{SSU}) and total body clearance (CL_{TU}) for plasma unbound drug, and species body weight ($r = 0{\cdot}98$ and $0{\cdot}96$, respectively) with the allometric equations as follows:

$$V_{SSU} = 1{\cdot}343 \times BW^{1{\cdot}014} \quad (3)$$

$$CL_{TU} = 1{\cdot}330 \times BW^{0{\cdot}690} \quad (4)$$

With the exception of mice, a significant linear relationship could be obtained between renal clearance (CL_R) and species body weight ($r = 0{\cdot}92$) with the allometric equation as follows:

$$CL_R = 0{\cdot}215 \times BW^{0{\cdot}864} \quad (5)$$

In addition, significant relationships were also observed between renal clearance for total plasma and unbound drug (CL_R and CL_{RU}) and species body weight ($r = 0{\cdot}91$ and $0{\cdot}93$, respectively), when the ratio of urinary excretion to dose of enprofylline in mice was taken to represent the average of all animals under study.

For MPX, the values of volume of V_{SS} and V_{SSU} also varied with dogs > rabbits > guinea-pigs > rats > mice. The values of CL_T ranged from $0{\cdot}009$ in guinea-pigs to $2{\cdot}44$ L h^{-1} in dogs, and the order was dogs > rabbits > rats > mice > guinea-pigs. The value of CL_T corrected for body weight in rats correlated well with that in dogs. In addition, CL_{TU} for unbound MPX among all the animal species studied was lower than the respective hepatic blood flow rates (Hanano et al 1985). No urinary excretion of unchanged drug was observed in any of the animal species, indicating that MPX is almost completely metabolized in the liver of the various animal species.

Allometric relationships between pharmacokinetic parameters of MPX, V_{SS} and CL_T, and species body weight are shown in Figs 6, and 7. A significant relationship was observed between V_{SS} and species body weight ($r = 0{\cdot}98$), with the allometric equation calculated as follows:

$$V_{SS} = 0{\cdot}238 \times BW^{1{\cdot}151} \quad (6)$$

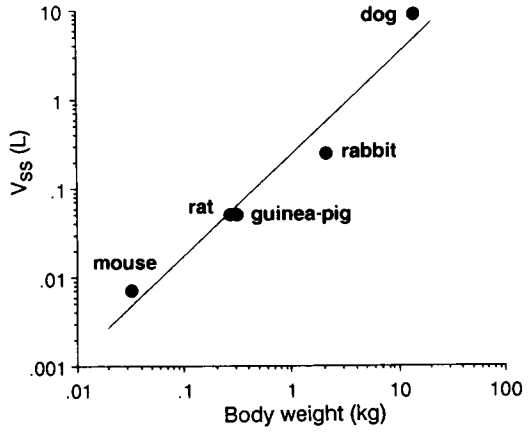

FIG. 6. Allometric relationship between steady state volume of distribution (V_{SS}) and species body weight for MPX.

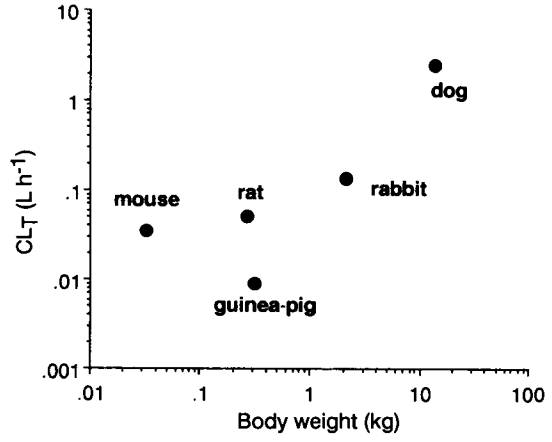

FIG. 7. Allometric relationship between total body clearance (CL_T) and species body weight for MPX.

A linear relationship was also obtained between steady state volume of distribution for plasma unbound MPX (V_{ssu}) and species body weight ($V_{SSU} = 2{\cdot}042 \times BW^{1{\cdot}151}$; $r = 0{\cdot}90$). However, a significant relationship could not be obtained between CL_T and species body weight (Fig. 7), although a linear relationship could be obtained between log CL_{TU} and log species body weight ($CL_{TU} = 1{\cdot}107 \times BW^{0{\cdot}743}$; $r = 0{\cdot}94$).

Discussion

The present study demonstrates that the kidney is the major route for the elimination of enprofylline in the various animal species investigated, and that significant relationships exist among total body clearance (CL_T) and species body weight. Assuming that the urinary excretion of enprofylline in mice is similar to that in rats, guinea-pigs, rabbits and dogs, allometric relationships were obtained between total and unbound renal clearance (CL_R and CL_{RU}) and species body weight. Various data for allometric exponentials in drugs excreted mainly via the kidney have been reported to range from 0·69 for creatinine to 0·89 for iodopyracet (Adolph 1949; Hanano et al 1985). In this study, the parameter for enprofylline was within this range and correlated well with the value for *p*-aminohippuric acid (PAH). With the exception of mice, the renal clearance of enprofylline among the four animal species exceeded the respective glomerular filtration rates (Adolph 1949; Lázníček et al 1990), indicating that enprofylline undergoes active tubular secretion. Such active tubular secretion of enprofylline has also been reported to occur in man (Borgå et al 1983, 1986; Lunell et al 1984) as well as in rat (Apichartpichean et al 1991; Nadai et al 1991).

Body weight affects the renal plasma flow rate and hepatic blood flow rate (Adolph 1949; Boxenbaum 1980). Dedrick et al (1970) have demonstrated interspecies relationships between total body clearance and volume of distribution of methotrexate, which is mainly excreted in the kidneys by a combination of glomerular filtration and active tubular secretion, and species body weight: the interspecies ratio of total body clearance of methotrexate to creatinine clearance is a constant. Recently, Ibrahim & Boudinot (1989) have reported a significant relationship between species body weight and renal clearance (CL_R) of AZT, a potent inhibitor of human immunodeficiency virus (HIV) replication, which is mainly excreted in the kidney as an active tubular secretion mechanism and the protein binding is relatively consistent among the various species. In the present study, a significant relationship was also observed between renal clearance and species body weight for enprofylline, although there are interspecies differences in the protein binding of enprofylline. The values of renal clearance for plasma unbound enprofylline (CL_{RU}) in rats, guinea-pigs, rabbits, dogs and man were nearly equal or somewhat lower than the respective renal plasma flow rates (Hanano et al 1985). These results suggest that the renal clearance of enprofylline is likely to depend on renal plasma flow rate with the possibility of no differences in affinity or capacity with relation to the tubular transport mechanism for enprofylline among the various animal species, since enprofylline possesses a high affinity and low capacity for the active tubular secretion transport mechanism in the tubular proximal cells of rats (Nadai et al 1991).

It is interesting to note that the correlation coefficients between both the total and unbound volume of distribution of enprofylline and body weight were also high and statistically significant ($r > 0{\cdot}98$). The slope of the calculated allometric equation was also nearly equal to unity, although the plasma unbound fraction of enprofylline in plasma was inconsistent among the various animal species. These results suggest that the steady-state volume of distribution is based on body weight regardless of the animal species. Therefore, the binding of enprofylline to the tissue may be considered low in each particular species, with the unbound fraction of enprofylline in the tissue unrelated to the unbound fraction in plasma as the unbound fraction in the intracellular compartment remains constant (Apichartpichean et al 1991).

Bonati et al (1984) and Gaspari & Bonati (1990) have demonstrated allometric relationships between the total intrinsic clearance of theophylline and caffeine, which are solely metabolized by the liver, and species body weight. In contrast to the results obtained for MPX, the plasma protein binding of both drugs has been found to be low and almost constant among species. Thus, the protein binding has no effect on scaling for the pharmacokinetics of either drug. A plot of total body clearance (CL_T) of MPX and species body weight showed a complicated relationship (Fig. 7). A strong correlation, however, was obtained between total body clearance for plasma unbound MPX (CL_{TU}) and species body weight, suggesting that the best pharmacokinetic parameter for drugs solely metabolized by the liver is intrinsic clearance (Boxenbaum 1980). These findings confirm the suggestion that protein binding is an important determinant in the total body clearance of drugs, including MPX. In addition, the values of CL_{TU} in all the animals seemed to be lower than the hepatic blood flow rate, indicating that MPX is a low hepatic extraction drug like theophylline (Rowland & Tozer 1989). However, new findings have been reported (Hasegawa et al 1990, 1991a) showing that enoxacin and its analogues decreased the total body clearance of MPX as well as theophylline (Nadai et al 1990). From these observations, it seems difficult to establish an interspecies pharmacokinetics scale for total MPX since it is mainly metabolized in the liver, whereas enprofylline is mainly excreted in an unchanged form in the urine. The present data for MPX, however, provide important suggestions for further studies on the structure-lipophilicity-biotransformation relationships of xanthine derivatives which are chemically related to MPX.

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