J. Pharm. Pharmacol. 1991, 43: 262–269
Received October 18, 1990

Structure-pharmacokinetic Relationships among the N^1, N^3-Alkylxanthines in Rats

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Abstract—The pharmacokinetics of four N^3-alkylxanthine and four N^1-methyl-N^3-alkylxanthine derivatives has been investigated in rats after intravenous administration of the individual alkylxanthines. The concentration of N^1, N^3-alkylxanthine in plasma and urine was determined by HPLC. A one-compartment model adequately described the plasma concentration time data. The steady-state volume of distribution (V_{ss}) was calculated using model-independent methods. The relation between V_{ss} and unbound drug fraction in plasma (f_u) was significantly correlated ($V_{ss} = 0{\cdot}844 f_u + 0{\cdot}119$; $r = 0{\cdot}999$, $P < 0{\cdot}01$), indicating that the differences in f_u among these xanthine derivatives is mainly responsible for differences in V_{ss}. The decrease in V_{ss} and increase in plasma protein binding with lipophilicity reflected a relatively constant tissue affinity. The total body clearance increased with lipophilicity with the exception of the first three lower congeners which were almost completely excreted unchanged in urine, mainly via active tubular secretion. Renal elimination was markedly reduced by the presence of a methyl group at the N^1-position. Renal clearance decreased with increasing lipophilicity, due to increased tubular reabsorption whereas non-renal (hepatic) clearance increased with increasing lipophilicity.

Despite its side effects theophylline is widely used for asthma treatment. In the search for xanthines with higher potency and lower adverse reactions, it has been reported that only N^1- and N^3-alkylsubstitutions may be essential for increasing bronchodilatory effects (Persson 1982). Extension of the alkyl chain at N^1- and N^3-positions increased bronchodilatory activity and the lipophilicity with a consequent affect on pharmacokinetic characteristics. Further structure-activity studies of N^1, N^3-alkylxanthines in guinea-pig smooth muscle in our laboratories indicated a linear relationship between lipophilicity and bronchodilatory activities (Miyamoto et al 1989). Lipophilicity has been shown to influence the pharmacokinetic characteristics of several drugs (Craig & Welling 1977; Toon & Rowland 1979, 1983; Rischel & Hammer 1980), thus affecting the biological activity in-vivo.

The aim of the present study was to evaluate the relationships between molecular structure, lipophilicity and pharmacokinetic characteristics of the N^1, N^3-alkylxanthines using the rat as a model, since the pharmacokinetic characteristics of xanthines have been widely investigated in rats (Aeschbacher & Wurzner 1975; Lohmann & Miech 1976; Williams et al 1979; Bonati et al 1984; Teunissen et al 1985; Bortolotti et al 1985; Wormald et al 1989). The relationships would be useful for further understanding the disposition and elimination of theophylline and for design of better bronchodilatory xanthines.

Materials and Methods

Materials

The N^1, N^3-alkylxanthine derivatives, 3-ethyl (EX), 3-propyl (enprofylline or PX), 3-butyl (BX), 1-methyl-3-ethyl (MEX), 1-methyl-3-propyl (MPX) and 1-methyl-3-butyl (MBX) xanthines were synthesized in our laboratory according to reported methods (Papesch & Schroeder 1951; Wooldridge & Slack 1962; Ohtsuka 1973; Sneddon 1982). 3-Methylxanthine (MX) and 1,3-dimethylxanthine (theophylline, TPH) were obtained from Sigma Chemical Co., St Louis, MO. All other reagents were of analytical grade.

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Animals

Seven-to-eight-week-old male Wistar rats (SLC, Shizuoka, Japan), 250–320 g, were used.

Apparent partition coefficient

These data were from our previous report (Miyamoto et al 1989): each xanthine derivative was dissolved at a concentration of 10 μg mL^{-1} in pH 7·4 phosphate-buffered saline solution. Five mL of the solution was added to an equal volume of n-octanol and equilibrated at 25°C by continuous shaking for 2 h. The concentration of each drug in the aqueous phase was determined by spectrophotometry at 278 nm. The apparent partition coefficient (P) of each compound was estimated as the ratio of the concentration in the organic phase to that in the aqueous phase, and lipophilicity was expressed as a logarithmic partition coefficient (log P).

Plasma protein binding

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 of 10000–20000. Plasma solutions (10 μg mL^{-1}, 400 μL) of each drug were freshly prepared from individual rat plasma (n = 4) and immediately (within 1 h after plasma preparation) dialysed against an equal volume of pH 7·4, isotonic phosphate buffer at 37°C for 5 h, by which time equilibrium had been shown to be established. The concentrations of drugs on each side of the membrane were then analysed by HPLC. Total protein concentrations in plasma samples before and after dialysis were determined (Lowry et al 1951) and the values were used

for correction of binding data. Experiments were carried out in duplicate.

Pharmacokinetic study

One day before the experiment, rats (n = 4–5) were cannulated in the right jugular vein, for blood sampling and drug administration, under light anaesthesia with sodium pentobarbitone (25 mg kg^{-1}). After overnight fasting with free access to water, each drug at a dose of 5 mg kg^{-1} (except TPH, MPX and MBX) was administered intravenously via the jugular vein. Saline (0·9% NaCl) (0·3 mL) was then injected to wash the remaining drug solution in the cannula. A small portion of blood (0·1 mL) was discarded before taking each blood sample and 0·1 mL of heparin solution (5 units mL^{-1}) was injected. TPH data were from our previous report (Nadai et al 1990), at a dose of 10 mg kg^{-1} at which linear pharmacokinetics still applies (Teunissen et al 1985). MPX and MBX were administered at a dose of 2·5 mg kg^{-1} because of their capacity limited elimination at 5 mg kg^{-1}. Blood samples of about 300 μL each were collected at appropriate intervals. The plasma samples were obtained by centrifugation at 11 000 rev min^{-1} for 5 min. Urine samples were also collected over a period of 24 h after dosing. Plasma and urine samples were stored at −40°C until analysis.

HPLC analysis

The concentrations of each drug were determined by appropriate modification of the HPLC method for theophylline (Ogura et al 1983) using an LC-4A system (Shimadzu, Kyoto, Japan). Separation was carried out on Zorbax ODS (Du Pont Instruments, USA). Chromatographic conditions are shown in Table 1. Internal standard was added in methanol at the appropriate concentration and the solution was added to the plasma sample to precipitate proteins. After centrifugation the supernatant was directly injected into the HPLC column. For MX and EX, the solvent was evaporated under a gentle stream of nitrogen and the residue was reconstituted with the mobile phase before analysis. For urine samples, the internal standard was added in the mobile phase to diluted urine before analysis. The assays were linear over the range studied, with a lower limit of quantitation of 0·2 μg mL^{-1}. The coefficients of variation for the assay were less than 5%.

Pharmacokinetic analysis

Plasma concentration-time data of each drug were analysed using model-independent methods and a nonlinear least squares method program, MULTI (Yamaoka et al 1981).

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) was determined by CL = Dose/AUC. The mean residence time (MRT) was calculated by MRT = AUMC/AUC. The apparent volume of distribution at steady state (V_{ss}) was calculated by $V_{ss} = CL \times MRT$.

Statistical analysis

The results were expressed as mean ± s.d. Statistical differences between the MX, EX, and PX experiments were examined by one-way analysis of variance (ANOVA), with Tukey's test to detect the differences among individual groups. Statistical significance was defined as $P < 0.05$. The regressions were performed using linear regression analysis.

Results

Lipophilicity and protein binding

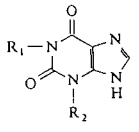
Table 2 shows the logarithmic partition coefficient, and plasma protein binding of each xanthine at concentrations of

Table 2. Lipophilicity and plasma protein binding of N^1, N^3-alkylxanthines.

Xanthine	Log P[a]	%Bound to rat plasma[b]
MX	−0·716 ± 0·051	23·90 ± 3·80
EX	−0·106 ± 0·019	39·40 ± 3·30
PX	0·331 ± 0·009	77·40 ± 2·00
BX	0·839 ± 0·023	78·60 ± 2·50
TPH	−0·042 ± 0·020	41·80 ± 3·90
MEX	0·516 ± 0·004	64·50 ± 1·00
MPX	1·022 ± 0·023	88·60 ± 1·50
MBX	1·286 ± 0·046	94·80 ± 0·04

Log P is the logarithmic partition coefficient in n-octanol. [a] Values represent the mean ± s.d. of three experiments at a concentration of 10 μg mL^{-1}. [b] Values represent the mean ± s.d. of in-vitro plasma protein binding of 4 rats at a concentration of 10 μg mL^{-1}.

Table 1. HPLC conditions of N^1, N^3-alkylxanthines.



Key	Xanthine	R_1	R_2	Mobile phase[a] (% CH_3CN)	Flow rate (mL min^{-1})	Temp. (°C)	I.S.[b]
1	MX	H	methyl	2	1·5	50	TPH
2	EX	H	ethyl	2	1·5	50	TPH
3	PX	H	propyl	12	1·0	40	8-Cl-TPH
4	BX	H	butyl	18	1·0	40	phenacetin
5	TPH	methyl	methyl	10	1·0	40	8-Cl-TPH
6	MEX	methyl	ethyl	12	1·0	40	8-Cl-TPH
7	MPX	methyl	propyl	18	1·0	40	phenacetin
8	MBX	methyl	butyl	18	1·0	40	phenacetin

[a] Mobile phase composed of acetonitrile in pH 4, 0·01 M sodium acetate. [b] Internal standard; TPH is theophylline; 8-Cl-TPH is 8-chlorotheophylline.

Table 3. Parameters describing plasma concentration-time profiles and distribution of N^1, N^3-alkylxanthines in rats.

Xanthine	Dose (mg kg^{-1})	V_{ss} (L kg^{-1})	$t_{\frac{1}{2}}$ (h)	AUC (mg h L^{-1})	MRT (h)
MX	5	0·78 ± 0·05	0·35 ± 0·00	2·99 ± 0·22	0·53 ± 0·01
EX	5	0·62 ± 0·06	0·32 ± 0·03	3·56 ± 0·30	0·44 ± 0·05
PX	5	0·31 ± 0·06	0·35 ± 0·05	8·60 ± 1·04	0·53 ± 0·10
BX	5	0·30 ± 0·02	0·73 ± 0·13	17·48 ± 3·04	1·06 ± 0·01
TPH	10	0·60 ± 0·02	2·79 ± 0·17	68·41 ± 2·68	4·19 ± 0·21
MEX	5	0·40 ± 0·08	0·95 ± 0·19	16·58 ± 3·56	1·19 ± 0·24
MPX	2·5	0·21 ± 0·03	0·43 ± 0·10	8·26 ± 1·58	0·68 ± 0·12
MBX	2·5	0·17 ± 0·00	0·32 ± 0·07	6·49 ± 1·39	0·45 ± 0·01

V_{ss}, $t_{\frac{1}{2}}$, AUC and MRT are volume of distribution at steady state, elimination half-life, area under the plasma concentration time curve and mean residence time, respectively. AUC and MRT were calculated using the trapezoidal rule with extrapolation to infinity. Values represent mean ± s.d. of 4–5 rats. V_{ss} is volume of distribution estimated using model-independent methods ($V_{ss} = CL \times MRT$).

10 μg mL^{-1}. Preliminary tests on concentration-dependent protein binding for all drugs in the series showed a modest change of percentage bound over the range 2·5–20 μg mL^{-1}, the plasma concentration range in the pharmacokinetic study. Binding to rat plasma within the series varied widely with values ranging from 24% for MX to 95% for MBX.

Pharmacokinetic studies

Pharmacokinetic data for each drug were obtained from 4–5 rats (Tables 3, 4). The plasma drug concentration-time profiles are shown in Fig. 1. As can be seen in Fig. 1, a one-compartment model adequately described the plasma concentration-time data for all drugs in the series and linear kinetics applied at the concentrations studied. The pharmacokinetics of MPX and MBX were studied at a dose of 2·5 mg kg^{-1} since preliminary tests showed capacity-limited elimination for MPX and MBX at a dose of 5 mg kg^{-1}. When normalized to the same dose, plasma concentrations at zero time (C_0) increased with increasing lipophilicity wheres V_{ss} decreased with lipophilicity, except for PX and MEX (Fig. 1, Table 3). Detection of unchanged drug in urine (f_e, Table 4) demonstrated that the first three lower cogeners (MX, EX, PX) were almost completely excreted unchanged whereas non-renal elimination is important for the higher congeners. Elimination parameters indicated that renal elimination decreases whereas non-renal elimination increases with increasing lipophilicity.

Table 4. Parameters defining the elimination of N^1,N^3-alkylxanthines in rats.

Xanthine	f_e[a]	Clearance (L h^{-1} kg^{-1}) CL[a]	CL_R	CL_{NR}	CL_R/f_u
MX	0·98 ± 0·04	1·68 ± 0·13	1·64	—	2·14
EX	0·95 ± 0·05	1·41 ± 0·13	1·33	—	2·16
PX	0·99 ± 0·02	0·59 ± 0·07	0·58	—	2·57
BX	0·55 ± 0·06	0·28 ± 0·05	0·16	0·12	0·75
TPH	0·37 ± 0·02	0·14 ± 0·00	0·05	0·09	0·09
MEX	0·07 ± 0·02	0·31 ± 0·08	0·02	0·29	0·06
MPX	N.D.[b]	0·37 ± 0·19	—	0·37	—
MBX	N.D.[b]	0·40 ± 0·07	—	0·40	—

[a] Values represent mean ± s.d. of 4–5 rats. [b] Not detected. f_e, CL, CL_R, CL_{NR} and f_u are fraction excreted unchanged in urine, total body clearance, renal clearance, non-renal clearance and unbound drug fraction in plasma, respectively. $CL_R = f_e$ dose/AUC. $CL_{NR} = CL - CL_R$.

Discussion

Lipophilicity and protein binding

Lipophilicity increases with increasing alkyl chain length both at N^1- and N^3-positions (Table 2). PX was thought to be less lipophilic than theophylline (Laursen et al 1988, 1989). The data from our study in the chloroform-buffer system indicated that the log P (chloroform) of PX is lower than that of TPH whereas in the n-octanol-buffer system, the log P (n-octanol) of PX is higher (Hasegawa et al 1990a).

Plasma protein binding of xanthine derivatives is affected by pH (Vallner et al 1979; Albary et al 1981; Hasegawa et al 1989). Binding of the higher congeners is also subject to displacement by free fatty acids (Hasegawa et al 1989). These effects were reduced by equilibrium dialysis with pH 7·4 buffer immediately after preparation of the drug-plasma solution. The pH of plasma before and after dialysis was close to the physiological pH (7·68 and 7·52, respectively). Although the binding data presented in this paper was obtained after correction for the change in volume, change in volume after dialysis was negligible (< 5%) and did not significantly affect the degree of binding for the drugs in this series.

As shown in Fig. 2, a linear relationship between percent of N^1,N^3-alkylxanthines bound to plasma protein and lipophilicity (log P) was observed ($r = 0·964$, $P < 0·01$). Similarly, a significant relationship was also found between logarithmic values of the ratio of fraction bound to fraction unbound in plasma ($\log f_b/f_u$) and log P ($r = 0·780$, $P < 0·05$, figure not shown). These are not unexpected, since binding to plasma protein simply reflects the partition of drug between a non-aqueous environment (mainly albumin) and an aqueous environment (plasma) and $\log f_b/f_u$ is the direct analogue of log P (Bird & Marshall 1967). The linear relationship between plasma protein binding and lipophilicity of other drug series such as barbiturates (Hansch et al 1967; Kakemi et al 1969; Toon & Rowland 1983), tetracyclines (Toon & Rowland 1979), penicillins (Bird & Marshall 1967) and sulphonamides (Seydel & Schapner 1981) has been reported.

The lipophilicity difference is greater when a carbon atom was added to the N^1-position than when it was added to the N^3-position of the same compound. Hansch et al (1965) suggested that the lower electron density on the nitrogen of the amine molecule reduces the chance of protonation of the

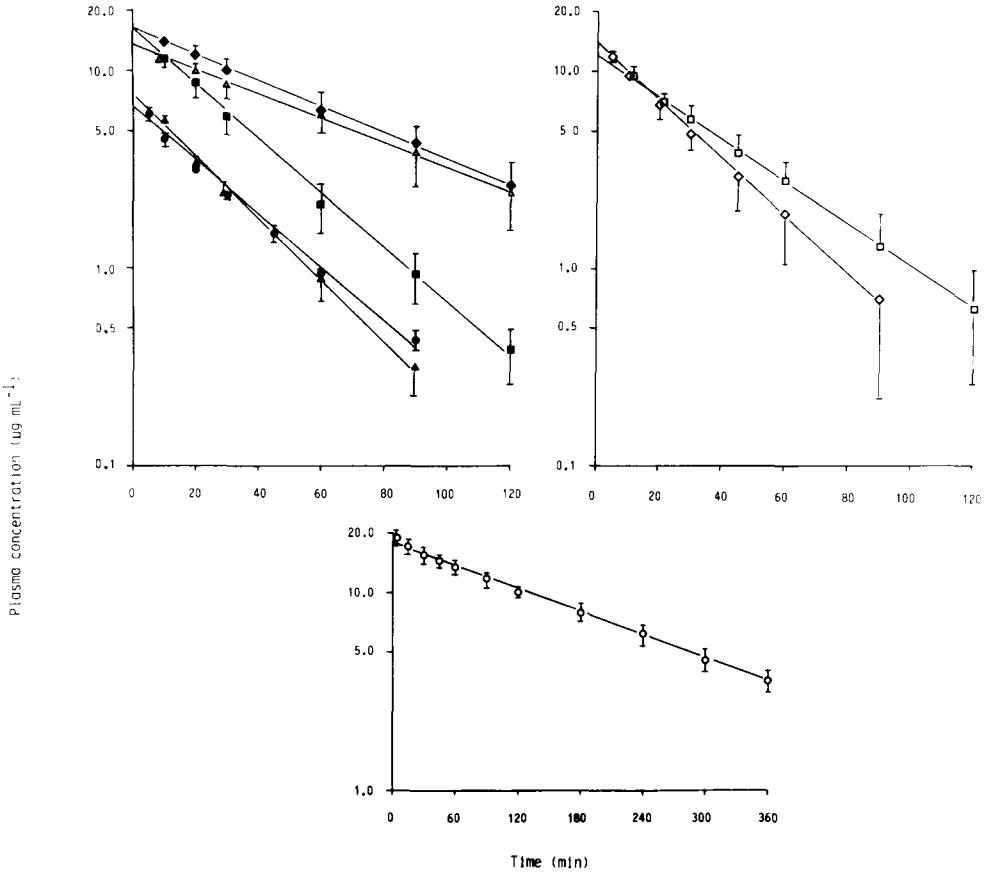


FIG. 1. Plasma drug concentration-time profiles after an i.v. bolus dose of each N^1, N^3-alkylxanthine. The doses were 10 mg kg^{-1} for TPH, 2·5 mg kg^{-1} for MPX and MBX, and 5 mg kg^{-1} for the others. The values are the mean ± s.d. of 4–5 rats. Key for symbols: ●, MX; ▲, EX; ■, PX; ◆, BX; ○, TPH; △, MEX; □, MPX; ◇, MBX.

nitrogen and hence the aqueous solubility of the amine. The substitution at both N^1- and N^3-positions thus reduces hydrophilicity of the xanthine by lowering the electron density on the nitrogen at both positions. Since N^1 is between two carbonyl groups, the electron density at N^1 would be lower than at N^3. The substitution of a methyl group at N^1 for hydrogen thus causes a greater effect. For protein binding the same trend as for lipophilicity applies except for MEX and PX. Plasma protein binding of TPH is similar in man (49%, Vallner et al 1979) and rats (42% in this study), whereas that of PX is different, i.e. 47% in man (Tegnér et al 1983) and 77% in rats (this study). It may be noted that, for higher congeners (PX and MPX, for example) the differences between affinity constants are large (Tegnér et al 1983; Apichartpichean et al 1989). However, the relationship between lipophilicity and plasma protein binding in man would be of the same trend as in rats although the order may be altered for some congeners.

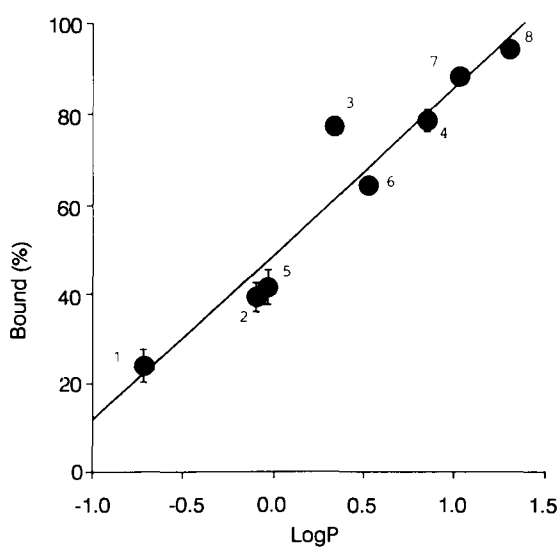


FIG. 2. Relationship between percentage of plasma protein binding and the lipophilicity (log P) for N^1, N^3-alkylxanthines. The values are the mean ± s.d. of 3 experiments. See Table 1 for key to the compounds.

Drug distribution

The volume of distribution at steady state (V_{ss}) ranged from 0·17 L kg^{-1} for MBX to 0·78 L kg^{-1} for MX (Table 3). V_{ss} was thought to increase directly with lipophilicity (Rischel & Hammer 1980), but an inverse relationship was found in this series of alkylxanthines (Fig. 3; $V_{ss} = -0{\cdot}316 \log P + 0{\cdot}548$; $r = -0{\cdot}960$, $P < 0{\cdot}01$). Lipophilicity may affect V_{ss} in various ways, depending on the specific nature of drug binding to plasma and tissue. For barbiturates, both plasma and tissue binding increased with increasing lipophilicity, resulting in a relative constancy of V_{ss} (Toon & Rowland 1983). For

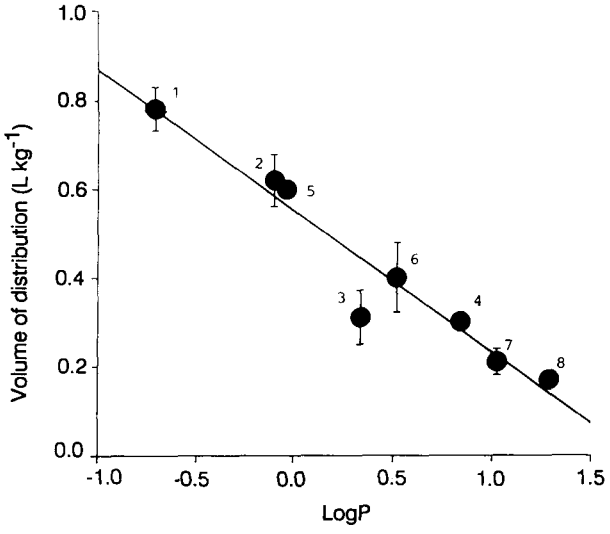


FIG. 3. Plot of volume of distribution at steady state and lipophilicity (log P) for N^1, N^3-alkylxanthines. See Table 1 for key to the compounds.

alkylxanthines, the binding was likely to be more specific to plasma protein than to tissue protein. A plot of V_{ss} versus f_u shows a highly significant correlation ($V_{ss}=0{\cdot}844\ f_u+0{\cdot}119$; $r=0{\cdot}999$, $P<0{\cdot}01$; Fig. 4). The intercept of 0·119 represents the V_{ss} of drug when $f_u=0$, which is equal to the sum of the volume of distribution of plasma albumin and extracellular albumin in rats. The volume of distribution for unbound drug (V_{uss}) may be calculated as $V_{uss}=(V_{ss}-0{\cdot}119)/f_u=0{\cdot}844$, which is constant throughout the series. This value is nearly equal to total body water in rats of 0·7 L kg^{-1} (Altman & Dittmer 1964a), indicating that the unbound drug distributes throughout body water. Oie & Tozer (1979) have developed a model of drug distribution which takes into account the known existence of plasma proteins within extracellular fluid outside of plasma. For drugs, such as

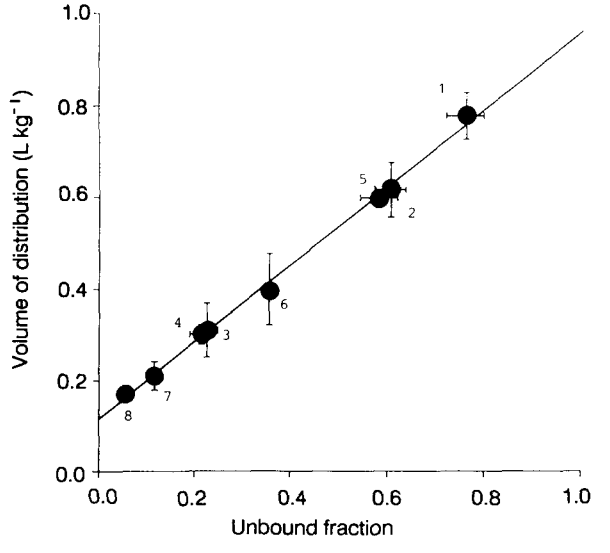


FIG. 4. Relationship between volume of distribution at steady state and unbound drug fraction in plasma for N^1, N^3-alkylxanthines. See Table 1 for key to the compounds.

alkylxanthines, which bind to albumin (Hasegawa et al 1989), the V_{ss} is related to f_u by the expression

$$V_{ss}=(V_E-R_{E/I}V_P+V_R/f_{uR})\ f_u+(1+R_{E/I})V_P \quad (1)$$

where V_P, V_E, V_R are the plasma volume, volume of extracellular fluid outside of plasma, and the rest of the fluid volume into which drug distributes, respectively; $R_{E/I}$ is the ratio of amount of binding protein in extracellular fluid outside of plasma to that in plasma, and f_{uR} is the fraction of drug unbound in the intracellular compartment. For the rat, $V_p=0{\cdot}04$ L kg^{-1}, $V_E=0{\cdot}26$ L kg^{-1} and $V_R=0{\cdot}40$ L kg^{-1} (Altman & Dittmer 1964b) as alkylxanthines distribute throughout body water. Furthermore, assuming that the distribution of albumin in the rat is the same as that in man, $R_{E/I}=1{\cdot}4$, upon rearrangement, equation 1 reduces to

$$V_{ss}=(0{\cdot}204+0{\cdot}40/f_{uR})f_u+0{\cdot}096 \quad (2)$$

Equation 2 is similar to the relationship of V_{ss} and f_u in Fig. 4, and the intercept, by assuming that $R_{E/I}=1{\cdot}4$, is nearly equal to the result from our study. From the similarity of the two equations, it may be assumed that f_{uR} of this series of alkylxanthines remains relatively constant. From the above assumption, it is likely that the free fraction in tissue, f_{uR}, is not related, at any instance, to lipophilicity, but rather is regulated by some other specific mechanism of the cell.

The tissue-to-plasma partition coefficent may be related to plasma and tissue unbound fraction by

$$K_P=C_R/C_P \quad (3)$$
$$f_u=C_u/C_P \quad (4)$$
$$f_{uR}=C_u/C_R \quad (5)$$
$$K_P=f_u/f_{uR} \quad (6)$$

where C_P and C_R are the total concentration of drug in and outside plasma, respectively and C_u is the unbound concentration of drug which is the same in all tissues including plasma, unless active transport occurs. Since f_{uR} is constant, it may be hypothesized that K_P correlates to f_u. The similar relationships between V_{ss} or K_p and f_u had been previously reported in a series of pyridonecarboxylic acids (Okezaki et al 1988).

Drug elimination

Excretion data (Table 4) show that for the first three lower congeners (MX, EX, PX), the kidney is the main elimination organ. For BX and TPH, elimination occurs almost equally by kidney and liver whereas the other higher congeners are eliminated mainly by the liver. A plot of total body clearance (CL) and log P shows a complex relationship (Fig. 5). With the exception of MX, EX, and PX, for which biotransformation may be negligible, the CL increases with increasing log P. The elimination half-life ($t_{\frac{1}{2}}$) decreases with increasing log P (Table 4). Non-renal clearance (CL_{NR}) increases with increasing log P. Renal clearance (CL_R) decreases with increasing log P, which may be due to increased tubular reabsorbtion. When corrected for protein binding, the unbound renal clearances of MX, EX, PX and BX (CL_R/f_u, Table 4) exceed the glomerular filtration rate in the rat of 0·24 L h^{-1} kg^{-1} (Orloff et al 1973), indicating that the lower congeners undergo active tubular secretion. Active tubular secretion of 3-MX and PX have been reported in man (Tang-Liu et al 1982; Borgå et al 1983, 1986, Lunell et al 1984) and in rats (Nadai et al 1991).

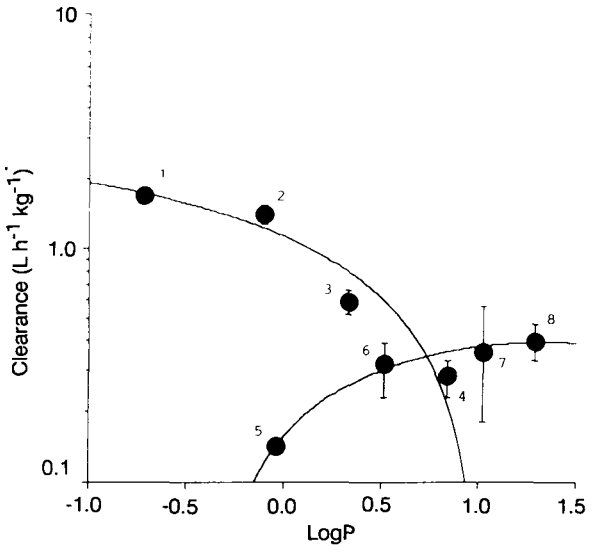


FIG. 5. Semilograthmic plot of total clearance against lipophilicity (log P) for N^1, N^3-alkylxanthines. See Table 1 for key to the compounds.

The biotransformation of methyl xanthines is catalysed by the cytochrome P450 mono-oxygenase system and leads to the formation of demethylated compounds or through oxidation to the urate and/or hydration to the diaminouracil (Bortolotti et al 1985). 3-Methylxanthine (3-MX) is an important metabolite of theophylline in man, but not in rat, and is not further metabolized (Jenne et al 1976; Lohmann & Miech 1976). It was reported that enprofylline (PX) is excreted unchanged (76–99%) after oral dosing in rats and because of the chemical resemblance to this metabolite it was hypothesized that PX might also be resistant to metabolism (Persson & Andersson 1977; Persson & Kjellin 1981). PX was also excreted almost unchanged in man (Lunell et al 1982; Borgå et al 1983, 1986). EX with its resemblance to both MX and PX, therefore, is eliminated by the same process. Our data for urine excretion supports this hypothesis, the fraction excreted unchanged (f_e) being almost equal to 1 for MX, EX, and PX (Table 4). Furthermore, statistical analysis of elimination rate constants (K) and half-lives ($t_{\frac{1}{2}}$) shows no significant differences among these three drugs. The total clearance (CL) or assumingly the renal clearance (CL_R) of MX, EX and PX was different due to their different degrees of plasma protein binding. The unbound clearances are similar among the three drugs.

BX, despite its resemblance to PX, was excreted only partially (55%) in urine. It may be concluded from the excretion data that, at N^3-position, only the substituents greater than propyl may be metabolized, via dealkylation and oxidation. According to Bergmann & Dikstein (1956), oxidation of xanthine at C^8 by xanthine oxidase is prevented by substitution at N^3, N^7, or N^9, but proceeds normally with the N^1-methyl derivative and the N^3, N^7, N^9 must be free for combination with xanthine oxidase. Furthermore, they suggested that only the 1-methyl derivative can be produced by direct action of xanthine oxidase and all other substituted uric acids must result from a different biochemical pathway. This may be the reason that MX, EX, PX and theobromine (3,7-dimethylxanthine) do not lead to uric acid metabolites in the urine. However, C^8-oxidation by microsomal systems in the presence of N^3-substitution is an important metabolic pathway of TPH in man and rats. Only 37% TPH, with the same molecular weight and similar lipophilicity as EX, was excreted in urine. Considering the structures of the whole series indicates that the presence of a methyl group at the N^1-position may contribute to a higher degree of biotransformation. Since N^3- demethylation and C^8-oxidation are negligible for MX, both in man (Tang-Liu et al 1983) and rats, it may be hypothesized that biotransformation by liver microsomal system and xanthine oxidase may be enhanced by the presence of N^1-substitution, as in TPH and caffeine. The effect of the N^1-methyl group on renal elimination and biotransformation may be explained by several mechanisms. It is possible that N^1-substitution prevents active renal secretion and hence provides a longer residence time for the substrate to be attacked by liver microsomal enzymes. According to Hansch et al (1965) the lower the electron density on the nitrogen of the amine molecule the greater the lipid solubility and the demethylation rate; the N^1-substitution may lower the electron density on both N^1- and N^3-nitrogen, enhancing lipid solubility, N^3-demethylation and/or the oxidation process. Gaudette & Brodie (1959) showed that the oxidative dealkylation of foreign *N*-alkylamines, including caffeine, theophylline and theobromine, by rabbit liver microsomes appeared to be limited to compounds which are lipid soluble as shown by high chloroform to water partition coefficients at physiological pH. Those authors suggested that the microsomal oxidative systems are protected by a lipid barrier which only fat soluble substances can penetrate or that the active sites on the microsomal enzymes can interact only with non-polar substances. McMahon & Easton (1961) studied the demethylation of amine derivatives by rat liver microsomes in-vitro and pointed out that the more lipophilic amines were, the more rapidly they were demethylated. Hansch et al (1965) found a high correlation of the logarithmic demethylation rate from the work of McMahon (1961) and McMahon & Easton (1961) with the logarithmic octanol–water partition coefficient. These observations may also apply for the N^3-dealkylation of the N^1, N^3-alkylxanthines. N^3-Dealkylation may lead to C^8-oxidation by xanthine oxidase. Furthermore, it is likely that the larger alkyl substituent will be subjected preferentially to metabolic attack, the smaller being sterically protected, as in barbiturates (Toon & Rowland 1983). The above observations may only partly explain the structure-lipophilicity-biotransformation relationships of these xanthine derivatives since the metabolic pathways of BX, MEX, MPX, and MBX remain unclear. Our previous studies (Hasegawa et al 1990b; Nadai et al 1991) have shown that enoxacin decreased the clearance of MPX and TPH in rats but did not increase the clearance of PX; its metabolite, 4-oxo-enoxacin, thought to competitively inhibit the elimination of theophylline via cytochrome P450 isozymes for *N*-demethylation (Beckmann et al 1987) had less effect on the pharmacokinetics of TPH and MPX.

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