0022-3565/89/2481-0315$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 248, No. 1
Printed in U.S.A.

Paraxanthine Metabolism in Humans: Determination of Metabolic Partial Clearances and Effects of Allopurinol and Cimetidine

AZNAN LELO, GUNNAR KJELLEN, DONALD J. BIRKETT and JOHN O. MINERS

Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, Adelaide, South Australia, Australia (A.L., D.J.B., J.O.M.) and Draco AB, Lund, Sweden (G.K.)

Accepted for publication September 6, 1988

ABSTRACT

Paraxanthine (PX; 1,7-dimethylxanthine) is the major metabolite of caffeine in humans. Despite the continuous exposure of a large proportion of the population to PX, little is known about PX disposition in humans. The present study was performed to define the metabolic partial clearances of PX in humans and, by determining the effects of cimetidine and allopurinol pretreatments on PX disposition, assess the relative importance of cytochrome P-450 and xanthine oxidase in PX biotransformation. The combined formation of the 7-demethylated products 1-methylxanthine (1-MX), 1-methyluric acid (1-MU) and 5-acetylamino-6-formylamino-3-methyluracil (AFMU) accounted for 67% of PX clearance. Formation of 7-methylxanthine (7-MX) and 1,7-dimethyluric acid and renal excretion of unchanged PX comprised 6, 8 and 9% of PX clearance, respectively. Allopurinol pretreatment had no effect on PX plasma clearance but decreased 1-MU excretion and increased 1-MX excretion, with the combined excretion of these metabolites remaining constant. Cimetidine pretreatment decreased PX plasma clearance by 30%. Metabolic partial clearances to 1-MX + 1-MU and to AFMU were reduced to a similar extent (*ca.* 40%) in the cimetidine treatment phase, but other pathways were not significantly affected. These data are consistent with 1-MX and AFMU being derived from a common intermediate, the formation of which is mediated by cytochrome P-450. Xanthine oxidase catalyzes only the secondary conversion of 1-MX to 1-MU.

PX is a dimethylxanthine which differs structurally from TB and TP only in the position(s) of N-methyl substitution. A recent study (Lelo *et al.*, 1986b) by this group demonstrated that PX is the major metabolite of CA in humans, with PX formation accounting for 80% of CA metabolic clearance. Moreover, we have demonstrated (Lelo *et al.*, 1986c) that appreciable concentrations (up to 2 mg/l) of PX are detectable in the plasma of coffee and tea drinkers. Thus, normal CA consumers are exposed continuously to PX. It is possible that PX may contribute to the pharmacological effects of CA but, with the exception of the reported use of PX as an antiasthmatic agent in the 19th century (Salter, 1859), there is no information relating to the effects of PX in humans. Available evidence (Bortolotti *et al.*, 1985) suggests that PX is more toxic than CA in the rat.

There is also little information relating to the disposition of PX. The pharmacokinetics of PX in human volunteers have been described recently (Lelo *et al.*, 1986a) and 1-MX, 1-MU, 7-MX, 7-MU, 1,7-DMU, and 1,7-DAU (the dimethylaminouracil metabolite) have all been identified in the urine of subjects administered PX (Arnaud and Welsch, 1980). Along with 1-MX and 1-MU the acetylated uracil derivatives AFMU and AAMU have been identified as major metabolites of CA, and therefore presumably of PX (Callahan *et al.*, 1982, 1983; Grant *et al.*, 1983a,b). AAMU was subsequently shown to be a decomposition product of AFMU; AFMU irreversibly deformylates in urine, spontaneously or by the action of dilute base of methanol (Tang *et al.*, 1983). Further studies (Grant *et al.*, 1983b, 1984) indicated that AFMU formation is mediated by the polymorphic N-acetyltransferase. Inasmuch as the urinary molar ratio of AFMU to 1-MX after CA administration appears to provide a measure of genetic polymorphism in acetylation capacity it has been postulated (Grant *et al.*, 1984) that the substrate for N-acetyltransferase is a ring-opened intermediate which in fast acetylators is converted rapidly to AFMU but ring-closes in slow acetylators to form 1-MX. On the basis of these data the scheme shown in figure 1 has been proposed for PX biotransformation in humans.

Although it appears that N-acetyltransferase is involved in AFMU formation, little is known about the other enzymes

Received for publication February 18, 1988.

ABBREVIATIONS: PX, paraxanthine (1,7-dimethylxanthine); TB, theobromine (3,7-dimethylxanthine); TP, theophylline (1,3-dimethylxanthine); CA, caffeine (1,3,7-trimethylxanthine); MX, methylxanthine; MU, methyluric acid; DMU, dimethyluric acid; DAU, 6-amino-5-(N-formylmethylamino)-3-methyluracil; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AAMU, 5-acetylamino-6-amino-3-methyluracil; HPLC, high-performance liquid chromatography.

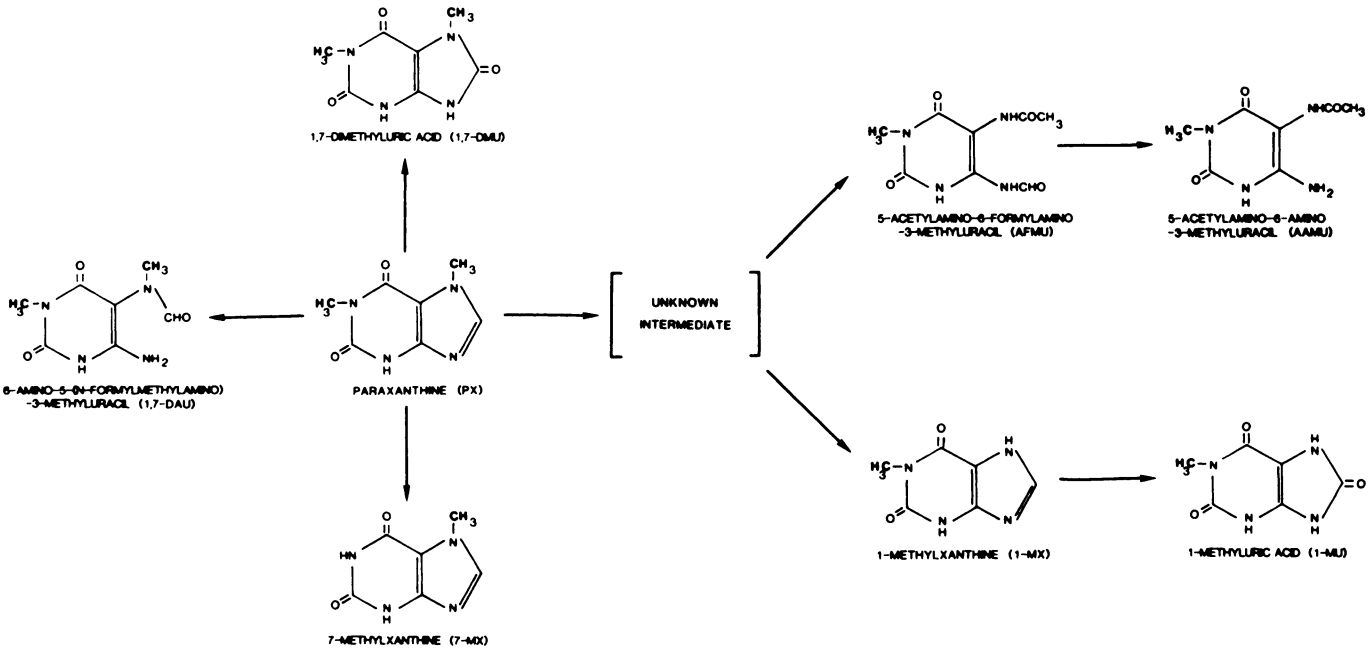

Fig. 1. Proposed scheme for PX biotransformation in humans. After Arnaud and Welsch (1980) and Grant *et al.* (1983a, 1984).

involved in PX metabolism. Inhibitors and inducers of the microsomal mixed function oxidase system have been particularly useful for demonstrating the involvement of cytochrome P-450 in the *in vivo* metabolism of CA, TB and TP (Birkett *et al.*, 1983a; Grygiel *et al.*, 1984; May *et al.*, 1982; Miners *et al.*, 1985a,b). Similarly, allopurinol has been instrumental in demonstrating the role of xanthine oxidase in the *in vivo* metabolism of 1-MX and 7-MX, biotransformation products of TP and TB, respectively (Grygiel *et al.*, 1979; Miners *et al.*, 1982). The aims of the present study were to determine the metabolic partial clearances of PX and to investigate the effects of allopurinol and cimetidine on PX metabolism in healthy volunteers to assess the relative importance of cytochrome P-450 and xanthine oxidase in PX biotransformation in humans.

Methods

Chemicals and drugs. 1-MU, 1-MX, 7-MU and 7-MX were purchased from Fluka AG (Buchs, Switzerland) and 1,7-DMU was obtained from the Adams Chemical Co. (Round Lake, IL). AAMU was synthesized according to the general method of Khmelevskii *et al.* (1958) using 1-MU as starting material. Allopurinol and cimetidine were administered in the form of Zyloprim (Wellcome Research Laboratories, Tuckahoe, NY) and Tagamet (Smith Kline and French Laboratories, Philadelphia, PA), respectively. PX was obtained from the Sigma Chemical Co. (St. Louis, MO). The PX used was shown to be > 99.8% pure by elemental analysis (C, H, N), melting point and HPLC (Lelo *et al.*, 1986a). Because PX is not an approved substance for use in humans, permission was obtained from the Australian Department of Health for its use in this study.

Protocol. Six healthy male nonsmokers between the ages 19 to 24 yr (mean ± S.D., 20.8 ± 1.0 yr) and with bodyweights in the range 63 to 82 kg (74.5 ± 7.6 kg) participated in the study. Subjects were healthy as determined by medical history, physical examination and standard biochemical and hemotological parameters. The subjects received no medications, other than those required for the study, for 2 weeks before and during the study. In addition, subjects abstained from all methylxanthine-containing foods and beverages for 4 days before and during the study. The study was approved by the Clinical Investigation and Drug and Therapeutic Advisory Committees of Flinders Medical Centre.

Subjects fasted from the evening before and until 4 hr after drug administration on each study day. A randomized three-way crossover study design was used, with 3 weeks separating each of the study days. In the control phase, subjects received a single p.o dose of PX, 250 mg (freshly prepared in 150 ml of orange juice). Subjects also received the same PX dose on day 7 of a 1-week treatment period with allopurinol, 100 mg every 8 hr and on day 7 of a 1-week treatment period with cimetidine, 400 mg every 8 hr. On each study day the dose of allopurinol or cimetidine was administered at the same time as the PX. Blood samples were collected *via* an indwelling cannula inserted in a forearm vein before and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hr after the PX dose. A blank urine sample was also collected before the PX dose and then a complete urine collection was performed for 24 hr after the PX dose. The separated plasma samples were stored at −20°C until analyzed but urine samples were assayed on the day of collection.

Analytical procedures. Plasma and urinary concentrations of PX were measured by a previously published (Lelo *et al.*, 1986b) HPLC procedure. AFMU was converted to AAMU by alkaline hydrolysis of urine and then quantitated by high performance exclusion chromatography (Tang *et al.*, 1986). Because AFMU deformylates quantitatively to AAMU (Tang *et al.*, 1986), results throughout this paper are expressed in terms of AFMU formation. Urinary concentrations of 1-MU, 1-MX, 7-MU and 1,7-DMU were measured by a modification of the published HPLC method for the determination of urinary metabolites of TB in humans (Miners *et al.*, 1982). Urine was injected directly onto an Altrex Ultrasphere C_{18} reversed-phase column (15 cm × 4.6 mm inside diameter; Beckman, Sydney, Australia) and eluted with 0.01 M acetate buffer, pH 5.2, at a flow rate of 2.0 ml/min. Under these conditions the retention times of 7-MU, 1-MU, 7-MX, 1-MX and 1,7-DMU were 6.7, 9.1, 13.1, 16.4 and 48.4 min, respectively. 1,3-DMU, added as internal standard, eluted with a retention time of 33.5 min. Peaks for each metabolite and internal standard were resolved from endogenous urine constituents but, immediately after 1,7-DMU eluted from the column, 0.25 ml of methanol was injected to facilitate the removal of PX and other lipophilic compounds. Quantitation was achieved using photometric detection at 275 nm. Unknown concentrations of each metabolite were determined by comparison of peak height ratios (to the internal standard) with those of calibration curves in the

range of 5 to 100 mg/l. There was an excellent correlation ($r = 0.98$) between 1-MX concentrations determined by the direct injection HPLC procedure and the high performance exclusion chromatographic method (Tang *et al.*, 1986), which also quantitates 1-MX. The limit of assay sensitivity for each metabolite, defined as the concentration which gave a peak height of analyte to background ratio of 10:1, was 2.5 mg/l. Mean intra- and interassay coefficients of variation for each metabolite were < 8%.

Analysis of results. Area under the PX plasma concentration curve (AUC) for each subject in each study phase was determined by the trapezoidal rule with extrapolation to infinite time. Elimination half-life ($T_{½}$) was calculated from the slope of the terminal portion of the plasma concentration-time curve by linear least-squares regression and volume of distribution by a model-independent procedure (Benet and Galeazzi, 1979). Apparent p.o. clearance (CL) of PX was determined as:

$$CL = D/(\text{AUC} \times b.wt.),$$

where D is the PX dose and $b.wt.$ the subject body weight. When the likely formation of 1,7-DAU is taken into account, the recovery of PX and its metabolites in the various study phases was essentially quantitative (see table 2 and "Results") and PX absorption was therefore considered to be complete for the purpose of the calculation of CL. Partial metabolic and renal clearances of PX were calculated as:

$$CL_m = f_m \times CL,$$

where f_m is the recovery of each metabolite or unchanged PX in urine as a fraction of the administered dose.

All results are expressed as mean ± S.D. Differences among the group means from each parameter were examined by analysis of variance, with the Newman-Keuls test being used to detect differences between individual study phases. Correlations between parameters were determined by linear regression analysis.

Results

The effects of allopurinal and cimetidine pretreatments on the pharmacokinetic parameters of PX are summarized in table 1. Representative plasma concentration-time profiles from one subject in each of the study phases are illustrated in figure 2. Allopurinol had no significant effect on any of the PX pharmacokinetic parameters. Cimetidine pretreatment significantly reduced PX total plasma clearance (by 30%) and prolonged PX elimination $T_{½}$ (by 41%) but did not alter volume of distribution.

Table 2 summarizes the effects of the allopurinol and cimetidine pretreatments on urinary PX metabolite excretion. The only metabolite profiles to show any significant changes were those of 1-MX and 1-MU in the allopurinol treatment phase. Allopurinol pretreatment enhanced the excretion of 1-MX and decreased the excretion of 1-MU compared to both the control and cimetidine phases. Similarly, when the excretion data are considered in terms of ratios, AFMU:1-MX in the allopurinol phase (0.34 ± 0.24) was significantly lower ($P < .01$) than in the control (0.92 ± 0.61) and cimetidine (0.83 ± 0.53) phases whereas AFMU:1-MU was significantly higher ($P < .05$) in the allopurinol phase (0.67 ± 0.45) compared to the control (0.48 ± 0.36) and cimetidine (0.40 ± 0.23) phases. However, the combined excretion of 1-MX + 1-MU (and that of 1-MX + 1-MU + AFMU) was not altered by allopurinol treatment and the ratio of AFMU to 1-MX + 1-MU (0.30 ± 0.23, 0.23 ± 0.16 and 0.27 ± 0.16 in the control, allopurinol and cimetidine phases, respectively) was not significantly altered by any of the treatments. There was a significant correlation ($r = -0.88$, P

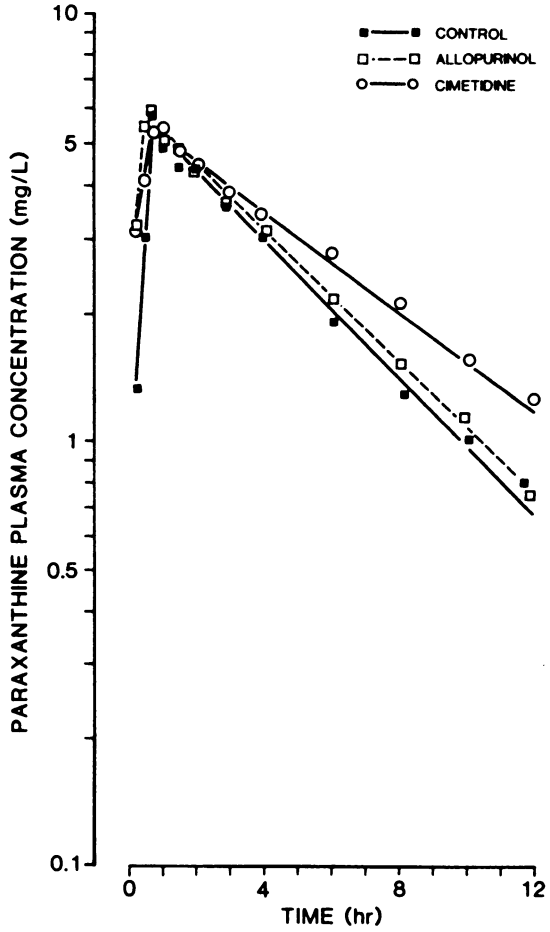

Fig. 2. Plasma PX concentration-time curves from a representative subject after p.o. administration of PX (250 mg) alone and after separate pretreatments with allopurinol and cimetidine. The first data point shown on the plots is 0.25 hr postdose; PX was not detected in the blank plasma of any of the subjects in any of the study phases.

TABLE 1

Effects of allopurinol and cimetidine pretreatments on the pharmacokinetic parameters of PX

Pharmacokinetic Parameter	Treatment Phase		
	Control	Allopurinol	Cimetidine
Clearance (ml/min/kg)	1.71 ± 0.30	1.67 ± 0.51	1.19 ± 0.23*
$T_{½}$ (hr)	3.9 ± 0.7	4.2 ± 1.1	5.5 ± 1.1*
Volume of distribution (l/kg)	0.60 ± 0.06	0.60 ± 0.10	0.58 ± 0.07

* $P < .05$ compared to both other treatment phases.

TABLE 2

Effects of allopurinol and cimetidine pretreatments on the urinary excretion of PX and PX metabolites

Results expressed as percentage of dose administered.

Metabolite(s)	Treatment Phase		
	Control	Allopurinol	Cimetidine
1-MX	17.8 ± 4.1	35.0 ± 7.2*	15.0 ± 3.1
1-MU	34.8 ± 10.1	18.5 ± 4.6*	30.4 ± 5.7
AFMU	14.2 ± 5.4	10.6 ± 4.5	11.2 ± 4.5
AFMU + 1-MX + 1-MU	66.8 ± 9.0	64.1 ± 8.8	56.6 ± 4.3
7-MX	5.2 ± 2.9	8.2 ± 4.3	4.8 ± 2.3
1,7-DMU	7.7 ± 0.8	6.1 ± 0.9	10.5 ± 1.1
PX	9.0 ± 3.3	10.3 ± 4.8	11.4 ± 3.0
Total	88.7 ± 2.2	88.7 ± 5.9	84.4 ± 3.7

* $P < .01$ compared to both other treatment phases.

< .01) between the excretion of AFMU and that of 1-MX + 1-MU using combined data from the three study phases. The total recovery of paraxanthine-derived products ranged from 84.4 to 88.7% (table 2) and when the likely formation of 1,7-DAU is taken into account (see "Discussion"), recovery of PX and its metabolites can be considered to be essentially quantitative.

The partial renal and metabolic clearances of PX are summarized in table 3. Neither the allopurinol nor cimetidine pretreatment significantly altered renal clearance of unchanged drug nor the metabolic clearances to 7-MX and 1,7-DMU. Cimetidine pretreatment reduced the metabolic clearances to 1-MX + 1-MU and to AFMU by a similar extent (41 and 44%, respectively), although the reduction in clearance to AFMU was only of marginal statistical significance (P = .10). The reduction in the combined metabolic clearance to AFMU + 1-MX + 1-MU in the cimetidine treatment phase was statistically significant (P < .01). Metabolic clearance to 1-MX + 1-MU and to AFMU (or the combination of these) in the allopurinol treatment phase were not significantly different to control values.

Discussion

This is the first study to define the partial metabolic clearances of PX in humans and to assess the involvement of cytochrome P-450 and xanthine oxidase in PX metabolism in humans *in vivo*. Grant *et al.* (1986) determined the effect of allopurinol on the excretion pattern of PX-derived metabolites in two human volunteers, but this was after administration of CA. Apart from the preliminary report of PX metabolism by Arnaud and Welsch (1980) and the pharmacokinetic study by this group (Lelo *et al.*, 1986a) no other investigations of PX disposition in humans appear to have been published, despite the continuous exposure of CA-consumers to PX (Lelo *et al.*, 1986b,c).

Metabolic partial clearance and urinary excretion data from the control phase of the present study indicate that N7-demethylation to form 1-MX, 1-MU and AFMU (assuming these are derived from a common intermediate; see later) is the major biotransformation pathway of PX, accounting for 67% of PX clearance. Renal clearance of unchanged PX and the N1-demethylation (to form 7-MX) and C8-oxidation (to form 1,7-DMU) pathways in total account for 22% of PX clearance. Other, unaccounted pathways presumably account for the remaining fraction of PX clearance. 1,7-DAU formation has been reported (Arnaud and Welsch, 1980) to comprise at least 4% of urinary PX metabolites; this compound was not measured in the present study as an authentic standard was not available.

TABLE 3

Effects of allopurinol and cimetidine pretreatments on the renal and metabolic partial clearances of PX

Clearance to:*	Treatment Phase		
	Control	Allopurinol	Cimetidine
1-MX + 1-MU	0.89 ± 0.26	0.90 ± 0.35	0.53 ± 0.12*
AFMU	0.25 ± 0.11	0.19 ± 0.10	0.14 ± 0.06
AFMU + 1-MX + 1-MU	1.14 ± 0.25	1.08 ± 0.42	0.67 ± 0.14*
7-MX	0.10 ± 0.06	0.14 ± 0.09	0.06 ± 0.04
1,7-DMU	0.13 ± 0.03	0.11 ± 0.04	0.14 ± 0.04
PX	0.15 ± 0.05	0.15 ± 0.03	0.13 ± 0.01

* Units of clearance, milliliters per minute per kilogram.
* P < .01 compared to both other treatment phases.

The urinary excretion profile of metabolites in the control phase of the present study is qualitatively similar to that reported by Arnaud and Welsch (1980), although there are quantitative differences in PX metabolite excretion between the two studies. In particular, approximately 50% of the PX dose was excreted as 1-MX + 1-MU in the present study whereas these metabolites accounted for 32% of the PX dose in the study reported by Arnaud and Welsch. The reason for the differences between the two studies is not clear, although obviously two different population groups (Australian and Swiss) were investigated. Moreover, details of sex, concomitant drug therapy, etc. for the subjects who participated in the Arnaud and Welsch study were not reported and the influence of such variables on PX metabolism cannot be discounted. It should be noted that 1-MX was measured independently by two procedures in the present study (see "Methods") and the agreement between the separate determinations was excellent. The excretion of PX-derived products has also been studied after CA administration (Callahan *et al.*, 1982, 1983; Grant *et al.*, 1983a,b, 1984, 1986) but it is not strictly valid to compare the excretion of monomethyl xanthines and urates between these studies and the present study as such compounds may be derived from monodemethylated metabolites of CA other than PX (*i.e.*, TB and TP). However, the mean urinary excretion of AFMU after PX administration reported here (*viz.* 14%) was similar to that (17%) after CA administration (Callahan *et al.*, 1982). Interestingly, the ratio of 1-MU to 1-MX excreted after PX administration is approximately 2 whereas the ratio after 1-MX (Birkett *et al.*, 1983b), CA (Callahan *et al.*, 1983) and TP (Grygiel *et al.*, 1979) administration is approximately 3.4, 1.3 and 11, respectively. It is unclear why the extent of 1-MX C8-oxidation should vary so markedly after administration of the various methylxanthines.

Allopurinol pretreatment did not alter the clearance (or any other pharmacokinetic parameter) of PX and did not affect metabolic partial clearances to AFMU, 1-MX + 1-MU, 7-MX or 1,7-DMU. These data indicate that xanthine oxidase is not involved in the primary biotransformation of PX. Allopurinol treatment, however, decreased 1-MU excretion markedly and enhanced 1-MX excretion without changing the combined recovery of these metabolites. These findings are consistent with the preliminary report of Grant *et al.*, (1986) which showed that allopurinol also inhibits the conversion of 1-MX to 1-MU after CA administration and indicate that 1-MU is formed by the xanthine oxidase catalysed C8-oxidation of 1-MX. Thus, the 1-MU to 1-MX ratio after CA administration may well provide a simple index of xanthine oxidase activity in humans *in vivo*. Allopurinol treatment at a dose of 300 mg/day has been shown previously not to affect the plasma clearances of TB (Miners *et al.*, 1982) and TP (Grygiel *et al.*, 1979), although allopurinol inhibited the secondary conversion of 1-MX to 1-MU and 7-MX to 7-MU in the TP and TB metabolic studies, respectively. The clearances of the dimethylxanthines PX, TB and TP are therefore dependent on enzyme systems other than xanthine oxidase, but xanthine oxidase is involved in the oxidation of secondary biotransformation products (7-MU was not identified in the urine of volunteers administered PX, presumably due to limitations of assay sensitivity rather than nonformation).

Cimetidine pretreatment resulted in a 30% reduction in PX total plasma clearance and a 40% prolongation in PX elimination $T_{1/2}$. The extent of inhibition of PX clearance due to

cimetidine is similar in magnitude to the effect of cimetidine on the clearances of CA (May *et al.*, 1982), TB (Miners *et al.*, 1985a) and TP (Grygiel *et al.*, 1984). Metabolic clearances to 1-MX + 1-MU and to AFMU were reduced to a similar extent (41–44%) during the cimetidine phase. Although the decrease in clearance to AFMU was only of marginal statistical significance, these data are nevertheless consistent with both AFMU and 1-MX being derived from a common intermediate whose formation is inhibited by cimetidine. Pretreatment with cimetidine did not significantly alter PX metabolic clearance by C8-oxidation (*i.e.*, 1,7-DMU formation) or by N1-demethylation (*i.e.*, 7-MX formation). However, it should be noted that there was a large degree of variability in clearance by N1-demethylation and this study only had the power to detect greater than 60% inhibition of this pathway.

Overall, data obtained in the present study are consistent with the metabolic scheme for PX initially proposed by Grant *et al.* (1984) and shown in figure 1. AFMU and 1-MX are apparently derived from a common intermediate, the formation of which is mediated by cytochrome P-450. Grant *et al.* (1984) have postulated that the intermediate is a ring-opened compound; whereas the structure of the putative intermediate has not been elucidated, it would seem that 5-amino-6-formylamino-3-methyluracil is necessarily the substrate for the N-acetyltransferase involved in AFMU formation (Grant *et al.*, 1983b, 1984). Neither AFMU nor AAMU have been identified in the urine of subjects administered 1-MX (J. O. Miners, unpublished results) and these compounds have not been identified as metabolites of TP. Thus, events during or proceeding PX N7-demethylation would appear to be essential for N7-C8 bond scission and AFMU formation. Data presented here also indicate that xanthine oxidase is not involved in the C8-oxidation of PX to form 1,7-DMU. Available evidence (Birkett *et al.*, 1983b; Grygiel *et al.*, 1984) indicates that cytochrome P-450 rather than xanthine oxidase mediates the C8-oxidation of TP to form 1,3-DMU. Despite the fact that cimetidine did not significantly inhibit the conversion of PX to 1,7-DMU or 7-MX, it would still seem most likely that cytochrome P-450 is the enzyme involved in these reactions. Indeed, cimetidine has been shown previously (Grygiel *et al.*, 1984; Miners *et al.*, 1985a) to differentially affect the various pathways of TP and TB metabolism in humans. If 1,7-DMU and 7-MX formation are, as expected, mediated by cytochrome P-450 then the differential effect of cimetidine on these pathways and on 1-MX + 1-MU + AFMU formation suggests the involvement of at least two isozymes of cytochrome P-450 in PX metabolism. There is some evidence from kinetic studies in human liver microsomes (Campbell *et al.*, 1987) to support the involvement of at least two forms of cytochrome P-450 in PX biotransformation.

It has been proposed by Grant *et al.* (1983b, 1984) that the molar ratio of AFMU to 1-MX in urine after CA administration provides a measure of acetylation capacity. These authors have demonstrated concordance in 20 subjects between the AFMU to 1-MX ratio and to sulphamethazine acetylation. However, the present study has demonstrated clearly that 1-MX is substantially converted to 1-MU by xanthine oxidase and the AFMU to 1-MX ratio is therefore likely to be dependent on both N-acetyltransferase and xanthine oxidase activities. Indeed, the AFMU to 1-MX ratio was reduced from 0.91 in the control phase to 0.34 in the allopurinol treatment phase. By contrast, the AFMU to 1-MX + 1-MU was minimally affected by allopurinol treatment. Thus, it would appear to be more valid use the AFMU to 1-MX + 1-MU ratio in the CA acetylation phenotyping test, as proposed recently by Tang *et al.* (1987).

Acknowledgment

The authors are grateful to Dr. B. K. Tang for providing a preprint of the AFMU analysis paper.

References

ARNAUD, M. J. AND WELSCH, C.: Caffeine Metabolism in Human Subjects, IX International Colloquium on Science and Technology of Coffee, Association Scientifique Internationale du Cafe, London, pp. 385–395, 1980.

BENET, L. Z. AND GALEAZZI, R. L.: Noncompartmental determination of the steady-state volume of distribution. J. Pharmacol. Sci. **68:** 1071–1074, 1979.

BIRKETT, D. J., MINERS, J. O. AND ATTWOOD, J.: Evidence for a dual action of sulphinpyrazone on drug metabolism in man: Theophylline-sulphinpyrazone interaction. Br. J. Clin. Pharmacol. **15:** 567–569, 1983a.

BIRKETT, D. J., MINERS, J. O. AND ATTWOOD, J.: Secondary metabolism of theophylline biotransformation products in man-route of formation of 1-methyluric acid. Br. J. Clin. Pharmacol. **15:** 117–119, 1983b.

BORTOLOTTI, A., JIRITANO, L. AND BONATI, M.: Pharmacokinetics of paraxanthine, one of the primary metabolites of caffeine, in the rat. Drug Metab. Dispos. **13:** 227–231, 1985.

CALLAHAN, M. M., ROBERTSON, R. S., ARNAUD, M. J., BRANFMAN, A. R., MCCOMISH, M. F. AND YESAIR, D. S.: Human metabolism of [1-methyl-^{14}C]- and [2-^{14}C]-caffeine after oral administration. Drug Metab. Dispos. **10:** 417–423, 1982.

CALLAHAN, M. M., ROBERTSON, R. S., BRANFMAN, A. R., MCCOMISH, M. F. AND YESAIR, D. W.: Comparison of caffeine metabolism in three non-smoking populations after oral administration of rabiolabeled caffeine. Drug Metab. Dispos. **11:** 211–217, 1983.

CAMPBELL, M. E., GRANT, D. M., INABA, T. AND KALOW, W.: Biotransformation of caffeine, paraxanthine, theophylline and theobromine by polycyclic aromatic hydrocarbon-inducible cytochromes P-450 in human liver microsomes. Drug Metab. Dispos. **15:** 237–249, 1987.

GRANT, D. M., TANG, B. K. AND KALOW, W.: Variability in caffeine metabolism. Clin. Pharmacol. Ther. **33:** 591–601, 1983a.

GRANT, D. M., TANG, B. K. AND KALOW, W.: Polymorphic N-acetylation of a caffeine metabolite. Clin. Pharmacol. Ther. **33:** 355–359, 1983b.

GRANT, D. M., TANG, B. K. AND KALOW, W.: A simple test for acetylator phenotype using caffeine. Br. J. Clin. Pharmacol. **17:** 459–464, 1984.

GRANT, D. M., TANG, B. K., CAMPBELL, M. E. AND KALOW, W.: Effect of allopurinol on caffeine disposition in man. Br. J. Clin. Pharmacol. **21:** 454–458, 1986.

GRYGIEL, J. J., MINERS, J. O., DREW, R. AND BIRKETT, D. J.: Differential effects of cimetidine on theophylline metabolic pathways. Eur. J. Clin. Pharmacol. **26:** 335–340, 1984.

GRYGIEL, J. J., WING, L. M. H., FARKAS, J. AND BIRKETT, D. J.: Effects of allopurinol on theophylline metabolism and clearance. Clin. Pharmacol. Ther. **26:** 660–667, 1979.

KHMELEVSKII, V. I., ABRAMOVA, E. I. AND VARIUKHINA, L. V.: Preparation of theophylline and caffeine from uric acid. J. Gen. Chem. (U.S.S.R.) **28:** 2016–2020, 1958.

LELO, A., BIRKETT, D. J., ROBSON, R. A. AND MINERS, J. O.: Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. Br. J. Clin. Pharmacol. **22:** 177–182, 1986a.

LELO, A., MINERS, J. O., ROBSON, R. A. AND BIRKETT, D. J.: Quantitative assessment of caffeine partial clearances in man. Br. J. Clin. Pharmacol. **22:** 183–186, 1986b.

LELO, A., MINERS, J. O., ROBSON, R. A. AND BIRKETT, D. J.: Assessment of caffeine exposure-caffeine content of beverages, caffeine intake and plasma concentrations of methylxanthines. Clin. Pharmacol. Ther. **39:** 54–59, 1986c.

MAY, D. C., JARBOE, C. H., VAN BAKEL, A. B. AND WILLIAMS, W. M.: Effects of cimetidine on caffeine disposition in smokers and non-smokers. Clin. Pharmacol. Ther. **31:** 656–661, 1982.

MINERS, J. O., ATTWOOD, J. AND BIRKETT, D. J.: Theobromine metabolism in man. Drug Metab. Dispos. **10:** 672–675, 1982.

MINERS, J. O., ATTWOOD, J., WING, L. M. H. AND BIRKETT, D. J.: Influence of cimetidine, sulfinpyrazone and cigarette smoking on theobromine metabolism in man. Drug Metab. Dispos. **13:** 598–601, 1985a.

MINERS, J. O., WING, L. M. H., LILLYWHITE, K. J. AND ROBSON, R. A. Selectivity and dose-dependency of the inhibitory effect of propranolol on theophylline metabolism in man. Br. J. Clin. Pharmacol. **20:** 219–223, 1985b.

SALTER, H.: Treatment and clinical history of asthma. Edinburgh Med. J. **IV:** 1109–1115, 1859.

TANG, B. K., GRANT, D. M. AND KALOW, W.: Isolation and identification of 5-acetylamino-6-formylamino-3-methyluracil as a major metabolite of caffeine in man. Drug Metab. Dispos. **11:** 218–220, 1983.

TANG, B. K., KADAR, D. AND KALOW, W.: An alternative test for acetylator phenotyping with caffeine. Clin. Pharmacol. Ther. **42:** 509–513, 1987.

TANG, B. K., ZUBOVITS, T. AND KALOW, W.: Determination of acetylated caffeine metabolites by high performance exclusion chromatography. J. Chromatogr. **375:** 170–173, 1986.

Send reprint requests to: Dr. J. O. Miners, Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, South Australia 5042, Australia.