Br. J. clin. Pharmac. (1986), **22**, 183–186

Quantitative assessment of caffeine partial clearances in man

A. LELO, J. O. MINERS, R. A. ROBSON & D. J. BIRKETT
Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, Adelaide, South Australia 5042

Five subjects who participated in an earlier study (Lelo *et al.*, 1986b) of the comparative pharmacokinetics of caffeine (CA) and its primary monodemethylated metabolites paraxanthine (PX), theobromine (TB) and theophylline (TP) were administered CA to steady-state. Using areas under the plasma concentration-time curves for each of the dimethylxanthines derived from CA in the steady-state study and individual plasma clearances of PX, TB and TP determined in the previous study, the fractional conversion of CA to PX, TB and TP and the individual partial clearances of CA have been defined. The mean (± s.d.) fractional conversion of CA to PX, TB and TP was 79.6 ± 21.0%, 10.8 ± 2.4% and 3.7 ± 1.3%, respectively. When only demethylation pathways are considered PX, TB and TP accounted for 83.9 ± 5.4%, 12.1 ± 4.1% and 4.0 ± 1.4%, respectively of the CA demethylations. The mean partial clearance of CA to PX was approximately 8-fold and 23-fold greater than those to TB and TP respectively. These data confirm earlier reports that PX is the major metabolite of CA in humans but suggest that PX formation is quantitatively more important than previously believed.

Keywords caffeine paraxanthine theobromine theophylline drug metabolism

Introduction

Caffeine (CA;1,3,7-trimethylxanthine) is one of the most widely consumed pharmacological agents throughout the world (Barone & Roberts, 1984). Available evidence (Arnaud & Welsch, 1980; Callahan *et al.*, 1982; Cornish & Christman, 1957; Lelo *et al.*, 1986a; Tang-Liu *et al.*, 1983) suggests that 1-, 3- and 7-demethylations to form theobromine (TB; 3,7-dimethylxanthine), paraxanthine (PX; 1,7-dimethylxanthine) and theophylline (TP; 1,3-dimethylxanthine), respectively, account for the major part of CA metabolism in humans. Minor pathways of CA metabolism in man include formation of 1,3,7-trimethyluric acid and the ring-opened derivative 6-amino-5-[*N* - formylmethylamino] - 1,3 - dimethyluracil (Arnaud & Welsch, 1980; Callahan *et al.*, 1982). Each of the primary demethylation products (PX, TB, TP) of CA are metabolised further to form a range of xanthines, urates and uracils (Figure 1).

To date three approaches have been adopted to characterise the relative conversion of CA to PX, TB and TP in humans. Initial estimates of relative CA-demethylations were based on urinary metabolite profiles after CA administration (Arnaud & Welsch, 1980; Callahan *et al.*, 1982). However, from Figure 1 it is apparent that many of the CA urinary metabolites are derived from more than one of the primary demethylation products and assessment of CA-demethylations from urinary metabolite profiles is unlikely to be valid. Kotake *et al.* (1982) measured expired labelled CO_2 after administration of [^{14}C]-methyl-CA but interpretation of the breath test data is difficult since the CO_2 exhaled may arise from either CA or its primary demethylated metabolites. In addition, this procedure provides a measure of elimination rate rather than formation clearance. Lelo *et al.* (1986a) determined the area under the plasma

Correspondence: Dr J. O. Miners, Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, South Australia 5042

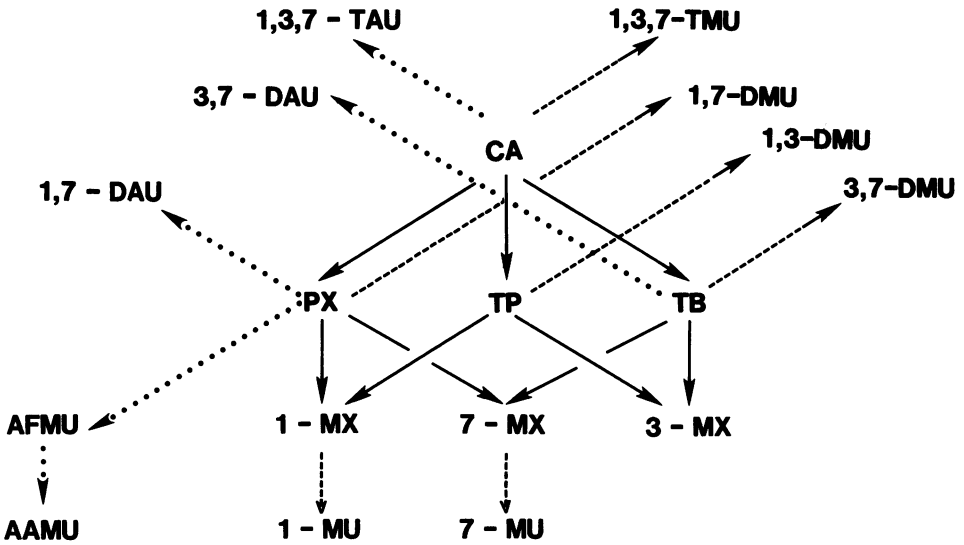

Figure 1 Biotransformation pathways of caffeine in humans. Solid lines (——) indicate demethylation pathways, dashed lines (---) indicate 8-oxidation, and dotted lines (.....) indicate uracil formation. CA = caffeine; PX = paraxanthine; TB = theobromine; TP = theophylline; MX = methylxanthine; MU = methylurate; TMU = trimethylurate; DMU = dimethylurate; 1,3,7-TAU = 6-amino-5-[*N*-formylmethylamino]-1,3-dimethyluracil; n, 7-DAU = 6-amino-5-[*N*-formylmethylamino]-*n*-methyluracil; AFMU = 6-formylamino-5-acetylamino-3-methyluracil; AAMU = 6-amino-5-acetylamino-3-methyluracil.

concentration-time curve (AUC) for PX, TB and TP in normal CA-consumers (tea and coffee drinkers). The plasma concentrations of these compounds however will be proportional to CA demethylation clearances only if the plasma clearances of each of the dimethylxanthines are the same. Tang-Liu *et al.* (1983) attempted to correct relative dimethylxanthine AUCs for differences in individual dimethylxanthine clearances by measuring clearances of CA and TP in the same subjects and by using a value of TB plasma clearance obtained from the literature. However, it was not possible to obtain a reliable estimate for conversion to PX since no pharmacokinetic data were available for this compound at the time.

In a recent comparative pharmacokinetic study of CA and its demethylated products in humans, Lelo *et al.* (1986b) demonstrated that the plasma clearance of PX is approximately two-fold greater than those of TP and TB. Previous estimates of the relative conversion of CA to PX, TB and TP based on plasma concentrations of these compounds after CA administration are therefore likely to have significantly underestimated the extent of CA 3-demethylation (i.e. PX formation). Thus, a CA steady-state (multiple dosing) study was performed in the subjects who participated in the earlier comparative pharmacokinetic study so that an AUC for each of the dimethylxanthines formed from CA could be determined accurately. Using these additional data we have been able to define the fractional conversion of CA to PX, TB and TP as well as to calculate the individual partial clearances of CA.

Methods

Subjects

Five of the six non-smoking volunteers, aged 19–21 years, weight 62–104 kg, who participated in the CA, PX, TB and TP comparative pharmacokinetic study (Lelo *et al.*, 1986b) took part in the present study. The additional study phase was approved by the Clinical Investigation and Drug and Therapeutics Advisory Committees of Flinders Medical Centre and written informed consent was obtained from each subject.

Protocol

CA (Hamilton Laboratories, Adelaide, South Australia), 200 mg in gelatin capsules, was administered every 8 h to the subjects for 3 days. On the third day of CA administration venous blood samples (5 ml) were collected into heparinised tubes through an indwelling cannula inserted in a forearm vein prior to and 0.5, 1, 1.5,

2, 3, 4, 6, and 8 h after the morning (08.00 h) dose. The cannula was kept patent with 0.9% w/v sodium chloride solution containing heparin, 5u ml^{-1}. Plasma was separated and stored at −20°C until analysed. The subjects abstained from all methylxanthine-containing foods and beverages for 4 days prior to and throughout the CA administration period. The CA multiple-dosing study was performed within 4 weeks of the final methylxanthine administration phase of the previous study (Lelo *et al.*, 1986b).

Analytical

Plasma concentrations of CA, PX, TB and TP were measured as described previously (Lelo *et al.*, 1986a).

Analysis of results

Area under the plasma concentration-time curve over the 8 h CA dosage interval (AUC) was determined for CA, PX, TB and TP using the trapezoidal rule. The fractional conversion of CA to each demethylated metabolite, DMX, was calculated (Houston, 1982; Tang-Liu *et al.*, 1983) according to the equation:

$$f_{DMX} = \frac{AUC_{DMX} \cdot CL_{DMX}}{\text{Dose (CA)}}$$

where AUC_{DMX} is the AUC for each dimethylxanthine formed from CA in the multiple dose study and CL_{DMX} is the total plasma clearance of the individual dimethylxanthine determined previously (Lelo *et al.*, 1986b) for each subject. This equation is valid if the systemic availability of the metabolite (DMX) is high (Houston, 1982). Available evidence (Blanchard & Sawers, 1983a,b; Drouillard *et al.*, 1978; Ogilvie, 1978) indicates that CA, TB and TP are essentially completely bioavailable. PX bioavailability is unknown, but it would not be expected to differ markedly from other methylxanthines. The partial clearance of CA to each dimethylxanthine ($CL_{CA \rightarrow DMX}$) was determined as,

$$CL_{CA \rightarrow DMX} = f_{DMX} \cdot CL_{CA}$$

where CL_{CA} is the total plasma clearance of CA determined in the steady-state study. CL_{CA} was calculated as,

$$CL_{CA} = \frac{\text{Dose}}{AUC(0,8)}$$

where AUC(0,8) is the AUC for CA over the 8 h dosage interval.

All results are expressed as mean ± s.d. Possible differences in CA clearance between the single and multiple dose studies were assessed by the paired Student's *t*-test. The correlation between the CA clearances was determined by linear regression.

Results

The attainment of steady-state in the present study was confirmed by the same pre-dose and 8 h plasma concentrations for CA, PX, TB and TP in each subject. There was no significant difference between CA total plasma clearance determined in the previous (Lelo *et al.*, 1986b) and present studies (2.20 ± 1.02 and 2.33 ± 1.02 ml min^{-1} kg^{-1}, respectively; $r = 0.95$).

The mean AUC values over the 8 h dosage interval for CA, PX, TB and TP were 20.64 ± 7.28, 16.52 ± 5.08, 3.76 ± 1.44 and 1.62 ± 0.54 mg l^{-1}h respectively. The mean fractional conversion of CA to PX, TB and TP was calculated to be 79.6 ± 21.0%, 10.8 ± 2.4% and 3.7 ± 1.3%, respectively. The unaccounted fraction (5.9%) presumably reflects other elimination pathways. When only demethylation pathways are considered, PX formation accounted for 83.9 ± 5.4% of the CA demethylations. TB and TP formation accounted for 12.1 ± 4.1% and 4.0 ± 1.4%, respectively of the CA demethylations. The mean partial clearance of CA to PX (1.84 ± 1.08 ml min^{-1} kg^{-1}) was approximately 8-fold greater than that to TB (0.24 ± 0.07 ml min^{-1} kg^{-1}) and approximately 23-fold greater than that to TP (0.08 ± 0.02 ml min^{-1} kg^{-1}).

Discussion

The necessity to perform the steady-state study described in this communication to determine PX, TB and TP AUC values after CA administration arose since individual dimethylxanthine AUC values could not be determined after administration of a single dose of CA. Limitations with assay sensitivity prevented measurement of an AUC for TP. Moreover, in the single dose study (Lelo *et al.*, 1986b) the log plasma concentration-time plots for PX and TB were apparently non-linear due to simultaneous formation (from CA) and elimination of each compound and this precluded calculation of an accurate AUC to infinite time. The non-linear log plasma concentration-time plots are to be expected when the elimination rate constants for the metabolite and parent drug are similar (Houston, 1982). There was, however, good agreement between total plasma clearance of CA determined in the single- and multiple-dose studies which supports the

validity of using clearance data from the previous study.

The results of the present study confirm that the 3-demethylation of CA to form PX is quantitatively the most important pathway of CA metabolism in humans. On average, this process is responsible for 84% of CA demethylations. The 1- and 7-demethylation pathways to form TB and TP respectively account for 12% and 4% of CA demethylations. Other pathways (presumably renal excretion of unchanged drug and formation of 1,3,7-trimethyluric acid and 6-amino-5-[*N*-formylmethylamino]-1,3-dimethyluracil) accounted for approximately 6% of total CA elimination. Previous estimates of CA 3-demethylation from urinary metabolite profiles (Arnaud & Welsch, 1980; Callahan *et al.*, 1982) and dimethylxanthine AUC values (Lelo *et al.*, 1986b; Tang-Liu *et al.*, 1983) following CA administration have ranged from 37% to 70%. Results from the first 2 h of the [^{14}C]-methyl-CA CO_2 breath test (Kotake *et al.*, 1982) showed that 80% of labelled CO_2 expired in the breath was derived from the 3-position, although at later times a lower proportion of exhaled CO_2 was derived from this position. However, as indicated in the introduction, there have been problems associated with the previous approaches used to characterise the relative conversion of CA to its monodemethylated metabolites and data presented here suggest that PX formation is quantitatively more important than previously believed.

The average formation clearance of PX from CA was approximately 8-fold and 23-fold greater than those of TB and TP, respectively. It is interesting to note that previous studies (Blanchard & Sawers, 1983a, 1983b; Lelo *et al.*, 1986b; Tang-Liu *et al.*, 1983) have reported considerable interindividual variability in CA elimination. With the demonstration here that PX formation on average accounts for 80% of CA plasma clearance, it is apparent that the interindividual variability in CA elimination will be due largely to quantitative and qualitative differences in the cytochrome P450 isozyme(s) responsible for CA 3-demethylation.

Although PX is the major metabolic product of CA very little is known about its pharmacological properties compared to the other methylxanthines. Furthermore, it is now apparent (Callahan *et al.*, 1982; Grant *et al.*, 1984) that the main metabolite of PX in humans is a uracil derivative (5-acetylamino-6-formylamino-3-methyluracil) also with unknown pharmacological properties. Thus, CA consumers are exposed continuously to these compounds about which little is known.

References

Arnaud, M. J. & Welsch, C. (1980). Caffeine metabolism in human subjects. *Ninth International Colloquium on Science and Technology of Coffee*, London, pp 385–395. Association Scientifique Internationale du Cafe.

Barone, J. J. & Roberts, H. (1984). Human consumption of caffeine. In *Caffeine: Perspectives from recent research*, ed. Dews, P. B., pp 59–73. Berlin: Springer-Verlag.

Blanchard, J. & Sawers, S. J. A. (1983a). Comparative pharmacokinetics of caffeine in young and elderly men. *J. Pharmacokin. Biopharm.*, **11**, 109–126.

Blanchard, J. & Sawers, S. J. A. (1983b). The absolute bio-availability of caffeine in man. *Eur. J. clin. Pharmac.*, **24**, 93–98.

Callahan, M. M., Robertson, R. S., Arnaud, M. J., Branfman, A. R., McComish, M. F. & Yesair, D. W. (1982). Human metabolism of [1-methyl-^{14}C] and [2-^{14}C]caffeine after oral administration. *Drug Metab. Disp.*, **10**, 417–423.

Cornish, H. H. & Christman, A. A. (1957). A study of the metabolism of theobromine, theophylline and caffeine in man. *J. biol. Chem.*, **228**, 315–323.

Drouillard, D. D., Vesell, E. S. & Dvorchik, B. H. (1978). Studies on theobromine disposition in normal subjects: Alterations induced by dietary abstention from or exposure to methylxanthines. *Clin. Pharmac. Ther.*, **23**, 296–302.

Grant, D. M., Tang, B. K. & Kalow, W. (1984). A simple test for acetylator phenotype using caffeine. *Br. J. clin. Pharmac.*, **17**, 459–464.

Houston, J. B. (1982). Drug metabolite kinetics. *Pharmac. Ther.*, **15**, 521–552.

Kotake, A. N., Schoeller, D. A., Lambert, G. H., Baker, A. L., Schaffer, D. D. & Josephs, H. (1982). The caffeine CO_2 breath test: Dose response and route of *N*-demethylation in smokers and nonsmokers. *Clin. Pharmac. Ther.*, **32**, 261–269.

Lelo, A., Birkett, D. J., Robson, R. A. & Miners, J. O. (1986b). Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br. J. clin. Pharmac.*, **22**, 177–182.

Lelo, A., Miners, J. O. & Birkett, D. J. (1986a). Assessing caffeine exposure: caffeine content on beverages, caffeine intake and plasma concentrations of methylxanthines. *Clin. Pharmac. Ther.*, **39**, 54–59.

Ogilvie, R. J. (1978). Clinical pharmacokinetics of theophylline. *Clin. Pharmacokin.*, **3**, 267–293.

Tang-Liu, D. D., Williams, R. L. & Riegelman, S. (1983). Disposition of caffeine and its metabolites in man. *J. Pharmac. exp. Ther.*, **224**, 180–185.

(Received 6 November 1985, accepted 2 April 1986)