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Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man

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1 The pharmacokinetics of caffeine (CA), paraxanthine (PX), theobromine (TB) and theophylline (TP) were studied in six healthy male volunteers after oral administration of each compound on separate occasions.

2 The total plasma clearances of CA and PX were similar in value (2.07 and 2.20 ml min^{-1} kg^{-1}, respectively) as were those for TP and TB (0.93 and 1.20 ml min^{-1} kg^{-1}, respectively). The unbound plasma clearances of CA and PX were also similar in magnitude (3.11 and 4.14 ml min^{-1} kg^{-1}, respectively) as were those of TP and TB (1.61 and 1.39 ml min^{-1} kg^{-1}, respectively).

3 The half-lives of TP and TB (6.2 and 7.2 h, respectively) were significantly longer than those of CA and PX (4.1 and 3.1 h, respectively).

4 The volume of distribution at steady state of TP (0.44 l kg^{-1}) was lower than that of the other methylxanthines (0.63–0.72 l kg^{-1}). The unbound volume of distribution of TP (0.77 l kg^{-1}) was however the same as that of TB (0.79 l kg^{-1}) whereas the unbound volume of distribution of PX (1.18 l kg^{-1}) was similar to that of CA (1.06 l kg^{-1}).

Keywords methylxanthines caffeine paraxanthine theobromine theophylline drug metabolism

Introduction

The structurally-related naturally occurring alkaloids caffeine (1,3,7-trimethylxanthine; CA), theobromine (3,7-dimethylxanthine; TB) and theophylline (1,3-dimethylxanthine: TP) (Figure 1) are among the most widely and frequently consumed compounds throughout the world. It has been estimated (Barone & Roberts, 1984) that the mean daily intake of CA in the USA from beverages such as coffee, tea and cola is 3 mg kg^{-1} for all adults in the general population. In addition, it has been estimated that about 1,000 prescription drug products and about 2,000 non-prescription drug products contain CA (Barone & Roberts, 1984). TB is a major constituent of cocoa and a minor constituent of tea and coffee; it has also been included in certain pharmaceutical preparations. TP has found widespread clinical use as a bronchodilator and also occurs as a minor constituent of tea. Along with the isomeric dimethylxanthine paraxanthine (1,7-dimethylxanthine; PX) (Figure 1), which does not occur naturally, TP and TB are primary metabolic products of CA. Arnaud & Welsch (1980) have recently demonstrated that PX is quantitatively the most important demethylation product of CA in humans, with PX formation accounting for approximately 70% of CA metabolism. Indeed, in a recent study (Lelo *et al.*,

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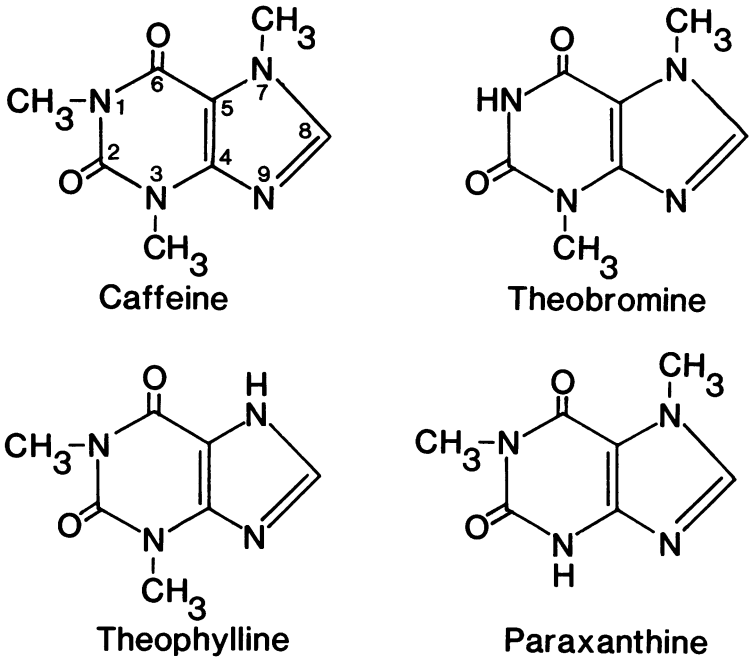

Figure 1 Structures of caffeine and its dimethylxanthine metabolites.

1986) in normal tea and coffee drinkers investigating the relationship of daily CA intake to plasma concentrations of CA and its dimethylxanthine metabolites, we showed that the average 24 h plasma concentration of PX was 2 mg l^{-1}, whereas those of TP and TB were 0.2 mg l^{-1} and 0.7 mg l^{-1} respectively. Thus, the indirect exposure of normal CA-consumers to PX may be considerable.

Consistent with the clinical importance of TP and the widespread use of CA, the literature relating to the pharmacokinetics of these agents is extensive (for reviews see: Bonati & Garattini, 1984; Ogilvie, 1978). However, there is no information relating to the pharmacokinetics of PX in man although some studies of TB pharmacokinetics and metabolism in humans have been reported (Birkett *et al.*, 1985; Drouillard *et al.*, 1978; Miners *et al.*, 1982, 1985; Shively *et al.*, 1985; Simons *et al.*, 1984; Tarka *et al.*, 1983). There has not been any direct comparison of the pharmacokinetics of all members of the methylxanthine group in humans. The aim of the present study was to define CA, TB, TP and PX pharmacokinetic parameters in the same individuals, thereby providing the first kinetic data for PX in man and enabling a direct comparison of the kinetics of these agents to be made.

Methods

Subjects

Subjects were six non-smoking male volunteers, aged 19–21 years, weight 62–104 kg. No subject was receiving any medications at the time of the study, although all were modest social drinkers of alcohol. The subjects were healthy as determined by medical history, physical examination and biochemical and haematological measurements. Written informed consent was obtained from each subject and the studies were approved by the Clinical Investigation and Drug and Therapeutics Advisory Committees of Flinders Medical Centre.

Preparations

CA and TB (both B.P.) were supplied by Hamilton Laboratories (Adelaide, S.A.) and administered in gelatin capsules. TP was administered in the form of Nuelin tablets (125 mg, Riker). PX was purchased from the Sigma Chemical Co. (St Louis, MO, USA) and administered in gelatin capsules. The PX used was shown to be > 99.8% pure by elemental analysis and chromatography. Permission was obtained from the Australian

Department of Health for the use of PX in this study since it is not an approved substance for use in humans.

Protocol

Equimolar oral doses of TP, TB, PX (250 mg of each) and CA (270 mg) were separately administered to each subject. Subjects fasted from the evening prior to and until 4 h following methylxanthine administration on each study day. The methylxanthine dose was administered with 150 ml water at approximately 08.00 h of the study day. The subjects abstained from all methylxanthine-containing foods and beverages for 4 days prior to and during the blood collection period following each methylxanthine administration. The order of the methylxanthine doses was randomised and at least 1 week separated each administration.

On each study day venous blood samples (5 ml into an heparinised tube) were collected through an indwelling cannula inserted in a forearm vein prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 10 h after the methylxanthine dose. The cannula was kept patent with 0.9% w/v sodium chloride solution containing heparin 5 u ml^{-1}. Additional samples were collected at 24, 28 and 32 h post-dose by venepuncture. Plasma was separated by centrifugation (3,000 *g* for 5 min) and stored at −20°C until analysed.

Analytical procedures

Plasma concentrations of CA, TB, TP and PX were measured by a high performance liquid chromatographic method (Lelo *et al.*, 1986). This method allows the separation of each methylxanthine in the presence of the others and has a limit of sensitivity of 0.1 mg l^{-1} for each compound. The plasma protein binding of CA, TB, TP and PX was determined in the blank (predose) plasma sample from each subject and was measured by equilibrium dialysis using a spectrapor-2 cellulose membrane (Spectrum Medical Industries). Plasma (1 ml) was introduced into one side of the cell (Dianorm) and dialysed at 37°C for 3 h against an equal volume of isotonic phosphate buffer (pH 7.4) containing either CA, TB, TP or PX. The binding of each methylxanthine was determined for added concentrations of 2, 4, 6 and 8 mg l^{-1}.

Analysis of results

The following parameters were estimated for each methylxanthine from the plasma concentration data. Area under the plasma concentration–time curve (AUC) was measured by the trapezoidal rule with extrapolation to infinite time. Elimination half-life ($t_{½,z}$) was calculated from the slope of the terminal portion of the concentration-time curve by linear least squares regression and volume of distribution at steady state (V_{ss}) by the model-independent procedure of Benet & Galeazzi (1979). Total plasma clearance was determined as:

$$CL = Dose/(AUC \times B.W.)$$

where B.W. is the body weight in kg. Unbound clearance was calculated as:

$$CLu = CL/fu$$

where *fu* is the fraction of unbound drug in plasma.

All results are expressed as mean ± s.d. The mean values of each pharmacokinetic parameter for the methylxanthines were compared by analysis of variance. Individual comparisons were performed using the Neuman-Keuls Studentised range test. Correlations between unbound clearances were determined by linear regression. The factors required for the conversion of plasma concentrations of the dimethylxanthines (TP, TB, PX) and CA from mg l^{-1} to S.I. units (μmol l^{-1}) are 5.55 and 5.15, respectively.

Results

Mean pharmacokinetic parameters for each of the methylxanthines are summarised in Figure 2. Representative plasma concentration–time profiles are shown in Figure 3.

The total plasma clearances of CA and PX were similar in magnitude (2.07 ± 0.96 and 2.20 ± 0.91 ml min^{-1} kg^{-1}, respectively) as were those for TP and TB (0.93 ± 0.22 and 1.20 ± 0.40 ml min^{-1} kg^{-1}, respectively). Although the total plasma clearances of CA and PX were significantly greater ($P < 0.025$) than that of TP, the differences between the total clearance of TB and those of CA and PX were not statistically significant. There was considerable interindividual variability in the total clearances of CA (3.2 fold), PX (2.7 fold) and TB (2.6 fold) but TP clearance was less variable (1.6 fold range). The half-lives of CA and PX were similar in value (4.1 ± 1.3 and 3.1 ± 0.8 h, respectively) and significantly shorter ($P < 0.01$) than those of TP and TB (6.2 ± 1.4 and 7.2 ± 1.6 h, respectively). The mean free fractions of CA, PX, TP and TB were 0.68 ± 0.04, 0.54 ± 0.04, 0.58 ± 0.06 and 0.86 ± 0.03, respectively. Protein binding of each of the methylxanthines was concentration independent over the range studied. As with

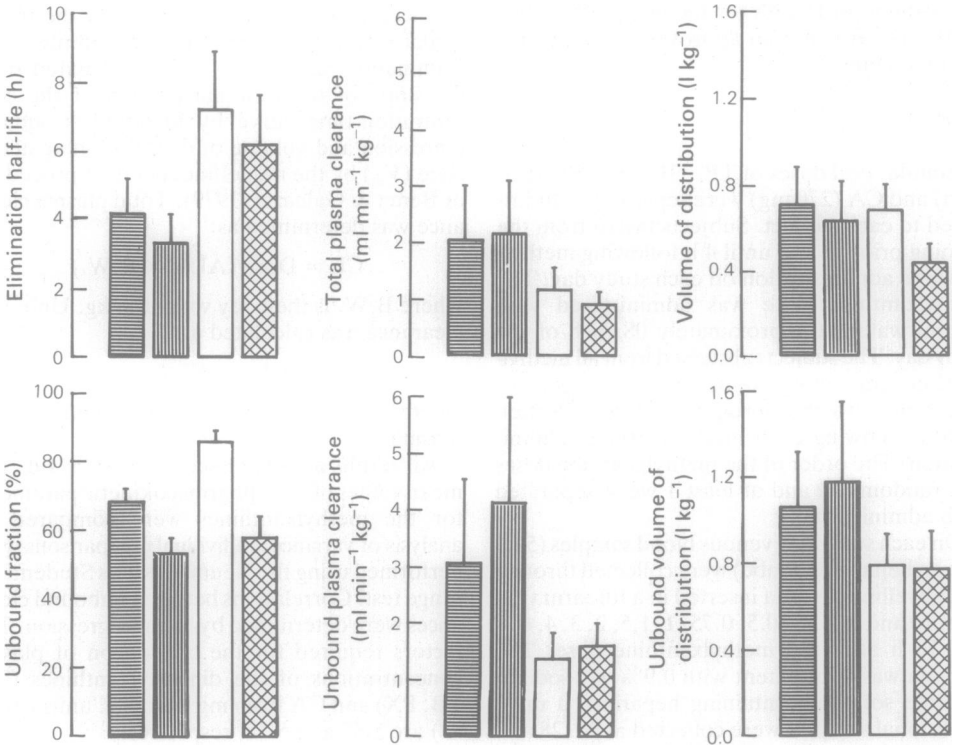

Figure 2 Mean ± s.d. pharmacokinetic parameters of caffeine (▤), paraxanthine (▥), theobromine (□) and theophylline (▩) in six healthy volunteers receiving each compound (250–270 mg p.o.) on four separate occasions.

total clearance, the unbound plasma clearances of CA and PX were similar (3.11 ± 1.50 and 4.14 ± 1.85 ml min^{-1} kg^{-1}, respectively) as were those of TP and TB (1.61 ± 0.41 and 1.39 ± 0.44 ml min^{-1} kg^{-1}, respectively). There was also a similar trend in the degree of variability in unbound clearance with that previously observed for total clearance, the variability in TP total

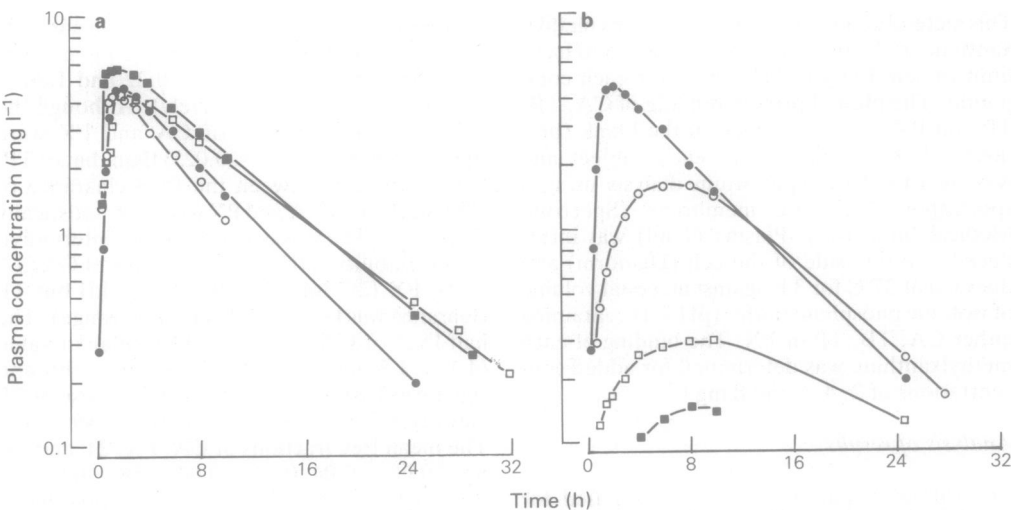

Figure 3 Plasma concentration–time curves of caffeine (●), paraxanthine (○), theobromine (□) and theophylline (■), (a) after oral administration of each compound (250–270 mg) on separate occasions to a single subject, and (b) after oral administration of caffeine (270 mg) to the same subject.

clearance (1.7 fold range) being less than that for TB (2.5 fold), PX (2.6 fold) and CA (3.6 fold). The differences in TP and TB protein binding reversed the order of TP and TB unbound clearance compared to that observed for total clearance. While the unbound plasma clearance of PX was significantly greater ($P < 0.025$) than those of TP and TB, the differences between the unbound clearances of CA and each of the other methylxanthines were not significant. The volume of distribution at steady state for TP (0.44 ± 0.08 l kg^{-1}) was lower ($P < 0.05$) than those of the other methylxanthines (0.63 – 0.71 l kg^{-1}). The unbound volume of distribution of TP (0.77 ± 0.17 l kg^{-1}) was however similar to that of TB (0.79 ± 0.15 l kg^{-1}), whereas the unbound volume of distribution of PX (1.18 ± 0.37 l kg^{-1}) was similar to that of CA (1.06 ± 0.26 l kg^{-1}) and it was significantly greater ($P < 0.05$) than those of TP and TB.

The unbound plasma clearance of CA was significantly correlated ($P < 0.05$) with those of TP ($r = 0.91$), TB ($r = 0.87$) and PX ($r = 0.90$). In addition, there was a significant ($P < 0.05$) correlation between the unbound plasma clearances of TP and TB ($r = 0.91$) but these were not significantly correlated with the unbound clearance of PX ($r = 0.75$ and 0.71 respectively).

Discussion

This is the first formal study of PX pharmacokinetics in man and the first direct comparison of the pharmacokinetics of CA, PX, TP and TB. Interestingly, PX total plasma clearance and unbound plasma clearance were two to three-fold greater than the values for the isomeric dimethylxanthines TP and TB. PX total and unbound plasma clearances were, however, similar to those of the trimethylxanthine CA. PX and CA plasma half-lives were correspondingly shorter than those for TP and TB. A similar trend was observed in a recent study of PX pharmacokinetics in the rat (Bortolotti *et al.*, 1985). For equivalent intravenous doses of the methylxanthines administered to rats, the plasma clearances and half-lives of PX and CA were similar whereas the plasma clearances of TP and TB were approximately half that for PX and the half-lives of TP and TB were approximately double that of PX. The pharmacokinetic parameters of CA, TP and TB determined in this study are similar to those generally reported in the literature (Birkett *et al.*, 1982, 1985; Blanchard & Sawers, 1983a; Bonati *et al.*, 1982; Miners *et al.* 1982, 1985; Ogilvie, 1978; Renner *et al.*, 1984; Tarka *et al.*, 1983).

Following CA administration the order of dimethylxanthine plasma concentrations was PX > TB > TP (Figure 3b). This is consistent with previous studies (Lelo *et al.*, 1986; Tang-Liu *et al.*, 1983) which have measured plasma concentrations of CA-derived dimethylxanthines. Tang-Liu *et al.* (1983) have reported that the log plasma concentration–time plots for each of the dimethylxanthines formed from CA are non-linear and there was a suggestion of the same effect in the present study. The non-linear metabolite concentration–time plots presumably arise from simultaneous formation (from CA) and elimination of each dimethylxanthine and are to be expected when the elimination rate constants for the metabolite and parent drug are similar in magnitude (Houston, 1982).

In this report it has been assumed that PX absorption from the gastrointestinal tract is complete. Previous studies (Blanchard & Sawers, 1983b; Miners *et al.*, 1982; Ogilvie, 1978) have demonstrated that CA, TB and TP are essentially completely absorbed and PX would not be expected to be different in this respect. In a PX metabolism study in humans Arnaud & Welsch (1980) demonstrated that approximately 60% of orally administered PX may be recovered in the urine as unchanged drug and known xanthine and urate metabolites. Callahan *et al.* (1982, 1983) have further shown that the formation of polar ring-opened uracil metabolites of PX accounts for up to 40% of PX metabolism in man. Available metabolic data therefore suggests that PX, like the other methylxanthines, is completely absorbed after oral doses.

In the present study there were positive correlations between the unbound clearances for all the methylxanthines but those for PX/TP and PX/TB did not quite reach statistical significance. This is consistent with our previous suggestion that a common group of cytochrome P450 isozymes under similar regulatory control is involved in the metabolism of the methylxanthines (Birkett *et al.*, 1985). At least two forms of cytochrome P450 appear to be involved in TP and TB metabolism (Birkett *et al.*, 1982, 1985; Miners *et al.*, 1985), although the relationship of these isozymes to those involved in CA and PX metabolism is less clear. Whereas *N*-demethylations and C(8)-oxidation are the primary metabolic pathways of CA, TB and TP metabolism, a major pathway for PX metabolism involves the formation of a ring-opened uracil derivative. The lower correlation coefficients for the comparisons between the unbound clearances of PX and the other dimethylxanthines (TB and TP) may be due to the involvement of a different enzyme(s) in uracil formation.

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