

Accumulation of caffeine in healthy volunteers treated with furafylline

E. TARRUS¹, J. CAMI², D. J. ROBERTS¹, R. G. W. SPICKETT¹, E. CELDRAN¹ & J. SEGURA^{1,2}

¹Research Institute, Laboratorios Almirall, Barcelona and ²Institut Municipal d'Investigació Mèdica Autònoma University of Barcelona, Barcelona, Spain

1 The pharmacokinetics and tolerance of repeated oral doses of furafylline were investigated in normal volunteers.

2 In accord with predictions from single dose studies, steady state was achieved on the first day following the administration of 90 mg and maintained by subsequent daily doses of 30 mg.

3 When corrected for body weight there were no significant differences in minimum and maximum plateau levels of furafylline between males (1.2–2.0 $\mu\text{g ml}^{-1}$; mean body weight 67.2 kg) and females (1.6–2.6 $\mu\text{g ml}^{-1}$; mean body weight 54.9 kg).

4 The half-life of elimination was less when the plasma concentration was lower than 600 ng ml^{-1} than during the stationary phase of treatment.

5 Despite constant plasma levels the repeated administration of furafylline appeared to be associated with the onset of adverse xanthine-like side effects, a finding which was subsequently traced to the presence of, and possible synergism with, accumulating serum levels of caffeine in those volunteers drinking caffeine containing beverages.

6 Subsequent studies showed that a single dose (90 mg) of furafylline results in a rapid accumulation of caffeine given orally (100 mg twice daily) and that this is accompanied by an elimination half-life of some 50 h and an abrupt decrease in metabolite levels.

7 The furafylline-induced accumulation of caffeine was not influenced by the smoking habits of the subjects, implying that the metabolite pathway blocked by furafylline is the demethylation of caffeine in position 3, an implication confirmed by the reduced formation of paraxanthine.

8 This demonstration of an unacceptable level of adverse side effects resulting from a potent inhibiting effect of furafylline on the metabolism of a normal dietary constituent has obvious implications in the interpretation of drug-induced toxicity.

Keywords furafylline caffeine metabolism inhibition

Introduction

Furafylline, 1,8-dimethyl-3-(2'-furfuryl)methyl-xanthine, and mexafylline, 1,8-dimethyl-3-(cyclohex-3-enyl)methyl-xanthine, are two new xanthine derivatives selected from a programme designed to find new drugs of potential use in the

treatment of chronic obstructive pulmonary disease. Both compounds are more potent than theophylline as bronchodilators and inhibitors of anaphylactic reactions in standard animal models and, unlike theophylline, appear to be

essentially devoid of stimulant effects on the central nervous system (Vega *et al.*, 1985). Mexafylline was subsequently withdrawn because of animal toxicity problems but phase 1 clinical studies were carried out with furafylline.

Pharmacokinetic studies have shown that furafylline has a long half-life (about 50 h) in healthy young male volunteers (Segura *et al.*, 1986) and a once daily dosage schedule was utilised for other studies in man.

The purpose of this investigation was to evaluate the pharmacokinetics and tolerance of furafylline after repeated administration to healthy volunteers at 24 h intervals. Nevertheless, as a consequence of the serendipitous discovery, during the study, of an inhibitory effect of furafylline on the metabolism of caffeine, additional experiments were performed in an attempt to obtain further information on this unexpected and potentially adverse effect.

Methods

Trial designs

The research protocol was approved by the Clinical Trials Committee of the Hospital Nuestra Sra. del Mar and authorized by the General Direction of Pharmacy and Health Products of the Spanish Department of Health (Study number 82/9101). All of the volunteers gave informed written consent for their participation in the study.

Multiple oral administration of furafylline Prior to the study all volunteers were subjected to a medical examination to confirm that they were in good health and as a consequence one of them

was eliminated because of abnormalities in blood hepatic enzyme levels. The characteristics of the remaining volunteers (five males and four females) chosen to take part in this study are given in Table 1. Female volunteers Nos. 6 and 7 continued to take their usual oral contraceptives during the trial and male volunteer No. 5 was withdrawn on the third day with a fever associated with influenza.

Since furafylline is only sparingly soluble, it was prepared as a fine suspension as follows. Sodium hydroxide (1N) was added dropwise to a suspension of the drug in water until completely dissolved (pH 12) and methyl cellulose was added to make a suspension of 20 mg ml⁻¹. Following degasification the pH was adjusted to 6.5–7.5 with hydrochloric acid (1N) and the final suspension made up to the desired volume (never greater than 20 ml) with water. This suspension was administered orally to fasted (12 h) volunteers and 2 h later they were allowed to eat a standard breakfast.

Following an initial loading dose of 90 mg, single doses of 30 mg furafylline were administered daily during the following 4 days. This schedule was chosen on the basis of the maximum blood levels obtained in previous human single dose studies (Segura *et al.*, 1986). In the present study, and in contrast to the above trial, no restriction was placed on the habitual ingestion of caffeine-containing beverages in order to obtain information on the pharmacokinetic behaviour and tolerance of furafylline under field conditions. The number of caffeine-containing drinks consumed during each day of the trial were registered (Table 1).

Blood samples were obtained from an appropriate arm vein (at 0, 2, 4, 8, 24, 26, 48, 50, 72,

Table 1 Characteristics of the volunteers and their ingestion of drinks containing caffeine

Subject characteristics	Volunteers								
	1	2	3	4	5	6	7	8	9
Age (years)	27	27	26	23	26	23	25	27	28
Weight (kg)	69.0	71.4	63.5	70.5	61.5	61.0	56.2	58.8	43.5
Height (m)	1.79	1.85	1.78	1.81	1.71	1.68	1.64	1.67	1.62
Sex	M	M	M	M	M	F	F	F	F
Smoking habit	No	No	Yes	No	No	Yes	No	Yes	No
SCN (nmol 100 µl ⁻¹ (1))	1.95 ±0.44	4.78 ±0.13	10.6 ±1.49	2.38 ±0.45	2.08 ±0.39	8.71 ±1.40	2.52 ±0.54	8.02 ±1.06	3.71 ±0.38
Caffeine drinks ingested (2)									
Coffees	12	8	12	5	0	5	9	15	5
Soft drinks	3	0	3	0	0	1	1	0	1

(1) Average (± s.d.) of three samples obtained on the first, fifth and ninth test days, except volunteer 5 (see methods).

(2) Total number of coffees and soft drinks ingested from the first until the fifth test day.

74, 96, 98, 100, 104, 168, 196, 336 h) using heparinized vacutainers. The samples were centrifuged and the separated plasma stored at -20°C until analysis.

Clinical evaluation for adverse effects, using an autoevaluation questionnaire designed for this study, was made daily for 9 days starting on the first day of furafylline administration, with a final evaluation on day 15. Each adverse effect in each volunteer was scored on a scale from 0 to 3 and his daily adverse effect value was calculated each day using the equation: $\Sigma \log_e (\text{score} + 1)$.

Oral administration of a single dose of furafylline

The levels of caffeine were determined in the preserved plasma samples of four volunteers who had received a single oral dose of 125 mg furafylline in a study originally designed to study its pharmacokinetic behaviour (Segura *et al.*, 1986). The coffee consumption in these volunteers had been restricted to 1 to 2 cups/day during the experiment.

Oral administration of a single dose of furafylline and multiple doses of caffeine

One healthy male volunteer (45 years, 79.5 kg) ingested 100 mg caffeine dissolved in 20 ml water every 12 h for 7 days. On the 3rd day of the experiment, and 2 h before the morning dose of caffeine, 90 mg of furafylline was administered as a fine suspension prepared as described above. The consumption of methylxanthine-containing foods or beverages was prohibited during the experiment. Blood samples were taken, prepared and stored as described above.

Analytical methods

Quantitative determination of furafylline and caffeine

Plasma concentrations of both drugs were measured by high performance liquid chromatography (h.p.l.c.). The plasma samples (stored at -20°C) were heated for 15 min at 40°C , subjected to ultrasound for a further 15 min and then thoroughly shaken to assure redissolution of the xanthines. Chloroform (7 ml) was added to an aliquot (1 ml) of each plasma sample and mixed by rocking during 35 min. The organic phase was separated by centrifugation and evaporated to dryness in a stream of nitrogen. The residue was taken up in 100 or 200 μl of methanol and a 20 μl sample injected into the h.p.l. chromatograph (Waters Assoc.) and the absorbance (280 nm) of the eluate was measured. The columns used were Radial Pack C_{18} (Waters Assoc.) compressed by an RCM-100 module (Waters Assoc.) and coupled to a precolumn of the same composition. The mobile phase (eluent

A) was a 2:3 mixture of methanol (Merck) and 0.02 M sodium acetate (Merck). The flow rate was 1.4 ml min^{-1} and the pressure 500 psi. The retention times found for caffeine and furafylline were 3.6 and 5 min respectively.

Measurement of thiocyanate in human plasma

The method described by Pettigrew & Fell (1972) was used. According to these authors, the thiocyanate plasma levels in non-smokers and smokers range from 2–5 and from 10–15 nmol $100 \mu\text{l}^{-1}$ of plasma respectively.

Identification of caffeine in human plasma

The extraction procedure used was identical to that already described for the quantitative determination of caffeine in human plasma.

Once the organic phase had been evaporated to dryness, the residues were successively redissolved in 30, 40 and 50 μl of chloroform and applied to thin layer chromatography (t.l.c.) plates of silicagel $\text{G}_{60} \text{F}_{254}$ (Merck). The developing solvents used were:

(solvent B) acetone: chloroform: n-butanol: 25% ammonia (3:3:4:1).

(solvent C) benzene: acetone (3:7) in an atmosphere of ammonia.

(solvent D) chloroform: ethanol: formic acid (44:5:1).

Spots were visualized under ultraviolet light (254 nm). The caffeine R_F values obtained were 0.82, 0.67 and 0.66 respectively for solvents B, C and D. Subsequently the spots corresponding to caffeine in the chromatograms of the test plasma extracts were eluted with methanol and subjected to ultraviolet-visible spectrophotometry. The spectra obtained were compared with those of methanol solutions of caffeine.

Identification of the caffeine metabolite

The h.p.l. chromatograms of the plasma extracts of volunteers drinking coffee showed several peaks corresponding to metabolites of caffeine (Figure 5). In the presence of furafylline one of these showed a clear reduction in peak height inversely related to that of the caffeine peak.

In order to separate and identify this metabolite, the h.p.l.c. method described above was followed. However the solvent system acetonitrile-sodium acetate 0.01 M (7:93) adjusted to pH 4 with concentrated acetic acid, (Solvent E) was used as the mobile phase (Orcutt *et al.*, 1977). The eluted fractions were subjected to ultraviolet-visible spectrophotometry (Perkin-Elmer, model 550) and the spectra obtained compared with those of 1,7-dimethylxanthine (paraxanthine) and 1,3,7-trimethyluric acid. The fraction con-

taining the metabolite was then evaporated to dryness in a stream of nitrogen and applied to t.l.c. plates, Silicagel G₆₀ F₂₅₄ (Merck). Solutions of 1,3-dimethylxanthine (theophylline), 3,7-dimethylxanthine (theobromine), 1,7-dimethylxanthine (paraxanthine), furafylline, caffeine and 1,3,7-trimethyluric acid were also applied as reference standards and the plates developed two-dimensionally with acetone: chloroform: n-butanol: 25% ammonia (3:3:4:1) (solvent B) followed by chloroform: heptane: methanol (2:2:1) (solvent F). Spots were visualized under ultraviolet light (254 nm). The R_F values obtained with all of these compounds are shown in Table 3.

Statistical analysis

The behaviour of each of the populations studied (smokers and non-smokers) with respect to the two variables (plasma concentration of caffeine and number of cups of coffee ingested) was defined by linear regression analysis. The equations were verified by calculating the correlation coefficient by the method of least squares and the test of significance of linearity and slope by analysis of variance. The lack of significant differences between the two populations was demonstrated using Student's *t*-test.

Results

Pharmacokinetics and tolerance of repeated oral doses of furafylline in human volunteers and the influence of caffeine ingestion

As had been predicted, plateau plasma levels of furafylline were achieved after the first dose in both males and females (Figure 1a). Furthermore, when these levels were corrected for body weight there were no significant differences between the sexes. Also, in agreement with the finding of the previous study (Segura *et al.*, 1986), when the plasma furafylline concentration was lower than 600 ng ml⁻¹ the approximate half-life of elimination was less than during the stationary phase of treatment.

Fifteen days after the initial dose plasma concentrations of furafylline were no longer detectable.

During the course of the experiment it became very evident that there was a progressive increase in the incidence of adverse effects during the period of drug administration. These adverse effects had disappeared by the 9th day of the study, 4 days after the last dose (Figure 1b). The two most important disorders observed were heartburn and mental confusion (Table 2).

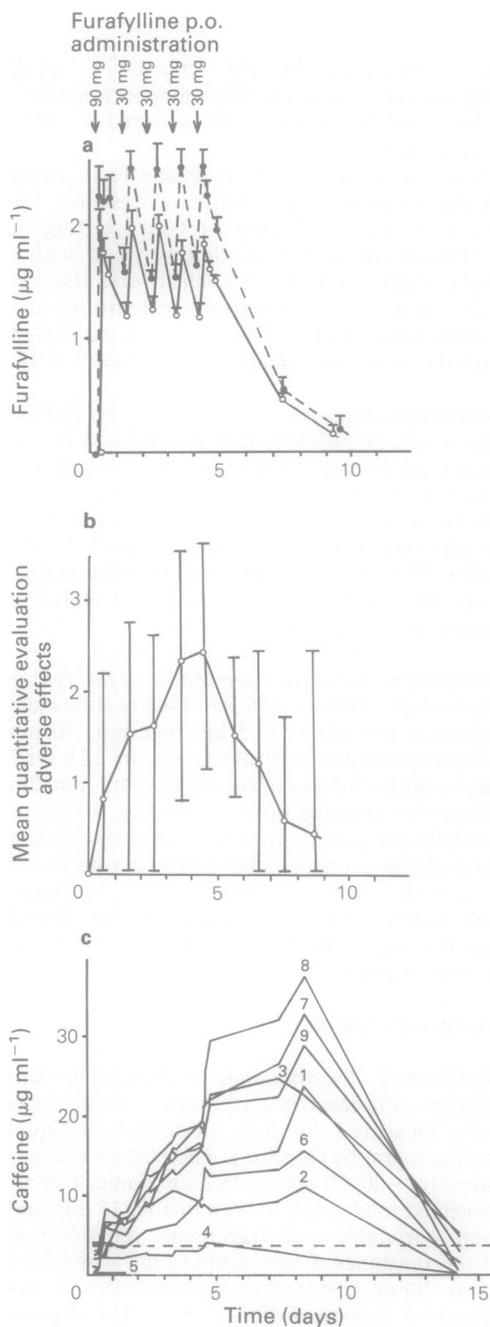


Figure 1 (a) Mean plasma levels of furafylline in male (○—○; $n = 5$, mean weight 67.2 kg) and female (●---●; $n = 4$, mean weight 54.9 kg) volunteers given repeated oral doses of furafylline as indicated. Vertical bars represent s.e. mean values. (b) Mean quantitative evaluation of adverse effects (as described in methods) which appeared during the trial. Vertical bars represent the extremes of the individual values. (c) Accumulation of caffeine in plasma due to simultaneous ingestion of caffeine-containing drinks. Each number refers to a different volunteer (see Table 1).

Table 2 Ranking of adverse effects in the furafylline multiple dose trial, (*) cumulative 9 day score recorded by participants (0–3 points item/day)

Effects	Score*	Effects	Score*
Heartburn	43	Insomnia	21
Mental confusion	42	Dry mouth	21
Headache	36	Thirst	16
Nausea	32	Belching	12
Flatulence	30	Vomiting	11
Anorexia	29	Accelerated pulse	10
Drowsiness	27	Sweating	9
Distal tremor	25	Fatigue	9
Indigestion	24	Restlessness	8
General weakness	24	Hot flushes	5

Volunteer no 5 is excluded, (see methods)

The levels of caffeine (identified by h.p.l.c., t.l.c. and ultraviolet-visible absorption spectrometry) found in the plasma samples of seven volunteers showed progressive increase during the study (Figure 1c), reaching maxima (10–40 $\mu\text{g ml}^{-1}$) on the 9th day and falling to normal pre-experiment levels (0–4 $\mu\text{g ml}^{-1}$) by the 15th day. The exceptions were volunteer no. 5, who did not drink any caffeine-containing beverages and no caffeine was detectable, and volunteer no. 4, whose caffeine levels never surpassed 4 $\mu\text{g ml}^{-1}$ (Figure 1c).

Plasma caffeine levels following a single oral dose of furafylline to human volunteers

In view of the surprising findings of the preceding experiment, stored plasma samples from a previous study with single doses (125 mg) of furafylline in four volunteers (Segura *et al.*, 1986) were evaluated for their caffeine content. The results (Figure 2) indicate that whereas the caffeine levels were low (0.1–0.8 $\mu\text{g ml}^{-1}$) and constant for up to 4 h after the administration of furafylline, from then onwards, and coinciding with the ingestion of coffee, they started to increase and continued to do so despite falling concentrations of furafylline. At the end of the experiment (96 h) caffeine concentrations ranged from 6–18 $\mu\text{g ml}^{-1}$.

Kinetic study of caffeine accumulation

Following the multiple administration of oral doses of caffeine (100 mg twice daily) a steady state concentration was reached (minimum 0.8–1.0 $\mu\text{g ml}^{-1}$, maximum 1.9 $\mu\text{g ml}^{-1}$) after 3 days (Figure 3). Nevertheless following the administration of a single oral dose (90 mg) of

furafylline the caffeine levels rapidly increased to reach 8.7–10 $\mu\text{g ml}^{-1}$ on the seventh day (4 days after the furafylline dose) and thereafter slowly declined (Figure 3). The furafylline concentrations reached a maximum (2.2 $\mu\text{g ml}^{-1}$) 2 h after administration and thereafter declined slowly over the following 4 days. The elimination half-life found for furafylline was 50 h, approximately the same as that found previously following single doses (Segura *et al.*, 1986).

The kinetics of caffeine accumulation after furafylline administration (Figure 3) may be described by the following equation (open monocompartmental model):

$$C = \frac{D}{V} \cdot \frac{k_a}{k_a - k_e}$$

$$\left[\left(\frac{1 - e^{-n \cdot k_e \cdot \tau}}{1 - e^{-k_e \cdot \tau}} \right) \cdot e^{-k_e t} - \left(\frac{1 - e^{-n \cdot k_a \cdot \tau}}{1 - e^{-k_a \cdot \tau}} \right) \cdot e^{-k_a t} \right]$$

The parameters used for the volume of distribution (V) and the absorption constant (k_a) were those found by Brazier *et al.* (1980) where $V = 351$ (for a body weight of 70 kg) and $k_a = 1.9 \text{ h}^{-1}$. Since the dose interval (τ , 12 h), the number of doses to be considered (n), the time since the last dose (t), the dose (D , 100 mg) and the plasma caffeine concentration (C) were known, the elimination constant (k_e) was calculated using successive approximations until a value was found which, when introduced into the equation, best described behaviour of the real plasma levels of caffeine (Figure 3). The elimination half-life of caffeine calculated using this value of k_e (0.014 h^{-1}) was 50 h whereas under normal circumstances the elimination half-life of caffeine is 5–7 h (Brazier *et al.*, 1980).

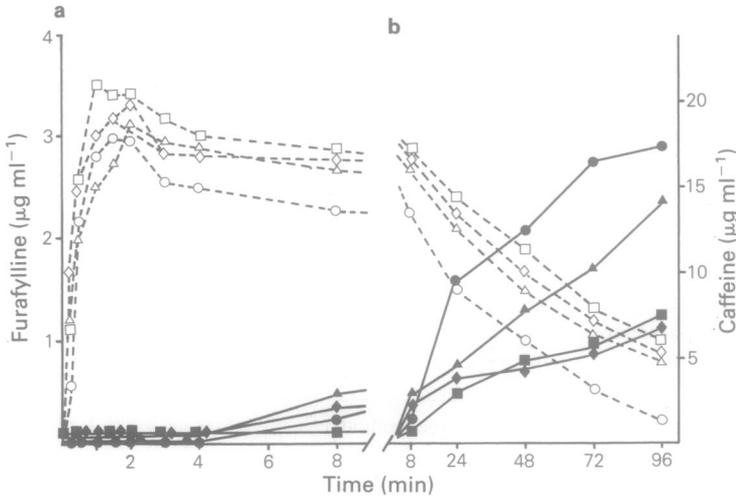


Figure 2 Plasma levels of furafylline (dotted lines) and caffeine (solid line) in four volunteers receiving one oral dose of furafylline (125 mg). The period 0–8 h has been expanded (a) for the sake of clarity.

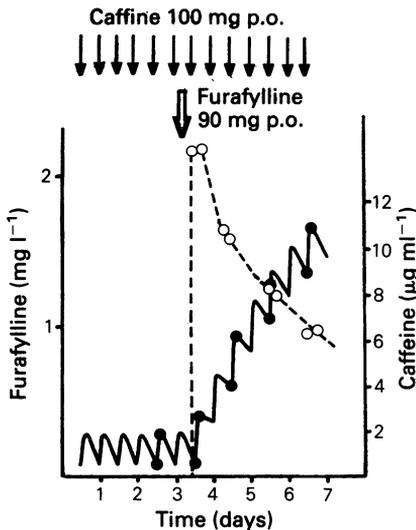


Figure 3 Plasma levels of furafylline (dotted lines) and caffeine (solid line) in one volunteer receiving repeated oral doses of caffeine and one single dose of furafylline as indicated. The elimination constants used for plotting caffeine concentration were (using an open mono-compartmental model and parameters described by Brazier *et al.* (1980)), $k_e = 0.100 \text{ h}^{-1}$ for the initial phase and 0.014 h^{-1} for the accumulation phase; the absorption constant was 1.9 h^{-1} in both instances (see Results).

Metabolic study of the accumulation of caffeine

In the present study the accumulation of caffeine in the blood was the same for both smokers and non-smokers suggesting that demethylation in

position 3 is the step inhibited by furafylline. Thus the accumulation is linearly related to the number of doses (cups of coffee) ingested, at least during the first 5 days (Figure 4) and a comparison of the regression lines allows a statistical comparison of the two populations (smokers and non-smokers). In the case of the single volunteer receiving caffeine alone, each 100 mg dose of caffeine was considered to be equivalent to 1 cup of coffee (Paoletti & Cataluppi, 1980).

Statistical analysis of the data presented in Figure 4 shows that the lines for smokers and non-smokers follow the equations $y = 1.32x + 3.511$ and $y = 1.48x + 2.267$ respectively where y is the caffeine concentration and x the number of cups of coffee. A comparison between the two lines using a test for parallelism based on Student's t -test showed that there was no significant difference ($0.40 > P > 0.30$) in the accumulation of caffeine between smokers and non-smokers. Each regression line was shown to fit the test of significance of linearity and slope.

In addition, the h.p.l. chromatograms of the plasma extract (Figure 5) show that as the caffeine concentrations increase in the presence of furafylline the metabolite concentrations decrease. When the same extracts were treated as described in **Methods** in order to identify the metabolite whose formation was being blocked by furafylline, the t_R value obtained by h.p.l.c. (eluent E) was 6 min which corresponds to the t_R value of paraxanthine. Furthermore u.v. spectroscopy of the eluent fraction corresponding to the metabolite showed a maximum at 270 nm and R_F

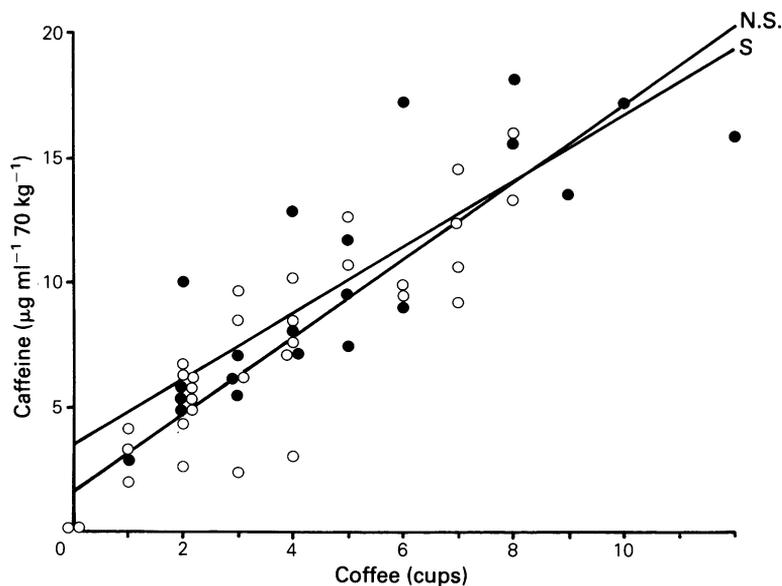


Figure 4 Caffeine levels reached in human plasma plotted against the number of cups of coffee ingested during the trial: (○) Smokers ($y = 1.32x + 3.511$, $r = 0.839$, $P < 0.05$) (●) Non smokers ($y = 1.48x + 2.267$, $r = 0.913$, $P < 0.05$). (See text).

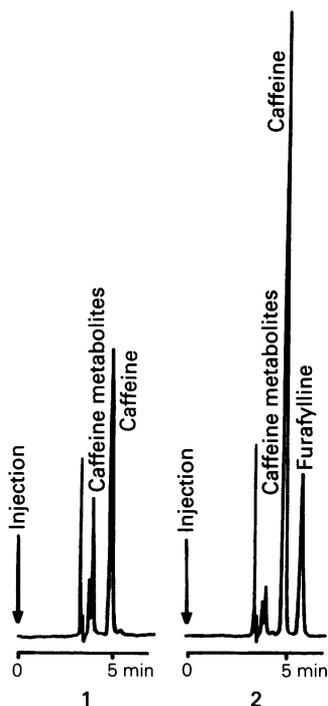


Figure 5 H.p.l. chromatograms of human plasma extracts after oral administrations of (1) caffeine alone and (2) caffeine plus furafylline.

values in t.l.c. using development solvents B and F were 0.24 and 0.30 respectively, coinciding with those of paraxanthine.

Table 3 The characteristics of xanthine derivatives extracted by HCl_3 from human plasma R_F^B value for solvent F, R_F^F value for solvent G.

Xanthine derivative	R_F^B	R_F^F
Theobromine	0.57	0.30
Theophylline	0.22	0.38
Paraxanthine	0.22	0.30
1,3,7-trimethyluric acid	0.09	0.46
Caffeine	0.80	0.44
Furafylline	0.73	0.41

Discussion

In agreement with the calculated predictions from single dose kinetic studies (Segura *et al.*, 1986), the oral administration of 90 mg of furafylline followed at 24 h intervals by 30 mg daily for 4 days, resulted in the steady state being reached after the first dose. The maximum and minimum plasma levels attained were 2 and 1.2 $\mu\text{g ml}^{-1}$ in males (mean body weight 67.2 kg) and 2.6 and 1.6 $\mu\text{g ml}^{-1}$ in females (mean body

weight 54.9 kg). Unfortunately, the repeated administration of furafylline appeared to be associated with the onset of adverse effects. The severity of the effects started to decline on day 6, when the drug was withdrawn, and all had virtually disappeared by day 9.

These findings were completely unexpected since the predicted and actual plasma levels on day 1 to 4 were the same and coincident with those obtained following a single dose of 125 mg of furafylline in a previous trial (Segura *et al.*, 1986) where no side effects were observed.

It seemed probable, therefore, that some other toxic substance was accumulating during the repeated administration of furafylline. A careful review of the h.p.l.c. traces (Figure 5) revealed the presence, in all but one of the chromatograms, of a peak whose magnitude increased during the period of the trial. The exception was volunteer no. 5, the only one not to have consumed coffee during the trial. Subsequent identification by h.p.l.c., t.l.c. and ultraviolet spectrophotometry proved that caffeine was the substance responsible for the extra chromatographic peak.

Nevertheless, although the adverse effects noticed are predominantly xanthine-like, it seems unlikely that caffeine accumulation *per se* can be the sole explanation of the observed effects. Thus, although the only volunteers (nos. 4 and 5) not showing such effects were those whose caffeine levels remained within normal dietary limits ($4\text{--}5\ \mu\text{g ml}^{-1}$, Christensen & Whittsett, 1976), in the remaining cases maximum caffeine levels ($10\text{--}40\ \mu\text{g ml}^{-1}$) were found on days 7–9 when the side effects had already passed their maximum intensities and had essentially disappeared.

It seems probable that the adverse effect phenomenon results from the simultaneous presence of caffeine and furafylline in the blood at concentrations which, when combined, exceed the tolerance limit. By superimposing the plasma concentration/times curves shown in Figure 1a (furafylline) and 1c (caffeine) and comparing with the incidence of adverse effects in Figure 1b, it can be seen that the furafylline levels are in steady-state and therefore the onset of adverse effects parallels the caffeine accumulation over the first 5 days. It would seem that these furafylline levels are just on the tolerance threshold of about $2.0\ \mu\text{g ml}^{-1}$ and at caffeine concentrations higher than $10\ \mu\text{g ml}^{-1}$ some additive effect results in the occurrence of signs of toxicity. Certainly, on withdrawing furafylline the incidence of adverse effects decreases in parallel with the falling furafylline blood levels in spite of a continued accumulation of caffeine. This would explain why no adverse effects were seen follow-

ing a single dose of furafylline (Segura *et al.*, 1986) since caffeine accumulation did not exceed $10\ \mu\text{g ml}^{-1}$ during the first 24 h when furafylline levels were approaching the tolerance limit and conversely when high caffeine were reached on days 3 and 4 the furafylline levels had fallen below $2.0\ \mu\text{g ml}^{-1}$ (Figure 2).

In the same study it was also evident that the basal caffeine levels remain constant during the first 4 h following the dose of furafylline, indicating negligible elimination, but start to accumulate on the 4th day of the experiment as coffee is ingested, reaching concentrations of $6.72\text{--}17.4\ \mu\text{g caffeine ml}^{-1}$.

Under normal circumstances, the renal excretion of caffeine is very low and its short elimination half-life in plasma is due to rapid biotransformation (Burg, 1975). It follows, therefore, that the accumulation of caffeine observed in the present work is most probably due to an inhibition of its metabolism. This supposition has been confirmed in the first instance by the experiment (Figure 3) showing that a single dose (90 mg) of furafylline results in a rapid accumulation of caffeine (given orally, 100 mg twice daily) and its elimination half-life, calculated from the accumulation pharmacokinetics, is some 50 h. The analysis of the h.p.l.c. graphs obtained with the plasma extracts from the same volunteer (Figure 5) show quite clearly that following the administration of furafylline, the increase in caffeine levels is accompanied by an abrupt decrease in metabolite levels.

Although some degree of interference in the metabolism of caffeine has been described for H_2 -histamine receptor blocking agents (Desmond *et al.*, 1980; Rendic *et al.*, 1982) and for oestrogens and progesterone (Knutti *et al.*, 1981; Tornatore *et al.*, 1982; Pathwardhan *et al.*, 1980), an effect thought to be due to the binding of such drugs to cytochromes P-450 and P-448, only one reference (Brazier *et al.*, 1980) has been found reporting an accumulation of caffeine of the same order as that found in the present work with furafylline. The drug involved is the muscle relaxant idrocilamide (*N*-(2-hydroxyethyl)-3-phenyl-2-propenamide).

The principal pathway of caffeine metabolism in man is via demethylation in position 3 and this pathway is induced by cigarette smoking resulting in lower plasma caffeine levels in smokers than in non-smokers (Kotake *et al.*, 1982; Weitholtz *et al.*, 1981). Since in the present work the accumulation of caffeine is clearly uninfluenced by the smoking habit (Figure 4), it may be implied that the inhibitory effect of furafylline on caffeine metabolism also occurs at the level of demethylation in position 3, thereby nullifying the ex-

pected difference between the two groups of volunteers. A similar situation has been described for idrocilamide, where it was also demonstrated that the accumulation of caffeine occurred at the expense of a reduced formation of paraxanthine (Brazier *et al.*, 1980). The reciprocal inhibition of paraxanthine formation is also evident in the present study where the inhibited metabolite is clearly distinguishable from the monomethylated xanthine derivatives (different t_R in h.p.l.c.) and from uric acid derivatives (different absorption maximum). Two dimensional thin layer chromatography also differentiates the metabolite from theophylline and theobromine and allows its identification as paraxanthine.

To our knowledge, this is the first demonstration of an inhibition of the metabolism of caffeine by a 1,3,8-trisubstituted xanthine in man, although using an animal model (rat) we have

also shown that the phenomenon is not exclusive to furafylline, but is common to a variety of such trisubstituted compounds (Segura & Tarrús, 1984). It seems probable, therefore, that the poor tolerance of verofylline (1,8-dimethyl-3-isobutyl-xanthine) in man (Cho *et al.*, 1981; Grassi *et al.*, 1980, 1981) is also due to the same phenomenon. In any event, the finding at rather late stages in their development, that some drugs are such potent metabolic inhibitors of a normal dietary constituent as to result in an unacceptable level of adverse effects, has obvious implications in the interpretation of drug-induced toxicity and emphasizes the value of a suitable model to allow early detection of the problem.

This work was presented in part by J. S. and E. T. at the 2nd European Congress of Biopharmaceutics and Pharmacokinetics, Salamanca, Spain, April 1984 (Abstr. 272).

References

- Brazier, J. L., Descotes, J., Levy, N., Ollangier, M. & Evreux, J. C. (1980). Inhibition by idrocilamide of the disposition of caffeine. *Eur. J. clin. Pharmac.*, **17**, 37-43.
- Burg, A. W. (1975). Physiological disposition of caffeine. *Drug Metab. Rev.*, **4**, 199-228.
- Cho, Y. W., Han, H. C., Oh, S. Y., Maines III, J. E. & Kuemmerle, H. P. (1981). A double-blind, placebo-controlled evaluation of (dl)-3,7-dihydro-1,8-dimethyl-3-(2-methylbutyl)-1H-purine-2,6-dione in exercise-induced bronchospasm. *Int. J. clin. Pharmac. Ther. Tox.*, **19**, 266-272.
- Christensen, H. D. & Whitsett, T. L. (1976). Pharmacokinetics of caffeine and coffee in the human. *Pharmacologist*, **18**, 177.
- Desmond, P. V., Pathwardhan, R., Parker, R., Schenker, S. & Speeg, K. V. Jr. (1980). Effect of cimetidine and other antihistaminics on the elimination of aminopyrine, phenacetin and caffeine. *Life Sci.*, **26**, 1261-1268.
- Grassi, C., Casali, L., Rampulla, C. & Rossi, A. (1980). Clinical efficacy trial: theophylline vs (dl)3, 7-dihydro-1,8-dimethyl-3-(2-methyl butyl)-1H-purine-2,6-dione. *Int. J. clin. Pharmac. Ther. Tox.*, **18**, 518-522.
- Grassi, C., Casali, L., Rampulla, C. & Rossi, A. (1981). Effect of (dl)3,7-dihydro-1,8-dimethyl-3-(2-methyl butyl)-1H-purine-2,6-dione on exercise-induced bronchospasm. *Int. J. clin. Pharmac. Ther. Tox.*, **19**, 297-302.
- Knutti, R., Rothweiler, H. & Schlatter, Ch. (1981). Effect of pregnancy on the pharmacokinetics of caffeine. *Eur. J. clin. Pharmac.*, **21**, 121-126.
- Kotake, A. N., Schoeller, D. A., Lambert, G. H., Baker, A. L., Schaffer, D. D. & Josephs, H. (1982). The caffeine CO₂ breath test: Dose response and route of N-demethylation in smokers and non-smokers. *Clin. Pharmac. Ther.*, **32**, 261-269.
- Orcutt, J. J., Kozak, P. P., Gillman, S. A. & Cummins, L. H. (1977). Micro-scale method for theophylline in body fluids by reversed phase, high pressure liquid chromatography. *Clin. Chem.*, **23**, 599-601.
- Paoletti, R. & Cantaluppi, S. (1980). New trends in the pharmacology of caffeine. *Colloq. Sci. Int. Caff. (C.R.)*, **9**, 41-53.
- Pathwardhan, K. M., Desmond, P. V., Johnson, R. F. & Schenker, S. (1980). Impaired elimination of caffeine by oral contraceptive steroids. *J. lab. clin. Med.*, **95**, 603-608.
- Pettigrew, A. R. & Fell, G. S. (1972). Simplified colorimetric determinations of thiocyanate in biological fluids, and its application to investigation of the toxic amblyopias. *Clin. Chem.*, **18**, 996-1000.
- Rendic, S., Alebic-Kolbach, T., Kajfez, F. & Ruf, H. H. (1982). Interaction of ranitidine with liver microsomes. *Xenobiotica*, **12**, 9-17.
- Segura, J., García, I. & Tarrús, E. (1986). The unusual pharmacokinetics characteristics of furafylline, a new 1,3,8-substituted xanthine, as compared to theophylline. *J. Pharm. Pharmac.*, **38**, 615-618.
- Segura, J. & Tarrús, E. (1984). Severe inhibition of caffeine metabolism by xanthine derivatives. *Proc. 2nd. Europ. Congress Biopharm. Pharmacokin.*, **2**, 520-528.
- Tornatore, K. M., Kanarkowski, R., McCarthy, T. L., Gardner, M. J., Yurchak, A. M. & Jusko, W. J. (1982). Effect of chronic oral contraceptive steroids on theophylline disposition. *Eur. J. clin. Pharmac.*, **23**, 129-134.
- Vega, A., Prieto, J., Moragues, J., Spickett, R. G. W., Llupiá, J., Llenas, J. & Roberts, D. J. (1985). Derivados de xantinas. Presented at the *IV Congreso Nacional de la Sociedad Española de Química Terapéutica*, Sitges (Barcelona), Spain, October, 1985.
- Wietholtz, H., Voegelin, M., Arnaud, M. J., Bircher,

J. & Preising, R. (1981). Assessment of the cytochrome P-448 dependent liver enzyme system by a caffeine breath test. *Eur. J. clin. Pharmac.*, **21**, 53-59.

*(Received 3 December 1985,
accepted 15 August 1986)*