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Effect of allopurinol on caffeine disposition in man

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Caffeine (5 mg kg^{-1}) was administered orally to two healthy, non-smoking subjects on three separate occasions—before, and during therapy with the xanthine oxidase inhibitor allopurinol at doses of either 300 or 600 mg daily. Plasma and urinary levels of methylxanthines, endogenous oxypurines and allopurinol and its metabolite oxypurinol were measured using h.p.l.c. analyses. Allopurinol treatment caused a specific, dose-dependent inhibition of the conversion of the caffeine metabolite 1-methylxanthine (1X) to 1-methyluric acid (1U). A good correlation was observed in both subjects between the urinary 1U/1X molar ratio and the ratio of endogenous urate to hypoxanthine + xanthine at the different allopurinol doses, supporting the proposal that the 1U/1X molar ratio after caffeine intake provides an *in vivo* index of xanthine oxidase activity in man.

Keywords caffeine allopurinol xanthine oxidase

Introduction

Caffeine (1,3,7-trimethylxanthine, 137X; abbreviations for methylxanthines are given at the bottom of Table 1) is biotransformed in man presumably by a number of distinct liver enzymes, which produce a variety of demethylated and 8-hydroxylated metabolites detectable in the plasma and/or urine (Bonati *et al.*, 1982; Callahan *et al.*, 1982; Tang-Liu *et al.*, 1983). In our studies (Grant *et al.*, 1983b) of variability in caffeine metabolism in human populations, we suggested that particular ratios of the five major urinary caffeine metabolites might serve as indices of specific liver enzyme activities, namely cytochrome(s) P-450, *N*-acetyltransferase and xanthine oxidase. The ratio of AFMU to 1X, for example, has been established as an index for the genetic polymorphism in acetylation capacity (Grant *et al.*, 1983a, 1984b), and is presently being used to distinguish 'rapid' and 'slow' acetylator individuals. However, the molar ratio of 1U to 1X, which we proposed as an indicator of xanthine oxidase activity, has yet to be verified as such.

Xanthine oxidase is a cytosolic enzyme which mediates the conversion of endogenous hypoxanthine and xanthine to uric acid (Coughlan, 1980). It may also take part in the detoxication (Rajagopalan, 1980) or activation (Krenitsky *et al.*, 1984; Kutcher & McCalla, 1984) of drugs and environmental chemicals. In methylxanthine metabolism, xanthine oxidase has been conclusively shown to catalyse the 8-hydroxylation of 1X to 1U after administration of 1X itself (Birkett *et al.*, 1983) and also after formation of 1X by 3-demethylation of theophylline (1,3-dimethylxanthine) (Grygiel *et al.*, 1979). *In vitro* studies indicate that all of the methylxanthines except for 1X are poor substrates for xanthine oxidase (Krenitsky *et al.*, 1972). Interestingly, the 1X formed from theophylline is almost completely converted to 1U in normal man, whereas roughly equal amounts of 1X and 1U are recovered in the urine after ingestion of caffeine (Grant *et al.*, 1983b) or paraxanthine (Grant, unpublished data). The reason for this difference is unknown at this time.

The intent of the present study was (1) to establish that the 8-hydroxylation of 1X to 1U after caffeine intake is in fact mediated by xanthine oxidase analogous to the situation after theophylline administration; (2) to determine whether the production of other caffeine metabolites *in vivo* is related to xanthine oxidase; and (3) to validate the use of the urinary 1U/1X molar ratio as an *in vivo* marker for this enzyme.

To do this, we have used the specific inhibitor allopurinol to alter *in vivo* xanthine oxidase activity, and have compared its effects on urine and plasma levels of both caffeine-derived and endogenous oxypurines in human subjects.

Methods

Chemicals and reagents

Allopurinol, oxypurinol, hypoxanthine, xanthine, uric acid, and 7-β-hydroxypropyltheophylline were obtained from Sigma Chemical Co., and sources of all other reference standards and analytical reagents have been detailed previously (Grant *et al.*, 1983b).

Subjects and drug administration

One male (Subject 1; 26 years, 63 kg) and one female (Subject 2; 30 years, 58 kg) participated in the study. Both were healthy, unmedicated non-smokers, and had previously been phenotyped for liver *N*-acetyltransferase activity as heterozygous rapid acetylators (Grant *et al.*, 1984b). Subjects were required to abstain from methylxanthine-containing foods and beverages for a total of 21 days. During each of three successive 7-day sessions, subjects received allopurinol (Zyloprim®) at daily doses of 0, 3 × 100 and 3 × 200 mg, given at 8-h intervals. On the sixth day of each session, after an overnight fast, subjects received a single oral dose of 5 mg kg^{-1} caffeine. Plasma samples were collected at 1.5, 4.5, 7.5, 10.5, 13.5, 16.5, 22.5, and 28.5 h after dosing. Urines (0–3, 3–6, 6–9, 9–12, 12–15, 15–18, 18–24, 24–27, and 27–30 h intervals) were collected, volumes measured, and aliquots were acidified to pH 3.5 with concentrated HCl and stored at −20° C until analysed. Allopurinol therapy was maintained throughout the period of caffeine administration and sample collection.

Analysis

Levels of caffeine and its detectable metabolites in plasma were determined by h.p.l.c. as follows: To a tube containing a 0.2 ml aliquot of plasma was added 120 mg of ammonium sulphate, followed by 50 μl of internal standard solution (7-β-hydroxypropyltheophylline, 20 mg l^{-1} in H_2O). The tube was vortexed vigorously for 15 s, and 6 ml of chloroform:isopropanol (90:10, v/v) was added, followed by vortexing for 30 s. After centrifugation to separate the phases, an aliquot of the organic layer was taken to dryness under N_2, and the residue was resuspended in 0.05% acetic acid. A volume of 20–50 μl was injected into a Hewlett-Packard Model 1084B liquid chromatograph equipped with a Waters Nova-Pak reversed-phase column. The column was eluted with methanol/0.05% acetic acid (14:86, v/v) at a flow rate of 1.0 ml min^{-1}, and compounds were detected by U.V. absorbance at 273 nm. Samples of blank plasma spiked with caffeine and its dimethylxanthine metabolites at concentrations up to 10 mg l^{-1} were taken through the above procedure, and standard curves relating peak heights of standards relative to that of the internal standard were used for quantitation.

Urinary caffeine metabolites were quantified as described previously (Grant *et al.*, 1983b), except that the extracting solvent was chloroform:isopropanol, 90:10, v/v. This allowed for the simultaneous determination of the methylxanthine and urate metabolites as well as the uracilic compound AFMU.

Plasma levels of allopurinol, oxypurinol, hypoxanthine, xanthine and uric acid were measured in each sample collected for caffeine analysis using the h.p.l.c. method of Miyazaki *et al.* (1983). Urinary levels of these compounds were determined by a modification of the method used by the above authors, as follows: Urine (0.5 ml) was mixed with 5 ml of 0.05% acetic acid, and 10 μl was injected onto an h.p.l.c. column (Beckman Ultrasphere ODS 5μ, 25 cm × 4.6 mm) which was eluted at 1.0 ml min^{-1} with a mobile phase of methanol/0.05% acetic acid (6:94, v/v). Compounds were detected by U.V. absorbance at 254 nm, and quantified by comparison of peak areas with those of authentic standards dissolved in water.

The elimination half-life ($t_{½}$) of unchanged caffeine was calculated from the log-linear decline of the plasma drug concentration by linear regression, and areas under the concentration-time curves (AUC) for caffeine and its metabolites were determined by the trapezoidal rule. Average renal clearances of endogenous and exogenous oxypurines were calculated, where possible, from plasma and urine concentrations as described (Gibaldi & Perrier, 1982).

Results

Plasma levels of oxypurinol, the metabolite of allopurinol contributing the major part of its inhibitory potency against xanthine oxidase (Spector, 1977), were relatively constant during allopurinol treatment, as shown by the small variance in eight measured concentrations during the 28.5 h period after each caffeine dose (Table 1). A linear relationship was also seen between

Table 1 Effect of allopurinol on plasma and urine parameters of endogenous and caffeine-derived oxypurines

	Subject 1			*Subject 2*		
Allopurinol dose (mg 24 h^{-1})	0	300	600	0	300	600
C_{plasma}[a]						
Oxypurinol (mg l^{-1})	0	5.0 ± 1.0	9.9 ± 1.1	0	4.7 ± 0.4	9.7 ± 0.6
Urate (mg dl^{-1})	4.09 ± 0.05	3.91 ± 0.10	4.02 ± 0.05	4.42 ± 0.11	4.42 ± 0.06	4.26 ± 0.05
Urinary excretion (μmol 24 h^{-1})						
Xanthine (X)	116	314	1043	46	983	1876
Hypoxanthine (HX)	122	265	487	82	430	691
Uric acid (U)	3657	2305	1762	2035	1230	565
U/(HX + X)	15.4	4.0	1.2	15.9	0.9	0.2
Caffeine $t_{½}$ (h)	5.4	5.5	5.8	3.9	3.4	4.0
Urinary excretion[b]						
1X	17.1	28.6	33.5	21.0	37.2	44.9
1U	13.5	9.5	3.8	21.7	5.5	3.3
AFMU	27.9	21.7	23.6	27.5	26.7	25.5
17X	12.6	13.4	14.8	6.8	8.3	6.9
17U	15.1	10.2	8.2	9.1	8.0	6.4
37X	3.5	3.0	3.5	2.1	2.0	1.4
7X	5.3	6.5	7.5	6.6	7.0	6.8
3X	2.8	5.0	2.7	3.0	3.2	2.7
13U	2.3	2.1	2.4	2.1	2.2	2.2
Recovery (% dose)	39.8	67.4	54.7	56.1	69.0	85.6
IU/1X	0.79	0.33	0.11	1.03	0.15	0.07

[a]Mean ± s.d. of 8 determinations over a 28.5 h period, on day 6 of allopurinol administration
[b]molar % of total quantified caffeine metabolites in 24 h
Methylxanthine
Abbreviations: 137X: caffeine; 1X: 1-methylxanthine, 1U: 1-methyluric acid; AFMU: 5-acetylamino-6-formyl-amino-3-methyluracil; 17X: 1,7-dimethylxanthine; 17U: 1,7-dimethyluric acid; 37X: 3,7-dimethylxanthine; 7X: 7-methylxanthine; 3X: 3-methylxanthine; 13U: 1,3-dimethyluric acid

the daily allopurinol dose and mean plasma oxypurinol levels. In view of the long plasma elimination half-life of oxypurinol (Hande *et al.*, 1978), which exceeds the 8 h allopurinol dosing interval used here, this constancy shows that steady state levels of the inhibitory metabolite had been reached by the sixth day of therapy at each allopurinol dose. Unchanged allopurinol was detectable only in plasma samples collected shortly after its intake, indicative of its rapid conversion to oxypurinol. Average oxypurinol renal clearances were 33 ml min^{-1} and 24 ml min^{-1} for subjects 1 and 2, respectively. These results are in good agreement with previously published data (Hande *et al.*, 1978).

Allopurinol therapy, as expected, caused a dose-dependent decrease in the renal excretion of endogenous uric acid (U) and a corresponding increase in excretion of hypoxanthine (HX) and xanthine (X), so that the molar ratio of U to HX + X fell markedly. Plasma urate, however, remained remarkably constant in both subjects during allopurinol, suggesting a decrease in the renal clearance of urate. Average (24 h) renal clearances of urate for the 0, 300 and 600 mg daily allopurinol doses were 11.0, 7.3 and 5.3 ml min^{-1} (Subject 1) and 5.2, 3.6 and 1.7 ml min^{-1} (Subject 2). Allopurinol also tended to cause an increase in the renal clearance of xanthine (from 40 ml min^{-1} to 66 ml min^{-1} in Subject 1 and from 27 ml min^{-1} to 113 ml min^{-1} in Subject 2).

Plasma kinetics of caffeine and its dimethylxanthine metabolites were not affected in any consistent fashion by allopurinol treatment, so that the plasma $t_{½}$ of caffeine (Table 1) and the AUCs for its primary demethylation products were essentially unchanged. However, an increase in plasma 1X from negligible levels to easily detectable concentrations was reflected in an increase in the plasma AUC for this metabolite. The major effect of allopurinol was to increase 1X and decrease 1U excretion in urine, as reflected by a marked decrease in the 1U/1X molar ratio. This is consistent with inhibition of xanthine oxidase-mediated conversion of 1X to 1U. Total recovery (as % of caffeine dose) was

increased with allopurinol. A slight decline in urinary excretion of 17U was also seen. Notably, there was no effect on AFMU excretion, providing supportive evidence for its formation via the pathway proposed previously (Grant *et al.*, 1983b).

Figure 1 shows the correlation in individual 3 h urine samples between the caffeine-derived 1U/1X ratio and the ratio of endogenous U to HX + X for both subjects at 0, 300 and 600 mg daily doses of AL. Correlation coefficients were 0.87 and 0.92 for Subjects 1 and 2, respectively ($P < 0.001$, $n = 26$).

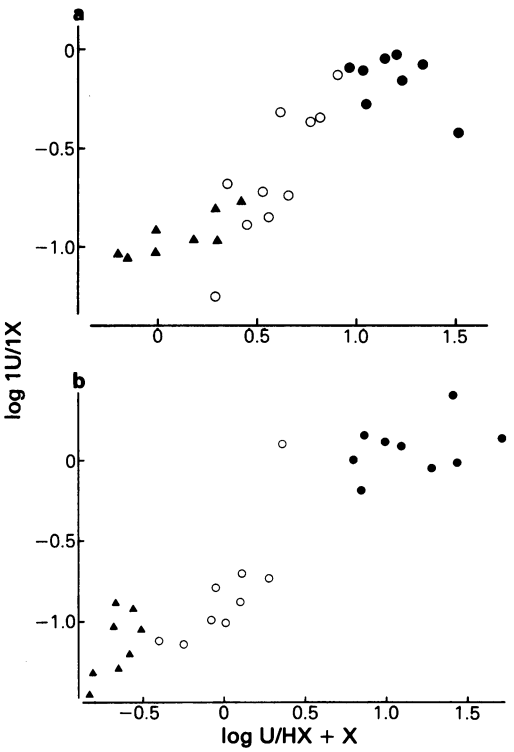

Figure 1 Correlations in individual urine samples between caffeine-derived 1U/1X ratio and endogenous U/(HX + X) ratio for (a) Subject 1, and (b) Subject 2, at allopurinol daily doses of 0 (●), 300 (○), and 600 (▲) mg.

Discussion

The lack of effect of allopurinol on caffeine plasma kinetics indicates firstly that xanthine oxidase activity does not govern to any appreciable extent the disappearance of caffeine from plasma, and secondly that allopurinol at the doses and duration used in this study does not inhibit the enzyme (probably a human cytochrome P_1-450 analogue) responsible for the primary demethylations of caffeine. Such an inhibitory effect had previously been noted with respect to theophylline plasma kinetics using a longer duration of allopurinol treatment (Manfredi & Vesell, 1981).

The dose-dependent effect of allopurinol upon urinary excretion of both the caffeine-derived and the endogenous oxypurines is consistent with the premise that xanthine oxidase is responsible for the conversion of endogenous xanthines and dietary methyl xanthines to their corresponding urates. However, the alterations in renal clearances observed in this study merit further comment. The constancy in plasma urate despite inhibition of its formation from xanthine can be attributed to a decrease in its renal clearance. Decreased renal urate clearance associated with allopurinol therapy has been noted previously in gouty patients (Gutman *et al.*, 1969). This effect may be pharmacological in nature due to an inhibitory effect of oxypurinol on urate secretory processes. Alternatively, it may be a physiological response by the kidney in an attempt to maintain plasma urate at some desired level in the face of decreased synthesis. Support for the latter interpretation was provided by Steele & Underwood (1976), who showed that decreased renal urate clearance in the rat during allopurinol administration was governed by plasma urate levels, and was not a result of the drug treatment itself.

In addition we found that the renal clearance of xanthine was elevated with allopurinol therapy. Such an effect has been noted in other studies after allopurinol and also in individuals genetically deficient in xanthine oxidase activity (for review, see Holmes & Wyngaarden, 1983) and is again considered to be a physiological response to elevated plasma xanthine levels. With this in mind, the increase in total recovery of caffeine metabolites seen in this study during allopurinol therapy may be due to an increase in the renal clearance of methylxanthine metabolites, especially 1X, which increased in urine by a greater amount than could be accounted for by a stoichiometric decrease in 1U. This may be occurring by an analogous mechanism to that operating for the endogenous xanthines.

The decrease seen in 17U excretion may be explained in two ways. Firstly, 17U renal clearance could be decreased by a mechanism similar to that for endogenous urate. On the other hand, preliminary *in vitro* data (Grant *et al.*, 1984a) using human liver tissue fractions suggest that a portion of 17U produced from paraxanthine is not via cytochrome P-450 and may thus be formed by xanthine oxidase. The decrease in 17U seen

here may then be due to inhibition of formation of that portion of the metabolite formed by this route.

In summary, the present study provides evidence that xanthine oxidase mediates the conversion of 1X to 1U after caffeine intake, and does not take part in the production of other caffeine metabolites with the possible exception of 17U. The molar ratio of 1U to 1X may then provide a valid *in vivo* index of xanthine oxidase activity in man.

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References

Birkett, D. J., Miners, J. O. & Attwood, J. (1983). Secondary metabolism of theophylline biotransformation products in man—route of formation of 1-methyluric acid. *Br. J. clin. Pharmac.*, **115**, 117–119.

Bonati, M., Latini, R., Galletti, F., Young, J. F., Tognoni, G. & Garattini, S. (1982). Caffeine disposition after oral doses. *Clin. Pharmac. Ther.*, **32**, 98–106.

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. Dispos.*, **10**, 417–423.

Coughlan, M. P. (1980). Aldehyde oxidase, xanthine oxidase and xanthine dehydrogenase: hydroxylases containing molybdenum, iron-sulfur and flavin. In *Molybdenum and molybdenum-containing enzymes*, ed Coughlan, M. P., pp. 119–185. Oxford: Pergamon Press.

Gibaldi, M. & Perrier, D. (1982). *Pharmacokinetics*, pp. 10–15. New York and Basel: Marcel Dekker, Inc.

Grant, D. M., Tang, B. K., Campbell, M. E. & Kalow, W. (1984a). Metabolism of paraxanthine by 9000 g supernatant from rabbit and human liver. 6th *International Symposium on Microsomes and Drug Oxidations*, Brighton, England, p. 97.

Grant, D. M., Tang, B. K. & Kalow, W. (1983a). Polymorphic *N*-acetylation of a caffeine metabolite. *Clin. Pharmac. Ther.*, **33**, 355–359.

Grant, D. M., Tang, B. K. & Kalow, W. (1983b). Variability in caffeine metabolism. *Clin. Pharmac. Ther.*, **33**, 591–603.

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

Grygiel, J. J., Wing, L. M. H., Farkas, J. & Birkett, D. J. (1979). Effects of allopurinol on theophylline metabolism and clearance. *Clin. Pharmac. Ther.*, **26**, 660–667.

Gutman, A. B., Yu, T-F. & Berger, L. (1969). Renal function in gout. III. Estimation of tubular secretion of uric acid by use of pyrazinamide (pyrazinoic acid). *Am. J. Med.*, **47**, 575–592.

Hande, K., Reed, E. & Chabner, B. (1978). Allopurinol kinetics. *Clin. Pharmac. Ther.*, **23**, 598–605.

Holmes, E. W. & Wyngaarden, J. B. (1983). Hereditary xanthinuria. In *The Metabolic Basis of Inherited Disease*, eds Stanbury, J. B., Wyngaarden, J. B., Frederickson, D. S., Goldstein, J. L. & Brown, M. S., pp. 1192–1201. New York: McGraw-Hill Co.

Krenitsky, T. A., Neil, S. M., Elion, G. B. & Hitchings, G. H. (1972). A comparison of the specificities of xanthine oxidase and aldehyde oxidase. *Arch. Biochem. Biophys.*, **150**, 585–599.

Krenitsky, T. A., Hall, W. W., deMiranda, P., Beauchamp, L. M., Schaeffer, H. J. & Whiteman, P. D. (1984). 6-deoxyacyclovir: a xanthine oxidase-activated prodrug of acyclovir. *Proc. Nat. Acad. Sci.* (USA), **81**, 3209–3213.

Kutcher, W. W. & McCalla, D. R. (1984). Aerobic reduction of 5-nitro-2-furaldehyde semicarbazone by rat liver xanthine dehydrogenase. *Biochem. Pharmac.*, **33**, 799–805.

Manfredi, R. L. & Vesell, E. S. (1981). Inhibition of theophylline metabolism by long-term allopurinol administration. *Clin. Pharmac. Ther.*, **29**, 224–229.

Miyazaki, H., Matsunaga, Y., Yoshida, K., Arakawa, S. & Hashimoto, M. (1983). Simultaneous determination of plasma uric acid, xanthine, hypoxanthine, allopurinol, oxipurinol, orotic acid, orotidine and creatinine by high-performance liquid chromatography. *J. Chromatogr.*, **274**, 75–85.

Rajagopalan, K. V. (1980). Xanthine oxidase and aldehyde oxidase. In *Enzymatic Basis of Detoxication* v. 1, ed Jakoby, W. B., pp. 295–309. New York: Academic Press.

Spector, T. (1977). Inhibition of urate production by allopurinol. *Biochem. Pharmac.*, **26**, 355–358.

Steele, T. H. & Underwood, J. L. (1976). Renal urate transport during variations in urate synthesis in the rat. *Pflugers Arch.*, **367**, 183–188.

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.

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