SECONDARY METABOLISM OF THEOPHYLLINE BIOTRANSFORMATION PRODUCTS IN MAN—ROUTE OF FORMATION OF 1-METHYLURIC ACID

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The route of formation of 1-methyluric acid (1MU) from theophylline has been investigated by i.v. administration of 1-methylxanthine (1MX) before and after allopurinol treatment and of 1,3-dimethyluric acid (1,3DMU) to healthy male volunteers. 1,3DMU was recovered unchanged in urine and was not demethylated to 1MU. By contrast after administration of 1MX the major part of the dose was recovered as 1MU. After allopurinol this was reversed with most of the 1MX dose being excreted unchanged and only a minor amount as 1MU. The results show that 1MU derived from theophylline is formed by an initial 3-demethylation to 1MX and then a rapid xanthine oxidase mediated 8-oxidation. No 1MU is formed by 3-demethylation of 1,3DMU.

Introduction

The three major products of theophylline biotransformation (Figure 1) are 3-methylxanthine (3MX), 1-methyluric acid (1MU) and 1,3-dimethyluric acid (1,3DMU) (Cornish & Christman, 1957; Thompson *et al.*, 1974; Jenne *et al.*, 1976; Grygiel *et al.*, 1979). 1MU could theoretically be formed either by an initial 3-demethylation followed by 8-oxidation or by the

same reactions in the reverse order. In the former case 1-methylxanthine (1MX) would be a metabolic intermediate in the formation of 1MU but most studies have failed to demonstrate 1MX as an excretion product following theophylline administration (Cornish & Christman, 1957; Thompson *et al.*, 1974; Jenne *et al.*, 1976). Recently, we (Grygiel *et al.*,

Figure 1 Theophylline biotransformation showing two possible routes for formation of 1-methyluric acid.

1979) have reported finding traces of 1MX in the urine of healthy subjects during chronic theophylline administration. We further showed that allopurinol treatment caused an increase in 1MX excretion and a close to stoichiometric decrease in 1MU excretion strongly supporting the concept that 1MU is formed by an initial 3-demethylation of theophylline followed by a rapid xanthine oxidase mediated 8-oxidation. However, Antal *et al.* (1981) recently reported they could find no evidence for excretion of 1MX after single oral doses of theophylline and suggested that 1MU is formed via 1,3DMU. They further suggested that our findings were due to metabolism of residual caffeine following too short a restriction of dietary methylxanthines. It should be noted, however, that the studies reported by us involved repeated analysis of urinary theophylline metabolites during chronic dosing with theophylline, with or without allopurinol, for a total of 16 days. A methylxanthine free diet was enforced for 2 days prior to and during each study so that the total duration of methylxanthine restriction was 18 days, making any interference from residual caffeine extremely unlikely. To resolve the question of the route of formation of 1MU, we have now administered 1MX and 1,3DMU on separate occasions to healthy volunteers and analysed urine to determine excretion of 1MX, 1MU and 1,3DMU.

Methods

1MX was purchased from Fluka AG and 1,3DMU from Adams Chemical Co. (Round Lake, Illinois, USA). Their composition was verified by elemental analysis and no impurities could be detected by high performance liquid chromatography. Sterile solutions of the two compounds were prepared for intravenous administration by dissolving 1MX or 1,3DMU

(75 mg) in a final volume of 9 ml of water alkalised with sodium hydroxide. The final pH of the solutions were in the range 9.5–10.5. Solutions were sterilised by filtration and used within 1 h although it was shown they were stable for at least 12 h.

The subjects were two (1MX) or three (1,3DMU) healthy male volunteers who were maintained on a methylxanthine free diet for 3 days prior to and during the studies. The solutions were administered over 1 h in 1.5 ml (12.5 mg) aliquots given at 10 min intervals via an indwelling cannula in a forearm vein. 1MX was administered on two occasions. One of these was after 3 days treatment with allopurinol 100 mg, 8-hourly, the last allopurinol dose being given at the start of 1MX administration. Urine was collected over an 8 h interval after beginning 1MX or 1,3DMU administration. Urine was analysed for 1MX, 1MU and 1,3DMU as previously described (Grygiel *et al.*, 1979). Heart rate and blood pressure were monitored before injection of each aliquot and at 15 min intervals for 1 h after the last injection. There were no changes in these parameters at any stage and no adverse effects were noted. The study was approved by the Australian Department of Health and The Clinical Investigation Committee of Flinders Medical Centre.

Results

Table 1 shows the percentage of the doses recovered over 8 h in urine as 1MX, 1MU and 1,3DMU after administration of 1MX (with and without allopurinol treatment) or 1,3 DMU. After administration of 1,3DMU only the unchanged compound was excreted in urine and no 1MU was detectable confirming that DMU is excreted unchanged and is not demethylated. By contrast, after 1MX the major product recovered in urine was 1MU with only 13.7%

Table 1 Urinary excretion products after intravenous administration of 1MX or 1,3DMU

Subject	1MX excretion products*		1,3DMU excretion products*	
	1MX	1MU	1,3DMU	1MU
A	—	—	91.0	ND
B	27.5 (29.7) ⁺	65.0 (70.3)	78.9	ND
B [†]	71.4 (90.1)	7.8 (9.9)	—	—
C	11.7 (13.7)	73.8 (86.3)	93.4	ND
C [†]	63.4 (86.5)	9.9 (13.5)	—	—

* Percentage of dose (75 mg) recovered in urine in 8 h

⁺ Figures in parentheses are expressed as percentage of total recovered 1MX + 1MU

[†] with allopurinol

and 29.7% being excreted unchanged by subjects B and C respectively. Allopurinol treatment had a dramatic effect on the excretion products after 1MX, the majority of the dose being excreted unchanged with only minor conversion to 1MU. For 1MX

$$CL_m = CL_T \times f_m \text{ and}$$

$$CL_T = CL_r + CL_m$$

so that $CL_m = CL_r (f_m/1 - f_m)$

where CL_m is the metabolic clearance to 1MU, f_m is the fractional recovery as 1MU in urine, CL_T is the total plasma clearance of 1MX, and CL_r is the renal clearance of unchanged 1MX. From the data in Table 1 it can be calculated that allopurinol caused a decrease in CL_m for 1MX from 2.35 CL_r to 0.11 CL_r for subject B, and from 6.3 CL_r to 0.16 CL_r for subject C. If renal clearance of 1MX is assumed to be constant in control and allopurinol studies this represents a 21.5-fold decrease in CL_m to 4.5% of control in subject B, and a 40.4-fold decrease to 2.5% of control in subject C.

Discussion

The results of this study confirm that the only pathway for formation of 1MU from theophylline is via 1MX and that 1,3DMU is excreted unchanged without demethylation. The very marked effect of allopurinol on the conversion of 1MX to 1MU confirms that this reaction is mediated by xanthine oxidase. This is consistent with *in vitro* studies which show that 1MX, unlike other methylxanthines, is a good substrate for mammalian xanthine oxidases (Bergmann & Dikstein, 1956; Lohmann & Miech, 1976; Krenitsky *et al.*, 1972). In the absence of allopurinol treatment the metabolic clearance of 1MX is three- to six-fold greater than the renal clearance. Only minor amounts are therefore excreted unchanged accounting for the difficulty in detecting 1MX in urine after dosing with theophylline.

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