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A metabolic and pharmacokinetic comparison of theophylline and aminophylline (theophylline ethylenediamine)

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The metabolism and pharmacokinetics of intravenously administered theophylline and aminophylline (theophylline ethylenediamine) have been studied in 3 volunteers, using ^{14}C-labelled theophylline. Both compounds were metabolized extensively and 1,3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine and two unknown minor metabolites were excreted in the urine, in addition to theophylline. The elimination of theophylline, 1,3-dimethyluric acid, 1-methyluric acid and the unknown metabolites followed first-order kinetics, but that of 3-methylxanthine followed Michaelis-Menten kinetics. When given as aminophylline, theophylline was metabolized more rapidly and extensively than when given alone. The recovery of ^{14}C in the urine was significantly higher after aminophylline than after theophylline. Abstention from intake of dietary methylxanthines for 7 days resulted in more rapid and extensive metabolism of aminophylline compared with results from the same subjects on their usual diets. The results indicate that, from a metabolic and pharmacokinetic viewpoint, aminophylline and theophylline are not equivalent.

Theophylline (1,3-dimethylxanthine) is a drug widely used in the treatment of respiratory disorders. It is sparingly soluble in water and therefore is not given intravenously. It does, however, form water-soluble derivatives with a number of agents including ethylenediamine, choline, adenosine and sodium salicylate and benzoate, to name but a few, and this property can be utilized pharmaceutically for preparing soluble delivery forms of theophylline. By far the most commonly used agent for this purpose is ethylenediamine, the combination being referred to as aminophylline, and this has been in use in therapeutics since 1908 (Dessauer 1908).

Despite its popularity and widespread use there appears to be some doubts as to the exact chemical nature of aminophylline. Thus, it is variously referred to as a salt (American Hospital Formulary Service 1978), a stable mixture or combination (Martindale 1977) or as a compound of two molecules of theophylline with one molecule of ethylenediamine (Merck Index 1976).

The pharmacological literature in general does not make a distinction between theophylline and aminophylline assuming them to be equivalent; this assumption may not be entirely justified.

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If aminophylline exists under physiological conditions as a molecular complex of theophylline and ethylenediamine then its pharmacokinetic and pharmacodynamic properties could differ from those of the parent methylxanthine. Alternatively, if aminophylline functions as a salt of a weak acid with a strong base then the latter (ethylenediamine), which has biological properties of its own, may affect the disposition and activity of theophylline. There are many examples in the literature of the effect of complexation and formulation-related differences on drug disposition (Groves 1979) so it would not be surprising if theophylline and aminophylline were not equivalent in this respect. We have therefore undertaken a study of the metabolism and pharmacokinetics of [^{14}C]aminophylline (labelled in the theophylline moiety) given intravenously to three volunteers. The findings are compared with those obtained in the same volunteers after intravenous injection of [^{14}C]theophylline. The influence of the withdrawal of all methylxanthine-containing foods from the diet for 7 days on the fate of intravenously administered aminophylline has also been investigated since this procedure has previously been shown to alter the disposition of theophylline (Monks et al 1979).

MATERIALS AND METHODS

8-[^{14}C]Theophylline (sp. act. 34mCi mmol^{-1}, radiochemical purity 99%) was purchased from the Radiochemical Centre, Amersham, U.K. and theophylline and ethylenediamine from Sigma London Chemical Co., Gillingham, Dorset, U.K. Other compounds were as described previously (Monks et al 1979).

[^{14}C]*Aminophylline*

[^{14}C]Aminophylline injection was prepared from 8-[^{14}C]theophylline and ethylenediamine as described in the British Pharmacopoeia (1973), to contain 125 mg aminophylline and 10 μCi ^{14}C in 10 ml (equivalent to 100 mg theophylline). This was sterilized by ultrafiltration before injection into the volunteers.

Volunteers and drug treatment

The participants were three healthy male volunteers, ages 21–30 years, on their normal and habitual diets, who gave their informed consent. The study had the approval of the Hospital and Medical School Ethical Committee and the MRC Isotopes Advisory Panel approved the administration of isotopes.

The study was divided into two parts. The subjects were first infused intravenously with [^{14}C]aminophylline (125 mg, 10 μCi; equivalent to 100 mg theophylline) over 10 min, and urine samples collected at regular intervals (see Fig. 1) over 48 h. After three months, two of the subjects abstained from the intake of all dietary methylxanthine-containing foods and beverages (coffee, tea, cola drinks and chocolate) for seven days before the intravenous infusion of [^{14}C]aminophylline as above. Urine samples were collected as before and the usual diet was not resumed until after the final urine collection.

Analysis of urinary metabolites

The ^{14}C content of urine, column eluates and other solutions was determined by liquid scintillation counting of 0·1–1 ml aliquots using a Triton X-100-toluene scintillator (5 ml) with a Packard Tri-Carb liquid scintillation spectrometer Model 3385, quench correction being by external standardization. ^{14}C on chromatograms was located by radiochromatogram scanning (Packard Model 7201) and quantitated by scintillation counting of sections of the chromatogram (see Monks et al 1979).

Urinary metabolites were assayed by ion exchange column chromatography (to separate xanthines from uric acids) followed by ion exchange paper chromatography to separate the individual metabolites in each fraction, as described by Monks et al (1979).

Pharmacokinetic analysis

Previous studies on theophylline metabolism (Caldwell et al 1977; Monks et al 1979) have shown that the elimination of theophylline, 1,3-dimethyluric acid, 1-methyluric acid and the unknown metabolites proceeds by apparent first-order kinetics while that of 3-methylxanthine is described by Michaelis-Menten kinetics. The rate constants describing the elimination of each metabolite were thus obtained by the use of the following linearized form of the Michaelis-Menten equation (Levy et al 1972):

$$\frac{S}{v} = \frac{K_m}{V_{max}} + \frac{S}{V_{max}}$$

where S is the substrate concentration, in this case the amount of theophylline remaining in the body (assumed to be unmetabolized), v is the rate of formation of the metabolite, here the rate of excretion of the metabolite in the urine (assumed to be limited by its formation) and K_m and V_{max} are the Michaelis-Menten parameters for each metabolic pathway. From this equation, when S/v is plotted against S, the slope of the line is $1/V_{max}$ and the intercept on the S/v axis is K_m/V_{max}. When data best described by apparent first-order kinetics are plotted in this fashion, then the slope of the line is zero and the intercept on the S/v intercept is 1/k, since the apparent first-order rate constant (k) is V_{max}/K_m. When the data are best described by Michaelis-Menten kinetics, then the line has a positive slope, permitting calculation of K_m and V_{max} as described.

RESULTS

Comparative elimination of aminophylline and theophylline

The cumulative urinary excretion of radioactivity by three volunteers after the intravenous injection of [^{14}C]aminophylline (125 mg ≡ 100 mg theophylline) is shown in Fig. 1. Also shown is the urinary excretion of radioactivity after the intravenous injection in the same three volunteers of an equivalent dose of theophylline (Monks et al 1979). After aminophylline injection some 90% of the dose was recovered in 24 h with a further 7% in the next 24 h. By comparison, an equivalent dose of theophylline was excreted significantly more slowly by the same three volunteers; 76% of the ^{14}C was eliminated in 24 h with a further 9% in the subsequent 24 h ($P < 0.05$). Table 1 shows the qualitative and quantitative aspects of the urinary elimination of aminophylline compared with theophylline. The urinary metabolites of aminophylline were identical with those found for theophylline, namely, 3-methylxanthine, 1,3-dimethyluric

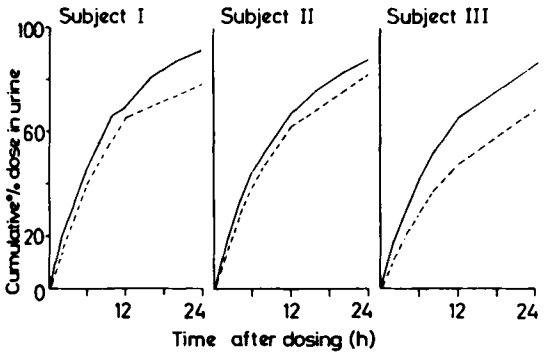

FIG. 1. Cumulative excretion of ^{14}C in the urine by three volunteers after the intravenous injection of [^{14}C]-aminophylline and [^{14}C]theophylline. Data for aminophylline from this study, for theophylline from Monks et al (1979). —— Aminophylline. - - - Theophylline.

acid and 1-methyluric acid. In addition, two further unknown metabolites were present which comprise the 'unidentified' material. Chromatographic evidence indicates that one of these unknown metabolites has the characteristics of a xanthine derivative and the other those of a uric acid.

Although the qualitative patterns of metabolism of aminophylline and theophylline are similar, there are small but significant differences in the amounts of two metabolites excreted, namely, 3-methylxanthine and 1,3-dimethyluric acid. The product of *N*-1 demethylation, 3-methylxanthine accounted for 21% of the dose when the subjects received aminophylline compared with 16·5% for theophylline ($P < 0.05$). 1,3-Dimethyluric acid excretion was also higher (37·0% of dose) after aminophylline when compared with theophylline (28%; $P < 0.05$). Theophylline accounted for 10.2% of the dose of aminophylline

Table 1. Comparative metabolism of aminophylline and theophylline in the same volunteers. [^{14}C]Aminophylline (125 mg; 10 μCi) was given intravenously to three volunteers (Fig. 1). Serial urine samples were collected and analysed as described in the text. The investigation was repeated four months later using [^{14}C]theophylline (100 mg i.v.; 10 μCi).

Drug Administered:	% dose excreted in 24 h as:		
Metabolite	Aminophylline	Theophylline†	P*
Theophylline	10·2 (6·8–14·7)	8·4 (6·5–11·1)	n.s.
3-Methylxanthine	21·2 (19·9–22·1)	16·5 (15·8–17·4)	<0·05
1,3-Dimethyluric acid	37·0 (34·4–40·1)	28·0 (26·0–30·1)	<0·05
1-Methyluric acid	17·3 (17·1–17·4)	18·9 (12·9–25·0)	n.s.
Unidentified	1·6 (1·1–2·0)	4·6 (2·3–6·3)	n.s.
Total	87·3 (85·0–89·7)	76·4 (68·6–82·0)	—
^{14}C excreted in urine	89·7 (87·5–91·2)	76·3 (68·6–82·0)	<0·05

*P**aminophylline *vs* theophylline, paired 2-tailed *t*-test.
† data taken from a previous study in the same volunteers (Monks et al 1979).

and 8·4% when administered as such; this difference is not statistically significant.

The pharmacokinetic parameters describing the elimination of aminophylline and theophylline are shown in Table 2. These figures were derived from the analytical values found for the urinary elimination of theophylline and its metabolites in serial urine samples by the graphical method described. Plots of amount theophylline unexcreted/rate of urinary

Table 2. Pharmacokinetic parameters describing the urinary elimination of aminophylline and theophylline in Volunteers (n = 3).

Parameter	Aminophylline	Theophylline*	P
k^{T}_{el} (h^{-1})	0·010 (0·009–0·011)	0·009 (0·005–0·016)	n.s.
k^{DMU}_{el} (h^{-1})	0·034 (0·031–0·036)	0·026 (0·025–0·027)	<0·05
k^{MU}_{el} (h^{-1})	0·016 (0·015–0·016)	0·018 (0·011–0·022)	n.s.
k^{U}_{el} (h^{-1})	0·001 (0·001–0·002)	0·005 (0·002–0·008)	n.s.
V^{3MX}_{max}(mg h^{-1})	1·66 (0·43–1·96)	1·06 (0·74–1·30)	<0·05
K^{3MX}_{m} (mg)	31·5 (25·6–42·7)	19·1 (3·1–29·6)	n.s.
$t_{\frac{1}{2}}$ (h)	7·4 (6·9–7·7)	10·0 (8·4–13·3)	<0·05

Parameters and the mode of calculation as described in the text.
* data taken from a previous study in the same volunteers (Monks et al 1979).

excretion of metabolite vs amount theophylline unexcreted for theophylline, 1,3-dimethyluric acid and 1-methyluric acid had slopes of zero, and the apparent first-order rate constants for the elimination of theophylline (k^{T}_{el}), 1,3-dimethyluric acid (k^{DMU}_{el}) 1-methyluric acid (k^{MU}_{el}) and the unknown metabolites (k^{U}_{el}) were calculated as described. When a similar plot was prepared for 3-methylxanthine elimination, it had a positive slope, and the Michaelis-Menten parameters for its elimination (V^{3MX}_{max} and K^{3MX}_{m}) were calculated as described.

In the three volunteers, the overall elimination half-life of radioactivity from [^{14}C]aminophylline was 7·4 h which was significantly shorter than that found for theophylline itself (10·0 h, $P < 0.05$). This was due to a small but significant ($P < 0.05$) increase in the apparent first order-rate constant for 1,3-dimethyluric acid formation compared with that for theophylline. The V_{max} for the formation of 3-methylxanthine was also increased in the case of aminophylline but although the K_m value for this demethylation was increased, this did not reach significance at the 5% level. There are thus small but significant differences between aminophylline and theophylline in terms of their metabolic disposition and elimination.

Influence of methylxanthine-deprived diet on disposition of aminophylline

Previous work has shown that theophylline disposition is influenced by dietary methylxanthines. Thus,

the overall urinary elimination half-life of theophylline, and other aspects of its elimination, change when subjects on their normal diets switch for a period to a diet containing no methylxanthines. Since the disposition of aminophylline differs significantly from that of theophylline we have determined whether its elimination is also affected by dietary methylxanthine deprivation. Accordingly, the disposition of [^{14}C]-aminophylline was investigated in two of the three volunteers who participated in the first study but after a seven day abstinence from all dietary methylxanthines.

Fig. 2 shows the cumulative excretion of ^{14}C by the volunteers following the intravenous injection of

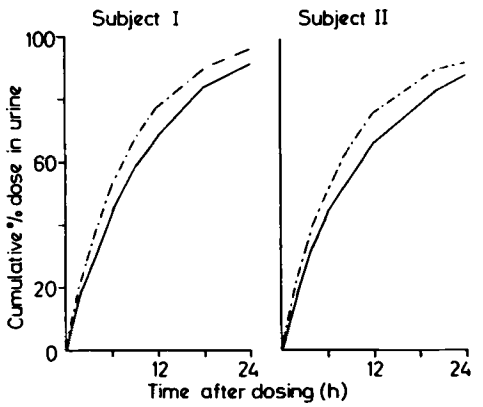

FIG. 2. Cumulative excretion of ^{14}C in the urine by two volunteers after the intravenous injection of [^{14}C]-aminophylline while on their usual (——) and methylxanthine deprived diets (– · –).

[^{14}C]aminophylline while on their usual diets and after abstention from all methylxanthine-containing foods and beverages.

Table 3 gives qualitative and quantitative results for the elimination of [^{14}C]aminophylline in the two subjects in the presence and absence of dietary methylxanthines. The recovery of ^{14}C in the 0–24 h urine was slightly higher (mean 93·4% of dose) on the methylxanthine-deprived diet compared with the normal diet (89·4%). The urinary metabolites were the same as those found previously, namely 3-methylxanthine, 1,3-dimethyluric acid, 1-methyluric acid and the two unknown minor metabolites, in addition to unchanged theophylline. The quantities of metabolites excreted were not appreciably changed by deprivation of dietary methylxanthines. The impact of this dietary manipulation on aminophylline metabolism is seen when the kinetics of its metabolism are examined. Table 4 shows the pharmacokinetic

Table 3. Metabolisms of aminophylline in two volunteers on normal and methylxanthine-deprived diets.

Subject	I		II	
	% dose excreted as			
Diet	Normal	Methylxanthine-deprived	Normal	Methylxanthine-deprived
Metabolite				
Theophylline	14·7	13·1	6·8	7·1
3-Methylxanthine	21·7	22·0	19·9	22·0
1,3-Dimethyluric acid	34·4	38·9	40·1	41·0
1-Methyluric acid	17·4	17·8	17·1	17·4
Unknown	0·7	0·2	1·1	0·9
^{14}C in 0–24 h urine	91·2	95·8	87·5	90·9
24–48 h urine	6·7	5·2	8·6	3·9

Dosage and other details as in Table 1 and the text.

parameters describing the fate of aminophylline in the two subjects on both dietary regimes, and it is apparent that the rate of conversion of aminophylline to its metabolites, notably 1,3-dimethyluric acid is increased by abstinence from methylxanthine-containing foods and beverages. The overall half-time for the elimination of ^{14}C was therefore reduced by deprivation of dietary methylxanthines from a mean of 7·2 to 5·8 h.

DISCUSSION

This paper gives comparative data for the metabolism of pharmacokinetics of theophylline in man, when given alone and when combined with ethylenediamine, as aminophylline. The qualitative aspects of the fate of theophylline given in these two forms are similar: the same metabolites are formed by two metabolic processes, that is *N*-demethylation and 8-hydroxylation, to give the uric acids. The only qualitative difference observed was that the unknown metabolite with the characteristics of a uric acid was formed from aminophylline on both the subjects on usual and methylxanthine-deprived diets, whereas after theophylline injection, it was only found when the subjects kept to their usual diets.

Table 4. Pharmacokinetic parameters describing the urinary elimination of aminophylline in two volunteers on normal and methylxanthine-deprived diets.

Subject	I		II	
Diet	Normal	Methylxanthine-deprived	Normal	Methylxanthine-deprived
Parameter				
k^{T}/el (h^{-1})	0·011	0·017	0·009	0·009
k^{DMU}/el (h^{-1})	0·031	0·046	0·036	0·051
k^{MU}/el (h^{-1})	0·015	0·022	0·016	0·019
k^{U}/el (h^{-1})	0·001	0·001	0·001	0·001
V^{3MX}/max (mg h^{-1})	1·96	2·04	1·43	3·13
K^{3MX}/m (mg)	42·7	33·8	25·6	77·0
$t_{\frac{1}{2}}$ (h)	6·9	5·9	7·5	5·7

Parameters and the mode of calculation as described in the text.

The quantitative data, both in terms of the amounts of each metabolite formed and of the kinetic parameters describing their formation, indicate that, from a dispositional viewpoint, theophylline and aminophylline are not entirely equivalent presentations of theophylline to the body. The combination of ethylenediamine with theophylline (to give aminophylline) results in its more extensive and rapid conversion to 3-methylxanthine and 1,3-dimethyluric acid, but not to 1-methyluric acid or the unknown metabolites. The total elimination of ^{14}C after aminophylline proceeds faster than after theophylline administration. The finding that the addition of ethylenediamine to theophylline speeds its metabolism and excretion is also supported by the work of Zuidema (1978) who studied the influence of ethylenediamine on the plasma pharmacokinetics of theophylline. The addition of increasing amounts of ethylenediamine caused progressive decreases in the plasma elimination half-life and apparent volume of distribution of theophylline, with consequent increases in its total body clearance.

The mechanism whereby ethylenediamine enhances the metabolic disposition of theophylline is obscure. Little is known about the disposition of pharmacokinetics and clinical toxicity of ethylenediamine itself despite its use as a component of aminophylline. Possibilities for the basis of the metabolic interaction must remain for the present speculative but it could involve a displacing effect of ethylenediamine on the protein binding of theophylline to alter the distribution of the latter or by increasing access to or by activation of the metabolizing enzymes.

We have previously shown that the metabolism and pharmacokinetics of theophylline may be influenced by the intake of methylxanthines in the diet. Thus, the rate and extent of its conversion to 3-methylxanthine and 1,3-dimethyluric acid are increased by 7 days abstinence from all methylxanthine-containing foods, and this is suggested to be due to the depletion of the pool of methylxanthines (of dietary origin) and their metabolites which normally compete with theophylline for the methylxanthine metabolizing enzymes.

Owing to the demanding nature of the studies described on the volunteers, it was only possible to examine the influence of deprivation of dietary methylxanthines on aminophylline metabolism in two individuals. The results obtained suggest that the fate of aminophylline is influenced by the pool of methylxanthines present in people consuming a normal Western diet containing tea, coffee and chocolate. The influence of these compounds on the relative proportions of aminophylline metabolites in urine is less marked than is the case for theophylline, but when the pharmacokinetics of aminophylline are considered, an appreciable difference is seen between results on the two diets.

The findings described show that small but significant differences exist in the metabolism and elimination of theophylline and theophylline-ethylenediamine (aminophylline) in man. This provides yet another, but perhaps more novel example, of a formulation-related change in drug disposition. Attention is drawn to the fact that little is known concerning the effects and disposition of the ethylenediamine component of aminophylline and further studies seem desirable in view of accounts of the toxicity of the diamine (White et al 1978).

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