Comparative plasma pharmacokinetics of theophylline and ethylenediamine after the administration of aminophylline to man

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Plasma concentrations of theophylline and ethylenediamine have been examined after the oral and intravenous administration of aminophylline to three healthy male volunteers who received on separate occasions 250 mg aminophylline i.v. or 300 mg aminophylline by mouth. Blood samples were taken at regular intervals and the plasma levels of theophylline and ethylenediamine were assayed by h.p.l.c. After i.v. injection, plasma concentrations of theophylline were described by a two-compartment open model, with $t\frac{1}{2}\alpha$ 6 min, V_c 191 ml kg^{-1}, $t\frac{1}{2}\beta$ 6·0 h and V_p 217 ml kg^{-1}. The plasma concentrations of ethylenediamine also exhibited a biphasic decline, the parameters of the two-compartment open model being $t\frac{1}{2}\alpha$ 7 min, V_c 214 ml kg^{-1}, $t\frac{1}{2}\beta$ 32 min and V_p 133 ml kg^{-1} (all values given are means of three subjects). The ratio of theophylline/ethylenediamine in plasma rose rapidly from the initial value of 6·2 (the ratio of the two compounds in aminophylline) over the first 10 min post injection to 47 at 120 min. After this time, ethylenediamine was not measurable in plasma, although theophylline was present for at least 6 h after dosing. Upon oral administration of aminophylline, plasma concentrations of theophylline rose to a peak value of 7·4 µg ml^{-1} at 1 h, falling thereafter with a $t\frac{1}{2}$ of 8·0 h. Ethylenediamine, by contrast, could only be detected over the first 2 h after dosing, in amounts below 0·4 µg ml^{-1}. Comparison of oral and i.v. data showed the bioavailability of ethylenediamine was approximately 34% in comparison with a value of 88% for theophylline. These data indicate that the two components of aminophylline are handled independently by the body and that there is no molecular association between theophylline and ethylenediamine in biological media.

There remains some doubt about the nature of the interaction between theophylline and ethylenediamine—the components of aminophylline. The drug has been referred to as a salt (Weinberger & Hendeles 1979), a stable mixture or combination (Martindale 1982) or a compound of one molecule of ethylenediamine with two molecules of theophylline (Merck Index 1976). Theophylline and aminophylline are generally assumed to be pharmacologically and therapeutically equivalent on a molar basis, but this may not be entirely justified. Monks et al (1981) have previously shown that, when given intravenously as aminophylline to volunteers, both the rate and extent of metabolism of theophylline increased compared with theophylline alone. Zuidema (1978) similarly showed that the addition of increasing amounts of ethylenediamine to theophylline caused progressive decreases in its plasma elimination half-life and apparent volume of distribution, with corresponding increases in total body clearance, after intravenous administration.

Ethylenediamine itself has marked biological effects. Prominent among these is the induction of both immediate and delayed hypersensitivity reactions, associated with its topical (White et al 1978) and intravenous (Elias & Levinson 1981), but not oral, administration. In-vitro, ethylenediamine shares many of the metabolic regulatory actions of the biogenic diamine putrescine (1,4-diaminobutane), and acts in the c.n.s. as a γ-aminobutyric acid mimetic and depressant (Perkins et al 1981).

To understand the comparative disposition of the two components of aminophylline, we report on the plasma pharmacokinetics of ethylenediamine and theophylline after the oral and intravenous administration of aminophylline to volunteers[†].

MATERIALS AND METHODS

Compounds

Anhydrous theophylline, m.p. 273 °C, etofylline (7-(2-hydroxyethyl)-theophylline) m.p. 158 °C,

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ethylenediamine and cadaverine (1,5-diamino-pentane), both as liquid free bases <99% pure, were purchased from Sigma. *m*-Toluoyl chloride, b.p. 86 °C, was purchased from Aldrich. H.p.l.c. grade dichloromethane and acetonitrile were purchased from Fisons, U.K. Aminophylline injection B.P. (250 mg/10 ml; Antigen Ltd., Eire, batch no. E104 52024) and aminophylline tablets B.P. (100 mg; Macarthy Ltd., Romford, U.K., batch no. BN A007194A) were supplied by the Pharmacy of St Mary's Hospital.

Volunteer studies

Three healthy male volunteers, ages 22–26 years, 65–95 kg, who gave their informed consent, participated in the investigation, which was approved by the Ethical Committee of St Mary's Hospital and Medical School. The volunteers abstained from all foods containing methylxanthines for 72 h before and during each study.

Oral administration. Each subject took 43 mg ethylenediamine in the form of 300 mg amino-phylline, taken as 3 × 100 mg aminophylline tablets B.P., with water at 9 a.m. on an empty stomach. Food was withheld for 3 h. Blood samples (5 ml) were taken from a vein in the antecubital fossa before dosing and at regular intervals up to 6 h. The 0–24 h urine was also collected.

Intravenous administration. 36 mg ethylenediam-ine in the form of 250 mg aminophylline (made by mixing a 10 ml ampoule of aminophylline injection 25 mg/ml B.P with 100 ml sterile isotonic saline) was infused into a vein in the right antecubital fossa, over a period of 8 min. Blood samples were withdrawn from a polyethylene cannula placed in the left median cephalic vein, immediately before, during and at the end of the infusion, and thence at regular intervals up to 6 h. The 0–24 h urine was also collected.

High pressure liquid chromatography

For this a Rheodyne 7120 valve loop injector, an HPLC Technology (Wilmslow, Cheshire, U.K.) RR 015 pump and an Altex 154 u.v. detector was used. The column was of stainless steel, 100 × 5 mm i.d. packed with ODS-Hypersil 5 μ (Shandon Southern Products, Runcorn, Cheshire, U.K.). For the assay of theophylline, the mobile phase consisted of sodium acetate (0.77 g), glacial acetic acid (5 ml), acetonitrile (53 ml) adjusted to 1 litre with distilled water, flow rate 0·8 ml min^{-1} and the u.v. detector had a 280 nm filter. In this system, theophylline and

etofylline had retention times 7·6 and 11·3 min respectively.

Assay of ethylenediamine in plasma and urine

Ethylenediamine in aliquots (1 ml) of plasma and urine was assayed by h.p.l.c. of its NN'-di(*m*-toluoyl)-derivative, using cadaverine as internal standard, as described by Caldwell & Cotgreave (1982a) and Cotgreave & Caldwell (1983a).

Assay of theophylline in plasma

Plasma (100 μl) was spiked with 2 μg etofylline (20 μl of a 100 μg ml^{-1} solution in 10% aqueous trichloroacetic acid) as internal standard, 80 μl 10% aqueous trichloroacetic acid added and the whole shaken gently for 20 min and centrifuged (1000 g 5 min). 10 μl aliquots of the supernatant were injected onto the h.p.l.c. column.

Quantitation was achieved by reference to a standard curve of the ratio of the peak heights for theophylline and etofylline, made daily over the range 0–20 μg ml^{-1} theophylline in blank plasma. The instrument response was linear, the lines passing through the origin with regression coefficients always >0·995. Reproducibility at 1 μg ml^{-1} was ±2·5% and at 15 μg ml^{-1} ±1·5% (n=4 in both cases). Day-to-day variability of the calibration curves was never greater than ±5% at all concentrations (n=6).

Storage of samples

Collected blood was placed in tubes containing lithium heparin and the plasma separated by centri-fugation (1000 g, 5 min). Plasma and urine were stored at −20 °C until analysis; there was no deterioration for at least 1 month.

Analysis of administered aminophylline

Four aminophylline tablets from the batch used were separately dissolved with shaking in 100 ml of distilled water. Two ampoules of aminophylline injection from the batch used were diluted to 100 ml with distilled water. Aliquots, suitably diluted, were assayed by h.p.l.c. The content found was within 2% of the B.P. values, and the dose of ethylenediamine was thus 14·4 mg/100 mg aminophylline tablet and 35·6 mg/250 mg ampoule of aminophylline injection and of theophylline 85 mg/100 mg aminophylline tablet and 215 mg/250 mg ampoule of aminophylline injection.

Pharmacokinetic analysis

The decline in plasma concentrations of theophylline and ethylenediamine after the intravenous injection

of aminophylline was analysed according to a two-compartment open model, using least-squares regression and the method of residuals. Terms were corrected for the duration of the infusion according to Loo & Reigelman (1970). Pharmacokinetic constants describing the plasma concentration-time curves for theophylline and ethylenediamine after the oral administration of aminophylline were obtained from least squares regression and the method of residuals (Greenblatt & Koch-Weser 1975). Areas under the plasma concentration time curves were calculated by the trapezoidal rule. Bioavailability was corrected for dose by the following equation:

$$\text{Bioavailability} = \frac{AUC_{oral}}{AUC_{i.v.}} \times \frac{Dose_{i.v.}}{Dose_{oral}} \times 100$$

RESULTS
Intravenous administration

After intravenous infusion of the drug the plasma concentrations of theophylline and ethylenediamine declined biphasically with time following the termination of the infusion (Fig. 1). The data for both compounds from three subjects were analysed according to a two-compartment open model and the various kinetic parameters are listed in Table 1. The results showed theophylline to have longer α- and β-half-lives and larger volumes of distribution than ethylenediamine, which could not be detected in plasma 3 h after aminophylline administration, while theophylline was present up to 6 h.

Oral administration

After the oral administration the drug, theophylline was rapidly absorbed, concentrations reaching a
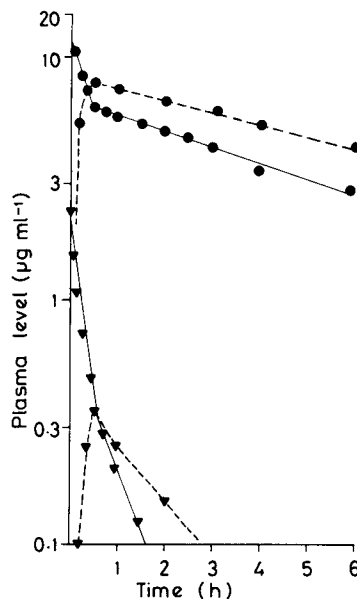

FIG. 1. Plasma concentration-time curves for theophylline (●) and ethylenediamine (▲) following the intravenous infusion of 250 mg aminophylline B.P. and the oral administration of 3 × 100 mg aminophylline tablets B.P. on separate occasions to subject 2.

maximum within 30 min, thereafter falling monoexponentially with a t½ of 6·5–9 h. Ethylenediamine was more slowly absorbed, with concentrations reaching a maximum at 45 min, thereafter declining monoexponentially with a t½ of 0·9–1·2 h (Fig. 1), and being undetectable 3 h after aminophylline administration. This prevented a full pharmacokinetic analysis. Again, the data (Table 2) show the independent in-vivo behaviour of the two compounds. Table 2 also presents estimates of the oral bioavailabilities of the two compounds.

Table 1. Pharmacokinetic parameters of theophylline and ethylenediamine after the intravenous infusion of 250 mg aminophylline to three healthy volunteers.

Subject	Theophylline				Ethylenediamine			
	1	2	3	Mean	1	2	3	Mean
A μg ml^{-1}	11·0	7·5	6·5	8·3	0·91	1·40	1·28	1·20
α (min^{-1})	0·160	0·114	0·085	0·20	0·05	0·165	0·204	0·140
T½α (min)	4·6	6·1	8·2	6·3	13·9	4·2	3·4	7·2
B (μg ml^{-1})	6·2	6·5	6·9	6·5	0·81	1·00	0·94	0·92
β (min^{-1})	0·0014	0·0023	0·0022	0·002	0·018	0·025	0·023	0·022
T½β (h)	8·0	5·0	5·1	6·0	0·67	0·47	0·50	0·55
V$_p$ (ml kg^{-1})	207	227	218	217	130	124	145	133
V$_c$ (ml kg^{-1})	122	207	245	191	210	190	243	214
CL (ml min^{-1})	108	120	113	114	587	556	580	574
AUC (μg ml^{-1} min^{-1})	1940	1751	1856	1849	59·6	63·0	60·3	61·0
K$_E$ (min^{-1})	0·0038	0·0048	0·0042	0·0043	0·029	0·038	0·037	0·035
% Dose unchanged in 0–24 h urine	—	—	—	—	18	24	12	18

Table 2. Pharmacokinetic parameters of theophylline and ethylenediamine after the oral administration of 300 mg aminophylline to three healthy volunteers.

Subject	Theophylline				Ethylenediamine			
	1	2	3	Mean	1	2	3	Mean
C_{MAX} (μg ml^{-1})	6·7	8·2	7·1	7·3	0·22	0·33	0·36	0·30
K_E (min^{-1})	0·0013	0·0018	0·0018	0·0016	0·012	0·009	0·013	0·011
T½ (h)	9	6·5	8·5	8·0	1	1·2	0·9	1·0
AUC (μg ml^{-1} min^{-1})	1663	2116	1965	1915	16·5	29·7	28·5	24·9
Bioavailability (%)	72	102	89	88	23	39	40	34
CL (ml min^{-1})	110	123	116	116	599	565	604	589
% Dose unchanged in 0–24 h urine	—	—	—	—	5	2	2	3

Comparative plasma concentrations of theophylline and ethylenediamine after aminophylline administration

The content of theophylline and of ethylenediamine in aminophylline is at a ratio of 2:1 on a molar basis, or 6:1 by mass. To illustrate the independent in-vivo behaviour of the two components the ratio of concentration of theophylline to ethylenediamine in plasma against time was plotted in Fig. 2. After i.v. infusion, the ratio is initially 6·3, and this rises rapidly as ethylenediamine disappears from plasma. In contrast, after oral administration, the ratio is initially high (90), and falls rapidly over 30 min to a minimum value of 26, rising thereafter until after 3 h no ethylenediamine can be detected. This indicates the slower absorption of ethylenediamine relative to theophylline, and since the ratio never approaches that in the administered aminophylline, the relatively poor extent of absorption of ethylenediamine.

Urinary elimination of unchanged ethylenediamine after aminophylline administration.

Tables 1 and 2 list the recovery of unchanged ethylenediamine in the 0–24 h urine following i.v. and oral administration of aminophylline to the subjects. The mean recovery was 3% of dose after oral and 18% after i.v. administration.

DISCUSSION

The availability of h.p.l.c. assays for theophylline and for ethylenediamine has permitted for the first time the simultaneous analysis of the pharmacokinetics of the two component molecules of aminophylline. The pharmacokinetics of theophylline reported here are broadly in accord with the findings of others (see Ogilvie 1978). The literature contains no information on ethylenediamine disposition in man. Following intravenous infusion of aminophylline, we have shown that the plasma concentrations of ethylenediamine follow a two-compartment open model, characterized by rapid distribution and clear-

ance, the two compartments having small volumes. The clearance of 574 ml min^{-1} approximates to renal blood flow in healthy humans, but an average of only 18% of the dose was recovered unchanged in the urine, suggesting that ethylenediamine is rapidly metabolized in man.

After oral administration of aminophylline the plasma concentrations of ethylenediamine are low, the maximum being achieved 30–60 min after dosage, and the decline thereafter is rapid. The mean bioavailability of ethylenediamine was 34%, and a mean of 3% of dose was recovered unchanged in the urine. The oral clearance, corrected for bioavailability, was 589 ml min^{-1} and therefore was similar to that seen after i.v. infusion.
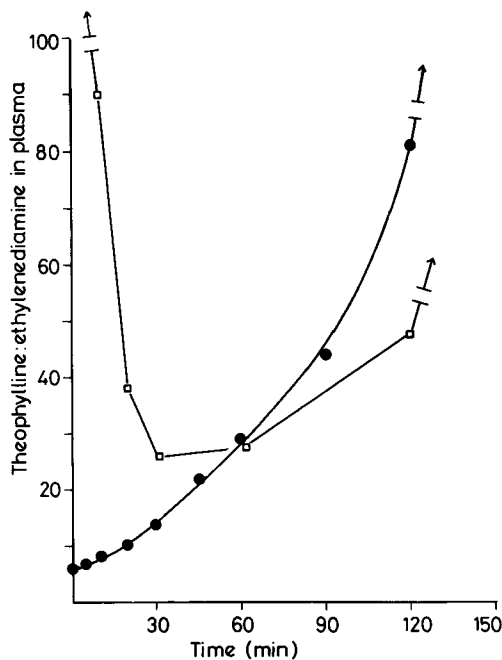

FIG. 2. Theophylline/ethylenediamine plasma concentration ratio vs. time curves following the oral (□) and intravenous (●) administration of aminophylline to subject 2.

Little is known of the human metabolism of ethylenediamine. It has been reported to be N-acetylated giving mono-N-acetylethylenediamine (Markiw 1975). We have shown in the rat its conversion to mono- and di-acetyl conjugates and deamination of some 40% of the dose to glycine (Cotgreave & Caldwell submitted for publication). The low bioavailability in man could arise from either poor absorption or first-pass metabolism. The short plasma elimination half-life makes it doubtful that this molecule could accumulate in patients using aminophylline on a chronic basis. Additionally, the low plasma concentration and poor bioavailability of ethylenediamine are perhaps responsible for the apparent lack of ethylenediamine-related toxicity of aminophylline given orally.

We have previously reported on the plasma protein binding and erythrocyte uptake of theophylline, ethylenediamine and mixtures thereof, and have demonstrated that these compounds do not influence each other's behaviour in such systems (Caldwell & Cotgreave 1981). From these findings, together with the present results it would appear that theophylline and ethylenediamine behave entirely independently in biological milieux, and that any differences between theophylline and aminophylline do not arise from an association between theophylline and ethylenediamine in the body.

Acknowledgements

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