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THE FATE OF TRIMETHYLAMINE IN THE RAT

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SUMMARY

The metabolism and excretion of [^{14}C]-trimethylamine has been investigated in seven strains of rat. Over 75% of the administered radioactivity was excreted via the urine within the first day, with up to 9% in the faeces. At this dose level (15mg/kg body wt) N-oxidation was the major metabolic pathway encountered (45% excreted dose) whilst demethylation was only of minor importance (3%). The remaining compound was excreted unchanged. No significant differences were observed between the strains studied.

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I. INTRODUCTION

Investigations into the clinical condition described in the literature as "Fish-Odour Syndrome" have shown that this problem results from an individual's inability to adequately metabolise trimethylamine, a natural food component with a pungent fish-like odour, into the relatively non-odorous trimethylamine N-oxide /1,2/. The consequences of this perturbation in intermediary metabolism are mainly psychosocial, arising from the episodic production of fish-smelling sweat and urine. Such a condition is either inherited (primary trimethylaminuria) or is secondary to other factors such as liver disease, renal failure or drug treatment (secondary trimethylaminuria). Within the general population this problem may be more prevalent at a sub-clinical level than previously thought.

This condition is not unique to man. A similar metabolic deficit has been described in the domestic fowl. The capacity of this animal to N-oxidize trimethylamine is inherited and is consistent with the involvement of a single, autosomal, semi-dominant gene. When in conjunction with other factors, this phenomenon expresses itself in the production of tainted eggs which are of little commercial value /3/.

In an attempt to find a more accessible animal model, to expedite the identification of pharmaceutical substrates which may share impaired N-oxidation, we have investigated trimethylamine metabolism in seven different rat strains. Such an approach has been successful previously in identifying an animal model for the debrisoquine 4-hydroxylation polymorphism /4/.

II. MATERIALS AND METHODS

Animals

Female adult rats (150-200g body weight) of seven different strains (Wistar, Lewis, Fischer, A/GUS, PVG, DA and BN) were obtained from various breeders (Oxford Laboratory Animals Centre, Bicester, U.K.; National Institute for Medical Research, London, U.K.; Bantin and Kingman, Hull, U.K.) and with the exception of Wistar, all strains were inbred. The animals were maintained on a standard pelleted diet (Labsure CRM pellets; K & K Greef Ltd., Croydon, U.K.) with free access to water.

[^{14}C]-Trimethylamine hydrochloride (New England Nuclear - Du Pont (UK) Ltd, Southampton, U.K.; 3.9μCi/mmol, radiochemical purity >99%) was administered orally as an aqueous solution to three rats of each strain (15mg/kg body weight; 4.1μCi/animal). The animals were housed in metal metabolism cages which permitted the separate collection of urine and faeces into vessels which contained 4M HCl (4ml) to prevent loss of volatile amines. Radioactivity which may have been excreted in the expired air was not measured. After the collection of 0-24 hour excreta, the cages were rinsed with 4M HCl and the volumes added to the urine. All samples were kept frozen at -20°C in the dark until analysis.

Measurement of radioactivity

Faecal samples were homogenised with 1M HCl (30ml) and centrifuged. Aliquots (0.1ml) of urine and faecal homogenates were added directly to a toluene-based scintillation fluid mixed with Triton X-100 (2:1 v/v) /5/ and counted by liquid scintillation spectrometry using a Packard Tri-Carb 4640 scintillation counter (Packard Instruments Ltd., Berks., U.K.) with external standards being employed for quench correction.

Metabolite identification

Thin-layer chromatography (t.l.c.) was performed on cellulose plates (0.1mm thick, 20 x 20 cm, aluminium-backed; Merck Darmstadt, FR Germany) using solvents A to C or on precoated silica gel G plates (0.2mm thick) using solvent D (Table 1). Compounds were visualised by spraying the dried plates with bromocresol green (0.2% w/v in ethanol) to give blue spots on a yellow ground /6/.

Untreated urine samples (2ml), neutralised faecal homogenates (2ml) or aqueous extracts of t.l.c. plates were reduced with titanous sulphate (0.2ml, 15% w/v in 23% w/v aq.H_2SO_4) in a sealed vessel at 30°C for 30 minutes /7/ and then examined by t.l.c.

The 2,4-dinitrobenzene derivatives of any methylamine or dimethylamine present in the urine were formed by reacting urine, made alkaline (pH 8 to 9) with solid $NaHCO_3$, with 2,4-dinitrofluorobenzene (Sanger's reagent) (0.1ml) in ethanol (1ml) at room temperature for two hours /8/. Any resulting derivatives were then investigated by t.l.c. on silica plates in solvent D together with standard compounds taken through the same procedure. Radioactive areas were located and quantified by cutting the t.l.c.

TABLE 1

Chromatographic properties of trimethylamine and related compounds

Compound	T.l.c. R_f value in solvent system A (cellulose plates)	B (cellulose plates)	C (silica plates)	D (silica plates)
methylamine	0.33	0.22	0.44	0.59
dimethylamine	0.38	0.27	0.46	0.64
trimethylamine	0.42	0.29	0.46	–
trimethylamine N-oxide	0.51	0.37	0.55	–

solvents:
A. butan-1-ol : formic acid (39% aq. v/v) [77:23 by vol.]
B. butan-1-ol : formic acid (22.5% aq. v/v) [1:4 by vol.]
C. butan-1-ol : ethanol : acetic acid : water [8:2:1:3 by vol.]
D. toluene : ethyl acetate [5:2 by vol.]

plates into 0.5cm strips and counting as described for urine samples above.

Duplicate samples (10μl) of urine and faecal homogenates were applied separately to filter paper (Whatman No. 1) and dried in a stream of cold air. In one case the dried urine spot was then covered with 0.1 ml of 4M NaOH and again dried to remove any volatile amines /1/. The same procedure with and without NaOH was repeated with chemically reduced samples (see above). The relevant areas of filter papers were then excised and counted as previously described.

Mass spectrometry

Electron-impact (E.I.) mass spectrometry was carried out on a Kratos MS80 instrument (Kratos Ltd, Urmston, Manchester, U.K.) with a source temperature of 200°C using a direct-insertion probe at 70 eV.

III. RESULTS

The results obtained for all radioactive balance studies were similar with no statistically significant differences existing between the rat strains investigated (Table 2). In general, over 75% of the administered radioactivity was excreted in the urine within the first 24 hours after dosing, whilst amounts of up to 9% were detected in the faeces.

Analysis of the urine by t.l.c. showed two major areas of radioactivity which co-chromatographed with trimethylamine and its N-oxide. Confirmation of identity was provided by mass spectrometry after elution of these areas and comparison with authentic standards. By means of derivatisation, small amounts of radioactive dimethylamine were detected, but no radioactive methylamine was present. Overall, the rats excreted 45% of the administered radioactive trimethylamine as its N-oxide and only 3% as dimethylamine; the remainder being trimethylamine. As excellent 0-24 hour recoveries were achieved (88%), it suggests that in the rat N-oxidation is the major metabolic route for trimethylamine, demethylation being a minor pathway. The

TABLE 2

Excretion of radioactivity (0-24 hours) after the oral administration of [^{14}C]-trimethylamine hydrochloride (15 mg/kg body weight; 4.1μCi/animal) to rats

rat strain	urine	faeces	total
Wistar	83.3 ± 4.9	4.7 ± 0.5	88.0 ± 5.2
Lewis	85.5 ± 8.8	4.2 ± 1.3	89.8 ± 7.4
Fischer	79.7 ± 5.6	4.2 ± 0.8	84.0 ± 5.7
A/GUS	83.1 ± 4.8	6.1 ± 0.7	89.1 ± 4.6
PVG	85.0 ± 4.2	4.4 ± 1.2	89.4 ± 5.4
DA	83.4 ± 9.2	4.8 ± 1.8	88.1 ± 9.3
BN	83.3 ± 5.5	4.6 ± 1.3	87.9 ± 5.6

Values are expressed as the mean ± 1 s.d. for 3 animals.

majority of the radioactivity present in the faeces was in the form of trimethylamine (94%) with the N-oxide accounting for the balance. No radioactive dimethylamine or methylamine were detected (Table 3). Again, no statistically significant differences in metabolism were evident between the rat strains investigated.

IV. DISCUSSION

In this study the use of [^{14}C]-trimethylamine permitted the demonstration of its efficient gastrointestinal absorption followed by its rapid removal from the body via the urine. The major metabolic pathway encountered was that of N-oxidation. The potential availability of unchanged trimethylamine as a substrate, indicated by its extensive excretion in the urine, suggests that the pathway of demethylation, which accounts for only a few percent of the administered dose, is of limited quantitative importance in the rat at this dose level. The presence of the majority of the radioactivity in the faeces in the form of the free amine presumably

TABLE 3

Radioactive metabolites in urine and faeces

rat strain	Urine			Faeces	
	DMA	TMA	TMAO	TMA	TMAO
Wistar	3.6 ± 1.2	49.6 ± 4.3	46.8 ± 2.2	95.5 ± 1.0	4.5 ± 0.9
Lewis	3.1 ± 0.9	52.3 ± 2.7	44.6 ± 3.4	97.9 ± 1.4	2.1 ± 1.1
Fischer	3.9 ± 0.8	49.0 ± 5.0	47.1 ± 5.8	91.9 ± 9.5	8.1 ± 4.8
A/GUS	2.8 ± 0.4	51.6 ± 5.4	45.6 ± 6.0	90.8 ± 5.6	9.2 ± 4.8
PVG	3.5 ± 1.2	52.2 ± 1.4	44.3 ± 1.7	90.3 ± 6.1	9.7 ± 4.0
DA	3.3 ± 0.6	51.5 ± 4.3	45.2 ± 2.7	94.7 ± 2.9	5.3 ± 3.0
BN	2.3 ± 1.3	53.5 ± 2.0	44.2 ± 1.3	97.8 ± 0.8	2.2 ± 0.6

Values are expressed (mean ± 1 s.d. for 3 animals) as a percentage of the total radioactivity excreted in the 0-24h urine or faeces.

reflects the reductive environment imposed by the gut microflora. The routes of excretion and metabolic pathways encountered are concordant with those found during previous investigations in the rat and other animals including man /9-12/.

Unlike the successful results achieved with debrisoquine 4-hydroxylation where the female DA rat showed a relative deficiency in this reaction and was subsequently proposed as a model for the human poor metaboliser /4/, the seven rat strains studied here showed no significant differences in their handling of trimethylamine. However, the use of such animal models has been criticised and whilst they may prove suitable in predicting other substrates which may also display impaired metabolism in man, studies into the underlying molecular mechanisms in animals may shed little or no light on the human problem /13/. Indeed, whilst trimethylamine has been shown to be a substrate for the microsomal FAD-containing monooxygenase isolated from pig liver /14-16/, it is also known to be oxidised by both this enzyme's counterpart and cytochrome(s) P450 derived from rabbit liver microsomes /17/. The relative contributions of these two enzyme systems (and others?) to the N-oxidation of trimethylamine in different animals species including man is not yet known.

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