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Preclinical toxicology, pharmacokinetics and formulation of N^2, N^4, N^6-trihydroxymethyl-N^2, N^4, N^6-trimethylmelamine (Trimelamol), a water-soluble cytotoxic *s*-triazine which does not require metabolic activation*

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Summary. N^2,N^4,N^6-Trihydroxymethyl-N^2,N^4,N^6-trimethylmelamine (Trimelamol) is a water-soluble synthetic *s*-triazine which, unlike hexamethylmelamine (HMM) and pentamethylmelamine (PMM), does not require metabolic activation. The physico-chemical characteristics of Trimelamol were studied with the aim of overcoming the problems of chemical instability, low solubility and polymerisation which had hindered the development of the drug for clinical use. Trimelamol had similar activity to PMM against the murine PC6 plasmacytoma, but enhanced activity with respect to PMM against the Walker 256 carcinosarcoma in the rat, a species which metabolises PMM less efficiently. Pharmacokinetic studies in mouse, rat and man did not show the major species differences characteristic of PMM. The drug exhibited similar toxicity to PMM against rodents, but had virtually no neurotoxicity. The potential advantages of Trimelamol over previously tested melamines are discussed.

Introduction

N^2,N^4,N^6-Trihydroxymethyl-N^2,N^4,N^6-trimethylmelamine (Trimelamol) is a water-soluble antitumour s-triazine synthesised by Cumber and Ross [6] and shown to have cytotoxic activity against a mouse PC6 plasmacytoma and a human lung tumour xenograft [5, 14]. An analogue of hexamethylmelamine (HMM), it differs from both HMM and pentamethylmelamine (PMM) in the crucial respect that it does not require activation by oxidative *N*-demethylation before expressing cytotoxic activity in vitro [13]. HMM is extensively *N*-demethylated in vivo [1, 15, 19, 20], which involves elimination of formaldehyde from *N*-hydroxymethyl intermediates [2]. In vitro HMM and PMM are only cytotoxic after prolonged exposure, unless activated by a liver microsomal preparation [13, 14], and the inhibitory effect of nonactivated HMM and PMM can be reversed by removing the drug [17]. In contrast, *N*-hydroxymethylmelamines are significantly more toxic in vitro [13], and their effect is not reversed by drug removal [17]. The in vivo studies of Rutty et al. [16] have shown that oxidative *N*-demethylation of PMM is much less efficient in man than in rodents. This suggests that a *N*-hydroxymethylmelamine such as Trimelamol would be a significantly more effective antitumour agent than PMM and probably HMM in man.

The development of Trimelamol posed a number of problems because of its relatively poor aqueous solubility, chemical instability and tendency to polymerise. The studies reported here form part of the preclinical evaluation of Trimelamol and include details of its physicochemical characteristics, antitumour activity, pharmacokinetic behaviour and toxicology in rodents. Similar antitumour activity to that of PMM was observed against murine tumours, but enhanced activity against a rat tumour was seen. This correlates with known species differences in the metabolic activation of PMM [16]. Trimelamol proved much less neurotoxic than PMM in rodents, which has important clinical implications.

Materials and methods

Chemicals. Some of the work reported here, including the toxicology, was carried out using melamines synthesised by Prof. W. C. J. Ross and colleagues at the Chester Beatty Research Institute. Other data, including the pharmacokinetics and the results of certain antitumour tests, were obtained using Trimelamol synthesised by Warner-Lambert (Ann Arbor, Michigan, USA) for the NCI (NSC 283162).

Analytical grade reagents used in HPLC and preparation of buffers were supplied by May and Baker Ltd (Dagenham, Essex, UK), BDH Chemicals Ltd (Poole, Dorset, UK) and Fisons Ltd (Loughborough, Leics., UK).

HPLC. Trimelamol and its metabolites were analysed using a Waters Associates Model ALC/GPC 204 chromatograph (Water Associates, Milford, Mass, USA) equipped with a model 480 variable wavelength UV detector. Separation was achieved using a 15 cm × 4.6 mm column containing Spherisorb 5 μm octyl packing (Phase Sep Ltd., Queensferry, Clwyd, UK) protected by a 6.5 cm × 2.1 mm precolumn containing CO : PELL ODS packing (Whatman Ltd., Maidstone, Kent, UK). Trimelamol estimations were carried out by isocratic elution with 30% methanol/70% 0.05 *M* ammonium bicarbonate pH 8.1 at a flow rate of 2 ml/min and a constant temperature of 16 °C. N^2,N^4,N^6-Trimethylmelamine (TriMM) (Fig. 1) was sepa-

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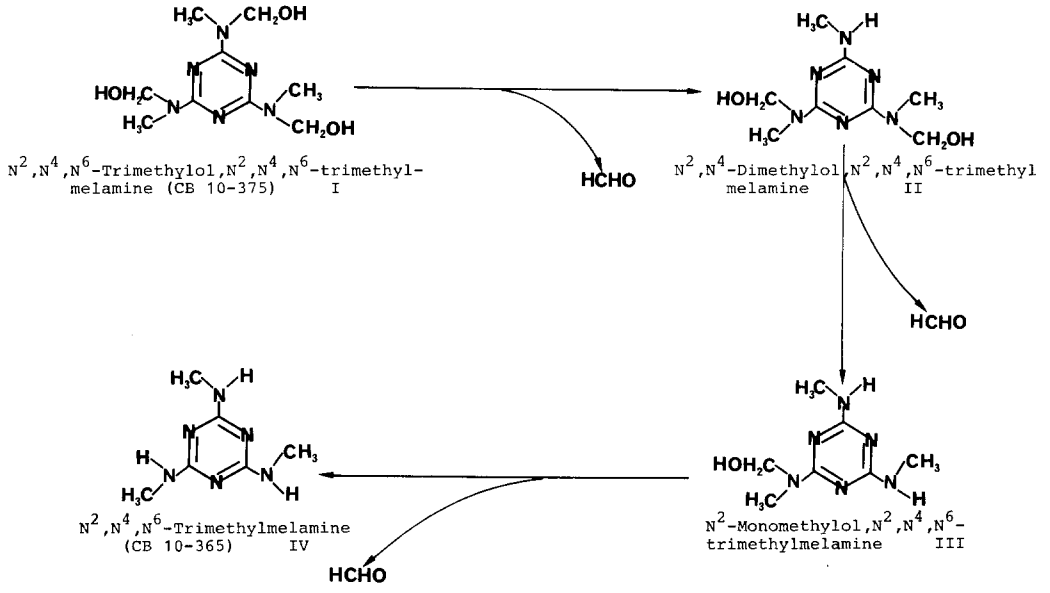

Fig. 1. The route of chemical decomposition of Trimelamol

rated from N^2-monohydroxymethyl-N^2,N^4,N^6-trimethylmelamine by elution with 9% acetonitrile/91% 0.05 *M* ammonium bicarbonate at 2 ml/min. Quantitation was achieved by measurement of the peak area at 225 and 254 nm using a Trilab II data analysis system (Trivector Scientific Ltd, Sandy, Beds., UK). Trimelamol and TriMM estimations were linear over the ranges 2–800 μ*M*, $r=0.9998$ and 3–1200 μ*M*, $r=0.9988$, respectively.

Solubility and stability. Although it is significantly more soluble than HMM (maximum aqueous solubility 0.09 mg/ml), the aqueous solubility of Trimelamol is nevertheless intrinsically low. A number of organic solvents were tested, and the effect of lyophilisation on solubility and rate of dissolution was also studied. The chemical stability of Trimelamol was measured over a wide range of aqueous media to determine the effects of variations in pH, ionic concentration and temperature, using the HPLC method described above. Polymerisation is known to occur under certain conditions, and the effects of concentration and temperature on this reaction were investigated using turbidity and HPLC analysis. The purity of the Warner-Lambert material was determined by HPLC, and an attempt to identify the impurities was made.

Antitumour activity. Trimelamol was tested for activity against the ADJ/PC6 plasmacytoma grown in Balb C^- mice and the Walker 256 carcinosarcoma grown in Wistar rats. Tumour inhibition was measured by percentage reduction in tumour weight compared with solvent controls. The initial tests were performed in comparison with pentamethylmelamine (PMM) using 2% ethanol/0.9% saline, this being the solvent used for the phase I clinical trial of PMM [11]. The PC6 test was performed in female Balb C^- mice (mean weight 21.6±0.9 g) according to published methods [4]. Five daily injections were given IP, starting 20 days after implantation over the dose range 6.25–200 mg/kg for both PMM and Trimelamol. The Walker carcinosarcoma 256 was routinely passaged in the ascitic form and for tests 2×10^6 cells were inoculated IM into male Wistar rats (mean body weight 156±5.4 g). Five daily injections were given IP, starting 24 h after implantation over the dose range 20–120 mg/kg. The test was terminated 7 days after the first treatment, and five rats were treated per dose level. The doses causing death in 50% of animals (LD_{50}) or 90% inhibition of tumour growth (ED_{90}) were calculated graphically [12]. The PC6 test was repeated for Trimelamol using the Warner-Lambert material, in 5% DMSO/dextrose as solvent, and alternative schedules and routes of administration, viz. 5× daily IV injections of 3.125–100 mg/kg, single dose of 6.25–200 mg/kg IV and single dose of 12.5–400 mg/kg IP, were investigated.

Pharmacokinetics. Groups of three male Wistar rats (150–200 g) and male Balb C^- mice (19–25 g) were treated with Trimelamol at a dose of 90 mg/kg IP in 5% dextrose, pH 7.4, using a lyophilised preparation, or 5% DMSO/dextrose and a volume of 0.03 ml/g or 0.02 ml/g body weight. Animals were anaesthetised with diethyl ether at various times after drug administration and exsanguinated by cardiac puncture. Blood was heparinised (10 IU/ml), placed on ice and spun at 1800 *g* for 10 min at 4 °C. Plasma was removed and the protein precipitated by addition of 2 vol. ice-cold methanol. Following further centrifugation at 4 °C the supernatant was removed for analysis by HPLC. It proved necessary to perform this the same day, because of the unstable nature of the drug. Total plasma *N*-hydroxymethylmelamine levels were estimated in the rat by the micro-Nash technique previously reported by Rutty et al. [16]. The plasma decay curve was monophasic. The exponential function $C=Ae^{-\beta t}$ (where *C* is the drug plasma concentration, A the constant, β the first-order rate constant, and *t* the time after the end of drug administration) was fitted to the data by a nonlinear least-squares method [7] to determine the half-life.

The areas under the Trimelamol and TriMM plasma concentration-versus-time curves (AUC) were determined by the trapezoidal rule.

This experiment was repeated in Balb C^- mice at 90 mg/kg IV and at 25 and 50 mg/kg IP.

The pharmacokinetics of Trimelamol in man was examined in patients at the Royal Marsden Hospital who

were receiving the drug by IV infusion during a phase I clinical trial. Informed consent was obtained for all procedures.

Toxicity. The toxicity of Trimelamol and PMM was studied in non-tumour-bearing male and female Balb C^- mice (mean body weights 25.2 ± 1.5 g and 23.6 ± 2.7 g, respectively) and in male Wistar rats (mean body weight 155.6 ± 3.9 g) using five daily IP injections. The drugs were administered in 5% ethanol/10 m*M* sodium bicarbonate pH 8.3 at a constant volume of 0.03 ml/g body weight. The mice received 30–120 mg/kg PMM, the same doses of Trimelamol and 150–240 mg/kg TriMM, while the rats were given 90–180 mg/kg PMM, 60–150 mg/kg Trimelamol and 180–270 mg/kg TriMM. Animals were weighed daily, and times of death recorded. The tests were concluded on the 25th day after the first injection.

Results

Solubility and stability

The aqueous solubility of the Warner-Lambert Trimelamol in its natural state was <1 mg/ml, but this could be increased to a maximum of 5 mg/ml by reducing the particle size. Freeze-drying proved very effective at enhancing the rate of dissolution, decreasing the dissolution time from about 15 min to <30 s, but did not increase the maximum solubility. HPLC analysis showed no significant breakdown of the drug during lyophilisation. Of the organic solvents tested Trimelamol was most soluble in dimethyl sulphoxide (DMSO), with a maximum solubility of 200 mg/ml. Ethanol, propylene glycol, and Cremophor EL were less effective. DMSO 5%–10% increased the solubility of Trimelamol in aqueous media, without apparently changing the toxicity or pharmacokinetic profile of the drug in rodents. Cremophor EL 10% also increased the aqueous solubility, but appeared to increase the neurotoxicity of Trimelamol in mice at the same time [10].

Table 1. Stability of Trimelamol in various buffers

Buffer	Molarity	pH	Temp	$t^1/_2$ (mins)
$NaHCO_3$	10 mM	8.3	R.T.*	485.6
$NaHCO_3$	150 mM	8.3	R.T.	54.2
Tris	1 M	7.4	R.T.	10.8
Tris	1 M	7.4	37°	4.92
Tris	0.1 M	7.4	R.T.	90.4
Tris	0.1 M	7.4	37°	36.9
Tris	0.01 M	7.4	R.T.	455.6
Tris	0.01 M	7.4	37°	141.5
Tris	0.1 M	8.0	37°	41.2
Tris	0.1 M	9.0	37°	71.9
Tris	0.1 M	10.6	37°	76.1
H_2O	–	5.0	4°	5340.0
H_2O	–	5.0	R.T.	820.6
H_2O	–	5.0	37°	200.7
NaCl	154 mM	4.9	R.T.	272.8
NaCl	154 mM	4.9	37°	92.4
Plasma	–	7.4	37°	40.2
5% Dextrose	–	3.9	R.T.	348.0
5% Dextrose	–	6.9	R.T.	542.1

* Room temperature 24–26°

The chemical stability of Trimelamol in a variety of aqueous solutions is shown in Table 1. As expected from the proposed mechanism of decomposition, stability was adversely affected by acid pH and was also inversely related to ionic strength and temperature. The breakdown of the drug was found to obey first-order kinetics. Stability was maximal in distilled water at 4 °C.

Polymerisation is known to occur under certain conditions, with formation of methylene bridges. This was found to be dependent on both concentration and temperature. The presence of undissolved drug particles also appeared to stimulate the process. When assessed by turbidity only, no polymerisation was detectable over 12 h in a 3 mg/ml aqueous solution at room temperature, whereas some polymer was detectable after 2 h at 4 mg/ml and after 90 min at 5 mg/ml. This reaction was much delayed by cooling to 4 °C and by raising the pH. In slightly alkaline 5% dextrose pH 7.6, a 5 mg/ml solution of Trimelamol only became opalescent after 5 h at room temperature and 8 h at 4 °C. Once formed, the polymer was insoluble in water and only poorly soluble in DMSO. During HPLC analysis it could be eluted with 100% methanol and appeared as a complex series of peaks.

The Warner-Lambert Trimelamol, NSC 283162, was analysed by HPLC and found to be only 91.3% pure, the remainder consisting of 8.1% N^2,N^4-dihydroxymethyl-N^2,N^4,N^6-trimethylmelamine and 0.6% N^2-monohydroxymethyl-N^2,N^4,N^6-trimethylmelamine. Chester Beatty Trimelamol was shown to be 99.5% pure by HPLC.

Table 2. Effect of PMM and Trimelamol on the growth of the PC6 tumour

PMM

Dose (mg/kg)	% Inhibition of growth	Toxicity
6.25	30.4	
12.5	63.9	
25	95.7	
50	96.2	
100	–	3/3 dead
200	–	3/3 dead
	LD_{50} 71 mg/kg	
	ED_{90} 21.8 mg/kg	
	T.I. 3.2	

Trimelamol

Dose (mg/kg)	% Inhibition of growth	Toxicity
6.25	38.2	
12.5	77.1	
25	95.3	
50	97.4	
100	–	3/3 dead
200	–	3/3 dead
	LD_{50} 71 mg/kg	
	ED_{90} 20.2 mg/kg	
	T.I. 3.5	

Drugs were given in 2% ethanol/saline daily x5

Table 3. Effect of Chester Beatty and Warner-Lambert Trimelamol on the growth of PC6 tumour

Chester Beatty

Dose (mg/kg)	% Inhibition of growth	Toxicity
3.125	2.2	
6.25	−5.7	
12.5	40.8	
25	92.5	
50	93.4	
100	–	3/3 dead
	LD_{50} 70 mg/kg	
	ED_{90} 24 mg/kg	
	T.I. 2.9	

Warner-Lambert

Dose (mg/kg)	% Inhibition of growth	Toxicity
3.125	10.5	
6.25	13.2	
12.5	75.0	
25	87.3	
50	94.1	
100	–	3/3 dead
	LD_{50} 70 mg/kg	
	ED_{90} 32.5 mg/kg	
	T.I. 2.2	

Drugs were given in 5% DMSO/saline daily x5

Table 4. Effect of single dose of Trimelamol on the growth of the PC6 tumour

Intra-peritoneal

Dose (mg/kg)	% Inhibition of growth	Toxicity
12.5	8.1	
25	4.5	
50	58.1	
100	90.4	
200	–	3/3 dead
400	–	3/3 dead
	LD_{50} 138 mg/kg	
	ED_{90} 98 mg/kg	
	T.I. 1.4	

Intra-venous

Dose (mg/kg)	% Inhibition of growth	Toxicity
6.25	−3.5	
12.5	7.8	
25	13.6	
50	27.7	
100	96.1	
200	–	3/3 dead
	LD_{50} 142 mg/kg	
	ED_{90} 94 mg/kg	
	T.I. 1.5	

Vehicle: 5% DMSO/Dextrose, Warner-Lambert Trimelamol, single dose only

Antitumour activity

Trimelamol was found to possess very similar activity to that of PMM against the PC6 plasmacytoma in mice (Table 2) and similar values for LD_{50} and ED_{90} were obtained when the drug was administered in 5% DMSO/dextrose. No significant differences in activity were observed between the 99.5% and 91.3% pure material (Table 3). Single-dose treatment was more toxic, giving an LD_{50} of 138 mg/kg, as opposed to 71 mg/kg × 5 = 355 mg/kg for

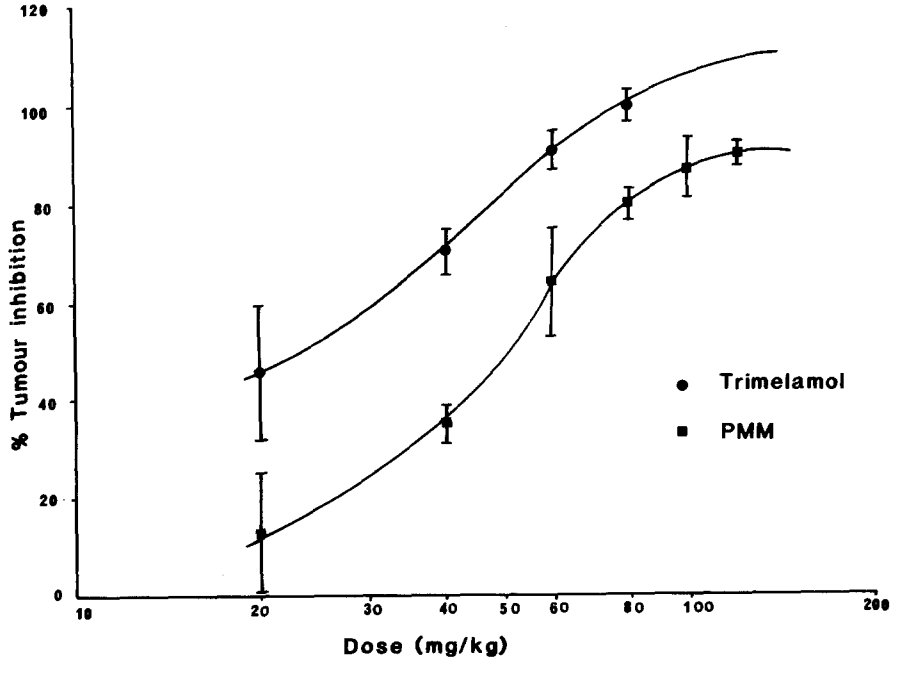

Fig. 2. The anti-tumour activity of Trimelamol and PMM against the Walker 256 carcinosarcoma in the Wistar rat

five daily doses. However, the ED_{90} was identical, at 98 mg/kg for single dose and 20 mg/kg × 5 for repeated administration. As with repeated doses, there was no significant difference between single-dose IP and IV administration (Table 4).

In the rat, however, Trimelamol proved to be both more toxic (LD_{50} = 103 mg/kg) and significantly, more effective (ED_{90} = 60 mg/kg) than PMM (LD_{50} > 120 mg/kg, ED_{90} = 113 mg/kg). Figure 2 illustrates the dose-response relationship of both drugs against the Walker tumour.

Pharmacokinetics

Mouse. Trimelamol is rapidly eliminated from the plasma in mice and, as in vitro, this disappearance follows first-order kinetics. At 90 mg/kg IP a peak plasma level of 125.5 ± 17.1 μ*M* was reached 10 min after injection, and the area under the concentration versus time curve (AUC) was 3381 μ*M* min^{-1}. The plasma TriMM concentration rose slowly and reached a peak of 74.8 ± 2.4 μ*M* 60 min after injection. The relative rates of elimination of Trimelamol and TriMM are shown in Fig. 3a. The AUC for TriMM was 6505 μ*M* min^{-1}.

The same dose of Trimelamol administered IV resulted in a slightly higher peak plasma level of 198.9 ± 26.1 μ*M* at 5 min, a shorter $t_{½}\beta$, 7.2 ± 0.33 min, and a smaller AUC of 2834 μ*M* min^{-1} (Table 5). Table 5 also shows the relationship between dose and plasma levels over the range 25–90 mg/kg. In general, a correlation is found between increasing dose and the peak plasma level or AUC obtained.

Rat. The rate of clearance of Trimelamol from the plasma of Wistar rats given the same dose of 90 mg/kg IP was slower than in Balb C^- mice. The half-life ($t_{½}\beta$) was 20.3 ± 1.2 min, and the lower peak plasma level of 38.9 ± 0.8 μ*M* was reached later, i.e., 20 min after injection. The AUC was also smaller at 1642 μ*M* min^{-1}. In addition to differences in the rates of distribution and elimination of the parent compound, a striking difference was seen in the rate of metabolism of TriMM between rats and mice. The slower rate of metabolism in the rat led to a much higher peak level (388.9 ± 19.5 μ*M*) after 240 min and an AUC of 85, 990 μ*M* min^{-1} (Fig. 3b).

The total *N*-hydroxymethylmelamine levels, as estimated by the micro-Nash method, which measures the formaldehyde released from these compounds, were nearly three times higher than those previously obtained following the same dose of PMM [15]. The peak plasma level was 551.3 ± 5.6 μ*M*, compared with 211.7 ± 14.7 μ*M* for PMM and the AUC 55 031 μ*M* min^{-1}, compared with 15 280 μ*M* min^{-1}.

Man. The pharmacokinetic behaviour of Trimelamol in man was similar to that in mice (Fig. 3c). The mean $t_{½}\beta$ was 6.5 ± 0.3 min. The peak plasma levels varied with dose and rate of IV administration. At 1500 mg/m^2 the mean peak plasma level in three patients was 69.3 ± 9.8 μ*M* and the mean AUC, 3238 ± 400 μ*M* min^{-1}. The latter figure is similar to that obtained following a dose of 90 mg/kg IV, in the mouse. At this dose the PC6 tumour is significantly inhibited in vivo. TriMM metabolism is extremely slow in man, and as shown in Fig. 3c, the concentration may continue to rise for up to 2 h following the start of treatment, so that genuine AUC values cannot readily be obtained.

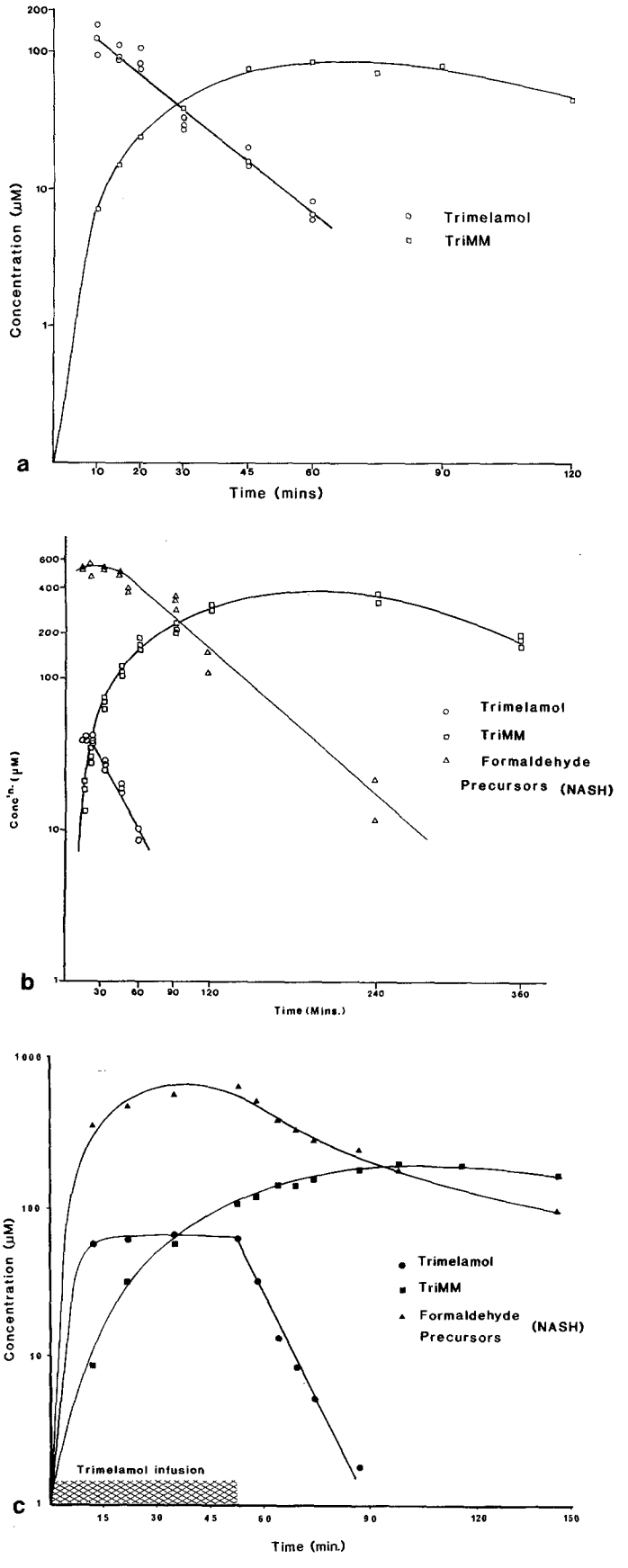

Fig. 3. a Plasma pharmacokinetics of Trimelamol 90 mg/kg i.p. in Balb C^- mice. **b** Plasma pharmacokinetics of Trimelamol 90 mg/kg i. p. in Wistar rats. **c** Plasma pharmacokinetics of Trimelamol in a patient receiving 1500 mg/m^2 by i. v. infusion over 50 min

Table 5. Comparative pharmacokinetics of Trimelamol in mouse, rat and man

Species	Dose (mg/kg)	Route	Peak plasma level μM		$t^1/_2 \beta$ min	AUC	
			Actual ±S.E.	At time 0 ±S.E.		Trimelamol μM. min	TriMM μM. min
Mouse	90	i.p.	124.5±17.1 (10 min)	229.7±22.1	11.7±0.55	3381	6505
Mouse	90	i.v.	198.9±26.1 (5 min)	249.4±28.5	7.2±0.33	2834	2692
Mouse	50	i.p.	66.4±11.9 (10 min)	132.3±18.2	10.2±1.4	1527	987
Mouse	25	i.p.	39.4± 3.0 (5 min)	71.9±10.1	6.7±0.52	602	–
Rat	90	i.p.	38.9± 0.8 (20 min)	76.3± 6.2	20.3±1.2	1642	85900
Man	1500 mg/m^2	i.v.	69.3± 9.8 (50 min)	–	6.3±0.6	3238	–

Toxicity. The toxicity of Trimelamol was compared with that of PMM and TriMM in mice and rats. The overall toxicity of Trimelamol and PMM was similar, whilst TriMM was such less toxic. The most striking difference was in neurotoxicity, in that PMM caused a rapid onset of sedation and hypokinesia at doses down to 30 mg/kg, a problem not seen with Trimelamol even at elevated doses. The LD_{50} values are summarised in Table 6.

Male and female Balb C mice

PMM. Mice receiving PMM became rapidly sedated within 5 min. This effect was dose-related in severity and duration. Animals receiving 120–180 mg/kg also lost their righting reflex for 1–2 h. At doses of 90 mg/kg and above the animals progressively lost up to 20% body weight, and at 120–180 mg/kg a marked drop in body temperature was noted. The days to death following treatment were dose-related, and the LD_{50} for PMM was calculated to be 70 mg/kg for male and 75 mg/kg for female mice.

Trimelamol. Unlike PMM, Trimelamol did not cause profound sedation, and at no dose level was a loss of righting reflex observed. Weight loss occurred, which was similar in severity to that induced by PMM (14%–20%). The days to death were less clearly related to dose and one fatality occurred at 60 mg/kg. The LD_{50} was 70 mg/kg for male and female animals.

Table 6. LD50 values for PMM, Trimelamol and TriMM

Species	Strain	Sex	Drug	LD_{50} (mg/kg) daily x5
Mouse	Balb C$^-$	♀	PMM	75
Mouse	Balb C$^-$	♀	Trimelamol	70
Mouse	Balb C$^-$	♀	TriMM	193
Mouse	Balb C$^-$	♂	PMM	70
Mouse	Balb C$^-$	♂	Trimelamol	70
Rat	Wistar	♂	PMM	187
Rat	Wistar	♂	Trimelamol	105
Rat	Wistar	♂	TriMM	225

TriMM. At the highest dose level, 240 mg/kg, TriMM also caused marked hypokinesia and a fall in body temperature. At 180 mg/kg only a small drop in body weight occurred, i.e. 14% by day 8 with full recovery by day 20, and no toxic deaths were observed. At 210–240 mg/kg all animals exhibited neurotoxic symptoms and died by day 9. The LD_{50} for females was calculated as 193 mg/kg.

Male wistar rats

PMM. A similar dose-related hypnotic effect was again seen, although it was more prolonged, reflecting the slower metabolic clearance of the drug. Weight losses of up to 22% occurred during the first 6 days, especially with above 150 mg/kg. Periorbital haemorrhage was noted after the 2nd day of treatment. The LD_{50} was 187 mg/kg.

Trimelamol. As in the mouse, no serious sedation comparable to that seen with PMM was observed. The overall toxicity, however, was greater than that caused by PMM. Periorbital haemorrhage and lowered body temperature were again observed; weight loss of 16%–26% was seen by day 6; and toxic deaths occurred at doses of 120 mg/kg and above. At 90–150 mg/kg diarrhoea was noted, which was severe after 4 days at 120 and 150 mg/kg. This problem had not been seen with PMM. The LD_{50} was lower than for PMM at 105 mg/kg.

TriMM. Unlike the mice, the rats did not become sedated following treatment with TriMM. They did, however, become notably aggressive at all dose levels. Periorbital haemorrhage was observed after the 2nd day at 240–270 mg/kg. The LD_{50} was 225 mg/kg.

These data were obtained using Chester Beatty material, with 5% ethanol/saline as solvent. In similar experiments using the Warner-Lambert Trimelamol in 5% DMSO/saline, tumour-bearing female Balb C$^-$ mice were treated on a five-times-daily schedule over the dose range 3.125–100 mg/kg. An identical LD_{50} of 70 mg/kg was obtained (Table 3).

Discussion

The clinical usefulness of HMM is limited by gastrointestinal toxicity (nausea and vomiting) and its poor aqueous solubility, which necessitates oral administration. PMM was chosen as a stable, water-soluble alternative to HMM, but unfortunately proved much more toxic in man. In phase I trials it caused severe dose-limiting emesis [8–10], sedation and even coma [3], and little evidence of antitumour activity was seen [3, 8, 9, 11, 18]. A possible explanation for this was given by Rutty et al. [16], who demonstrated that PMM is relatively poorly metabolised to cytotoxic *N*-hydroxymethylmelamine intermediates in man compared with rodents. Having identified this problem Trimelamol was considered for clinical development because of its lack of requirement for metabolic activation [12].

In the initial selection of a water-soluble alternative to HMM, *N*-hydroxymethylmelamines were largely overlooked because of predictable problems of formulation. Although the relatively low aqueous solubility of Trimelamol can be improved by the addition of DMSO, this solvent is avoided in clinical practice if possible, because of severe halitosis and potential hepatotoxicity. A lyophilised formulation was therefore developed, which was rapidly soluble in aqueous media. Trimelamol breakdown was found to be catalysed by acid pH and high ionic strength solutions. Dextrose 5% pH 7.4 proved to be a suitable isotonic aqueous vehicle for this drug. Polymerisation was avoided by keeping the concentration below 4 mg/ml. The bulk material synthesised by Warner-Lambert was found to be only 91.3% pure, but the impurities are also *N*-hydroxymethylmelamines, and as such would be expected to contribute to the antitumour activity [14]. No significant differences in efficacy or toxicity have been observed between the 91.3% and 99.5% pure preparations.

Antitumour activity and toxicity were found to be very similar to those of PMM when tested against the PC6 tumour in mice. In the rat, however, Trimelamol was both more toxic and significantly more effective against the Walker 256 tumour. This may be explained by the lower ability of the rat than the mouse to activate PMM, as shown by the Nash assay, which measures total *N*-hydroxymethylmelamine levels [16]. This explanation is supported by the much higher peak plasma level and AUC for Nash-positive material recorded in the rat following the same dose of Trimelamol and PMM [16]. These differences would be even more striking if the two drugs were compared on an equimolar basis, in which case the dose of PMM would be only 68 and not 90 mg/kg.

Trimelamol was rapidly cleared from the plasma of mouse, rat and man, and only small species differences in plasma half-life were observed. Distribution and clearance were slower in the rat than in the mouse, giving a delayed time to peak level and a longer plasma half-life. Furthermore, a striking difference was observed in the handling of TriMM. The TriMM concentration represents a balance between formation due to chemical decomposition of Trimelamol via the lower *N*-hydroxymethylmelamines (Fig. 1) and by metabolic degradation to yield predominantly N^2,N^4-dimethylmelamine. By analogy with HMM and PMM, this is thought to proceed via oxidative *N*-demethylation, since TriMM is on the same metabolic pathway and N^2,N^4-dimethylmelamine is one of the major urinary metabolites of HMM in rodents and man [15, 19, 20]. Although incompletely separated, an early running peak was seen on HPLC, which co-eluted with an N^2,N^4-dimethylmelamine standard. This occurs only very slowly in the rat and in man, leading to much higher peak plasma levels of TriMM and a very large AUC. The differences in peak plasma level and AUC between IV and IP administration presumably reflect the slower distribution rate following IP injection. A reasonably good correlation was observed between dose and plasma levels in the mouse, and these values can be compared with the single-dose PC6 test results to provide an estimate of the drug concentration required for cytotoxicity. In this system a peak plasma level $>50\,\mu M$ and AUC $>1500\,\mu M\ min^{-1}$ appear to be required for significant single-dose activity. Patients receiving a dose of 1500 mg/m^2 by IV infusion over 1 h had a mean peak plasma level of 69.3 μM and mean AUC of 3238 $\mu M\ min^{-1}$, indicating that potentially cytotoxic plasma levels of the drug can be achieved in man. This is in marked contrast to PMM [16].

The toxicity data were encouraging, in that Trimelamol was clearly less neurotoxic in rodents than PMM. Overall toxicity was similar in the mouse, as expected from the known rate of PMM activation, although Trimelamol did not cause marked sedation. In the rat a similar difference in neurotoxicity was seen, but lethal toxicity was observed at lower doses with Trimelamol than with PMM.

In summary, the problems of stability and solubility associated with the administration of Trimelamol can be overcome by the use of a neutral pH aqueous solvent of low ionic strength and a lyophilised preparation of the drug. Its antitumour activity is similar to that of PMM in the mouse but enhanced in the rat, which is less able to activate PMM. Given the even poorer metabolic activation in man Trimelamol might be expected to be significantly more active clinically than PMM and probably HMM. It is also much less neurotoxic than PMM in rodents. Since parenteral administration of PMM in man actually resulted in an increase in gastrointestinal toxicity compared with HMM, this was clearly a centrally mediated effect. We may therefore hope that the reduced neurotoxicity of Trimelamol will be reflected in a reduced emetic potential in man. There is good evidence to support the clinical evaluation of Trimelamol, and this study has commenced.

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