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Dose-dependent Pharmacokinetics of PMM in the Rat

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Summary. *Concentrations of pentamethylmelamine (PMM) and some metabolites were determined in plasma of rats treated with 10 and 50 mg PMM/kg IV. The areas under the plasma levels curve after these doses were 241 and 1,827 μg/ml × min; plasma clearances were 0.042 and 0.027 l · kg^{-1} · min^{-1}, respectively.*

These data suggest that PMM pharmacokinetics is dose-dependent in the rat. The N-demethylated metabolite levels were not proportional to the administered dose.

Introduction

Pentamethylmelamine (PMM) is a water-soluble metabolite of hexamethylmelamine (HMM), which appears to have the same spectrum of activity of the parent compound [7], with the advantage of being injectable IV. Because of the low bone marrow toxicity seen during phase I clinical trials, PMM doses have been escalated from 80–2,500 mg without any appreciable myelotoxicity having been observed; gastrointestinal and CNS side effects were the dose-limiting drawbacks [5].

Renal elimination of the unchanged drug is negligible in animals and man [1, 3]. The drug is in fact cleared metabolically, substantially through successive N-demethylations by liver microsomal enzymes [1, 2].

Since dose-dependent kinetics have been reported [9] for other drugs undergoing oxidative N-demethylation, it seemed worth investigating the influence of the dose on the plasma disposition of PMM and of some metabolites, in view of the high doses that can be used clinically.

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This report shows that with increasing doses of PMM, the elimination capacity diminishes in the rat.

Materials and Methods

Male CD-COBS rats (Charles River, Italy), weight 180–200 g, were divided into groups of four and received IV PMM at doses of 10 and 50 mg/kg (in saline). The animals were killed at various intervals up to 6 h after drug administration. Plasma was extracted at pH 11.5 once with n-hexane and once with ethylacetate, and analyzed as previously described [4] by gaschromatography with a NPSD detector. PMM, $N_2N_2N_4N_6$-tetramethylmelamine (TMM), and $N_2N_4N_6$-trimethylmelamine (TriMM) were generously provided by NCI, NIH, Bethesda, Md., USA.

A one-open-compartment model was applied for pharmacokinetic analysis, and pharmacokinetic parameters were calculated by conventional methods. Confidence limits of the slope values were calculated to allow statistical comparison of the kinetic curves.

Results

Table 1 shows the concentrations of PMM and of its metabolites TMM and TriMM in the plasma of rats treated with 10 and 50 mg PMM/kg IV. The concentration of PMM was proportional to the dose administered after 1, 5, and 15 min, but not at 30 min and later. After 30 and 60 min the levels after the higher PMM dose (50 mg/kg) were more than 11 times those after 10 mg/kg. At both doses highly significant fitting ($r = 0.995$ and 0.999, respectively) was observed when a one-open-compartment model was used (see Fig. 1).

The disappearance curve after the lower dose appears to be steeper, and the difference was statistically demonstrable ($P < 0.05$) on comparison of their elimination constants (K_{el}), which were 0.054 (0.048–0.060) and 0.0415 (0.039–0.043), respective-

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Table 1. Plasma levels of PMM, TMM, and TriMM after IV injection of PMM[a] in rats

	μg/ml ± SE after (min)							
	1	5	15	30	60	120	180	360
PMM (10 mg/kg)	17.06 ± 1.4	9.64 ± 0.95	4.52 ± 0.63	2.16 ± 0.21	0.68 ± 0.17	0.02 ± 0.004	ND	ND
TMM from PMM 10	ND[b]	0.70 ± 0.27	1.61 ± 0.25	2.34 ± 0.22	3.09 ± 0.49	ND	ND	ND
TriMM from PMM 10	ND	ND	1.68 ± 0.42	2.50 ± 0.87	6.01 ± 2.34	7.78 ± 0.69	4.17 ± 0.81	0.81 ± 0.29
PMM (50 mg/kg)	78.58 ± 10.13	57.90 ± 4.96	30.51 ± 1.47	23.83 ± 3.97	7.70 ± 1.31	0.53 ± 0.06	0.05 ± 0.01	ND
TMM from PMM 50	ND	2.89 ± 0.46	12.35 ± 1.84	17.09 ± 1.09	14.46 ± 2.43	2.18 ± 0.24	1.25	ND
TriMM from PMM 50	ND	ND	ND	6.69 ± 1.48	20.27 ± 3.81	25.51 ± 4.50	23.89 ± 2.07	13.13 ± 1.52

[a] PMM dissolved in saline was injected IV at the doses of 10 mg/kg and 50 mg/kg
[b] ND, not detectable (< 0.01 μg/ml)
Each figure is the mean of four determinations

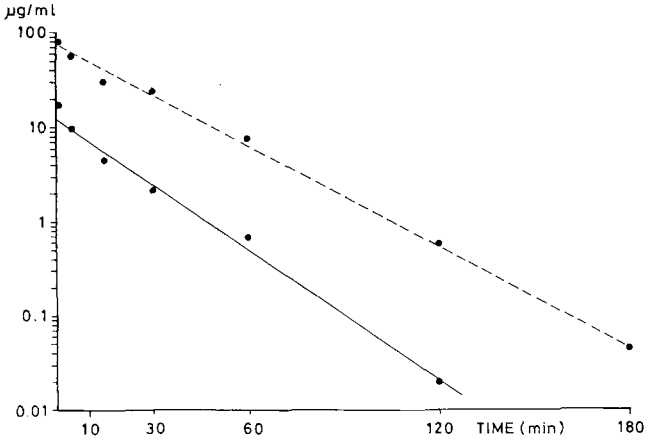

Fig. 1. Disappearance curve of PMM from plasma of rats after IV injection. 10 mg/kg (●——●) ($y = 2.562 - 0.052 \times$); 50 mg/kg (●– – –●) ($y = 4.310 - 0.041 \times$)

Table 2. Plasma pharmacokinetic parameters of PMM injected IV

	10 mg/kg	50 mg/kg
Co[a] (μg/ml)	12.97	74.45
$T_{1/2}$[b] (min)	13 (12–15)	17 (16–18)
Vd[c] ($l \cdot kg^{-1}$)	0.77 (0.55–1.09)	0.67 (0.56–0.81)
Cl[d] ($l \cdot kg^{-1} \cdot min^{-1}$)	0.042	0.027
AUC[e] (μg/ml × min)	241	1,827

[a] Co, drug level extrapolated at time 0
[b] $T_{1/2}$, half-life
[c] Vd, volume of distribution
[d] Cl, body clearance
[e] AUC, area under the concentration versus time curve (theoretical)

ly. The pharmacokinetic parameters for PMM at the two dose levels are reported in Table 2.

The metabolites TMM and TriMM appeared in the plasma 5–15 min after PMM injecton and their levels rose, but not in proportion to the dose, more evidently after 180 min (see Table 1).

Discussion

The rate of elimination of PMM by the rat appears to be influenced by the dose. The plasma clearance value, which provides a measure of elimination, is lower when the dose is higher. This different disappearance rate is reflected by the different half-life and by the lack of proportion of the AUC (area under the plasma level curve) values at the two doses. In a previous study in mice [3], after doses of 100 or 50 mg PMM/kg the clearance values were 0.284 and 0.758 $l \cdot kg^{-1} \cdot min^{-1}$ and the AUC 352 and 66 μg/ml × min, respectively, suggesting that the kinetics are dose-dependent in mice too.

As the drug is eliminated mainly through metabolic biotransformation [1, 2, 3] it seems reasonable to assume that saturation of N-demethylating enzymes is at the basis of the slower elimination of PMM. This is consistent with the observation that TMM and TriMM also appear to be eliminated more slowly after the high dose of PMM.

The findings reported may possibly be important in assessment of the pharmacological effects of PMM in the rat and mouse; even though the mechanism of action of PMM has not yet been elucidated, it seems to be related to its metabolic biotransformaton [6, 8].

If such saturation also occurs in man, raising the dose will not necessarily result in increased activity.

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