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Non-linear pharmacokinetic behaviour of dapsone in the rabbit

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Although the capacities of man to acetylate and deacetylate drugs differ substantially from those of other species (Gordon et al 1973; Tannen & Weber 1979), the rabbit shows several resemblances. This species clearly demonstrates a bimodal distribution of acetylation capacity for sulphonamides and the slow acetylator trait is a recessive genetic characteristic. Substrate specificity of the rabbit *N*-acetyltransferase also parallels that of man for sulphamethazine, sulphanilamide, sulphadiazine, sulphalene, isoniazid and *p*-aminosalicylic acid (Frymoyer & Jacox 1963; Gordon et al 1973; Weber et al 1976). Although it has been suggested that dapsone is bimodally acetylated in the rabbit (Gordon et al 1973), the study now described suggests that the interpretation of plasma dapsone and monoacetyldapsone (MAdapsone) concentrations may be complicated by other factors. In man, dapsone metabolism demonstrates a clear bimodality which is correlated with fast and slow acetylation of isoniazid and sulphamethazine. Unlike these latter drugs, however, the half-life of dapsone is similar in both fast and slow acetylator groups and the difference in genotype is manifested by extensive or reduced metabolism to MA dapsone. Thus the ratio of MAdapsone/dapsone may be used to classify acetylator-type individuals as fast or slow acetylators (Gelber et al 1971). We have examined the pharmacokinetics of dapsone and MAdapsone in rabbits after different doses of dapsone.

Eight adult male New Zealand white rabbits, 4·2–4·75 kg were fasted overnight but allowed free access to water before each drug administration. A solution of dapsone in 0·90% NaCl (saline) containing 5% ethanol was administered as single oral doses of 5, 10 or 20 mg kg^{-1} in random order, separated by an interval of at least one week. At 0 and 0·5, 1, 2, 4 and 6 h after dosing, 2 ml blood samples were drawn into heparinized tubes. Plasma was separated by centrifugation and stored at −20 °C pending analysis.

Concentrations of dapsone and MAdapsone in plasma were estimated by quantitative high performance thin layer chromatography (Ahmad & Rogers 1980) which has a minimum level of detection of 20 ng ml^{-1} and a within-assay coefficient of variation of 7·8% at 100 ng ml^{-1} and 3·5% at 1200 ng ml^{-1}.

Plasma protein binding of drug and derivative was determined by equilibrium dialysis against 0·07 M sodium phosphate buffer (pH 7·4) at 37 °C using Visking tubing. Preliminary experiments showed that neither bind significantly to this membrane. The 1 h plasma sample was used for ex vivo studies.

Examination of the plasma concentration, time plots revealed that the data could be adequately represented by a one compartment open pharmacokinetic model. The plasma elimination half-life was calculated by linear least squares regression of ln (plasma concentration) upon time during the elimination phase to obtain the first order elimination rate constant k. The area under the plasma concentration, time curves (AUC) was estimated by the linear trapezoidal rule with suitable extrapolation for the infinite part of the curve. Total body clearance was calculated from Cl = Dose/AUC and the apparent volume of distribution from V_d = Cl/k. These equations assume complete bioavailability as found in rats, in which the recovery of radioisotope is similar following oral and intraperitoneal administration of radiolabelled dapsone (Andoh et al 1974).

Statistical comparisons were made by Friedman's non-parametric analysis of variance. Data are presented as the median and its dispersion indicated by median absolute deviations.

Table 1 shows the derivative/drug ratios found at various times after different drug doses. The range of ratios was wide but varied with the dose, being lower at higher doses. There was also a significant ($P < 0.05$) variation with time at the 5 and 10 mg kg^{-1} doses but with 20 mg mg^{-1} the ratio in the plasma was not significantly different over the time studied.

Table 2 gives the derived pharmacokinetic parameters for drug and derivative at the various doses of drug. With increasing dose of drug clearance is decreased and the half-life correspondingly lengthened. There is a significant increase in the apparent volume of distribution and this may be associated with the decrease in dapsone binding to plasma protein that occurs with increasing dosage of drug. No significant change was detected in the elimination rate constant of MAdapsone but with increasing dapsone dosage the normalized AUC does not commensurately increase. This is associated with a significant increase in the normalized AUC for the drug. These AUC changes are another facet of the changing ratio of derivative/drug with increasing dose.

Fig. 1 shows that dapsone binding to rabbit plasma protein is non-linearly related to drug concentration.

Table 1. Medians (median absolute deviation) of MAdapsone/dapsone ratios at different times following oral administration of 5, 10, 20 mg kg^{-1} dapsone.

Dapsone dose (mg kg^{-1})	Time (h) after dapsone administration 0·5	1	2	4	6
5	0·87 (0·16)	1·37 (0·72)	1·53 (0·67)	1·63 (0·57)	1·64 (0·30
10	0·82 (0·43)	0·74 (0·31)	0·80 (0·42)	0·87 (0·47)	1·44 (0·91)
20	0·47 (0·26)	0·58 (0·35)	0·32 (0·17)	0·53 (0·27)	0·58 (0·25)

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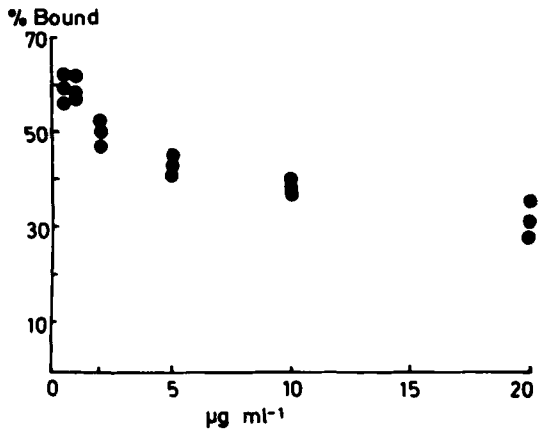

FIG. 1. Relationship of binding of dapsone by rabbit plasma in vitro to total concentration. Plasma from 3 animals was used.

Table 2. Median (median absolute deviation) of pharmacokinetic parameters of dapsone and MAdapsone after administration of indicated dapsone dose.

Dapsone	Dose of dapsone (mg kg⁻¹) 5	10	20	Significance of change in parameter with dose
k (h^{-1})	0·42 (0·06)	0·30 (0·01)	0·30 (0·01)	0·01
Dose normalized*				
AUC (μg ml^{-1} h)	0·83 (0·08)	0·92 (0·20)	1·22 (0·09)	0·001
Vd (*litre* kg^{-1})	2·75 (0·31)	2·85 (0·29)	3·07 (0·32)	0·01
Cl (ml min^{-1} kg^{-1})	20·4 (2·8)	18·1 (2·9)	13·5 (0·5)	0·0001
Protein binding (%)	59·6 (0·4)	59·1 (1·5)	53·7 (0·1)	0·01
MAdapsone				
k (h^{-1})	0·31 (0·06)	0·29 (0·02)	0·26 (0·03)	N.S.
Dose normalized*				
AUC (μg ml^{-1} h)	1·01 (0·29)	1·09 (0·54)	0·87 (0·44)	0·01
Protein binding (%)	83·3 (0·1)	80·8 (1·5)	79·8 (0·5)	N.S.

* Normalized to correspond with a dapsone dose of 5 mg kg^{-1}.

These findings are consonant with the ex vivo findings (Table 2). Reduction in plasma protein binding at increasing plasma drug concentrations could result in an increased apparent volume of distribution and renal drug clearance.

The rabbit clearly differs from man in whom, after the drug, the derivative/drug ratio is constant and is attained almost immediately (Gelber et al 1971). It has been suggested that the percentage of acetylation of the administered drug is the most consistent parameter for demonstrating the acetylator phenotype (Gordon et al 1973). Our results would suggest that this distinction cannot be easily made with dapsone in the rabbit because of the dependence of this fraction both on dapsone dosage and the time after drug administration. In this group of rabbits there was a wide range of acetylation rates, e.g. with 5 mg kg^{-1} drug, the derivative/drug ratio varied from 0·64 to 3·26 at 2 h which because of the small number of animals did not allow identification of acetylator population groups as described by Gordon et al (1973). Whilst the correlation coefficients between the ratio and the 2 h plasma drug concentration were 0·005, 0·30 and 0·46 at 5, 10 and 20 mg kg^{-1} doses respectively, in contrast, the corresponding correlations for derivative were 0·91, 0·99 and 0·80. The extent of acetylation (which is not the only metabolic fate of dapsone) may therefore be more easily determined from measurement of the formation of MAdapsone. However, deacetylation of MAdapsone to dapsone occurs in the rabbit (Gordon et al 1973), although its extent is uncertain. In man, that deacetylation is extensive and a steady state of dapsone acetylation and MAdapsone deacetylation is attained almost immediately after administration of drug (Gelber et al 1971), the differences in the ratios characterizing fast and slow acetylators probably resulting primarily from acetylation rather than differences in deacetylation. In the rabbit, some of the variation in the ratios could be due to a different pattern of deacetylation.

With increasing dapsone dose, the dose-normalized AUC increased significantly and was associated with a prolonged half-life, decreased clearance and increased apparent volume of distribution of the drug. The AUC for dose-normalized MAdapsone was significantly decreased at the highest dose of drug. Such changes in pharmacokinetics could possibly be related to saturation of the *N*-acetyltransferase enzyme.

The data presented for dapsone would be consistent with saturation of *N*-acetyltransferase, although non-linear changes in plasma protein binding have also been demonstrated.

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