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Acetylator phenotype determination in the rabbit: sulphamethazine or sulphadiazine, the choice of a substrate

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The use of diverse phenotyping methods has become a current clinical practice in order to predict and/or avoid some drug related diseases (Ellard 1976). To study drug acetylation, the closest animal model to man is the rabbit, as this animal presents a polymorphic pattern of acetylation (Frymoyer & Jacox 1963a,b). Several substrates have been proposed to segregate fast and slow acetylator rabbits, sulphadiazine (SDZ) being the most accepted. However, SDZ kinetics are non-linear (Souich et al 1978a,b) and as a consequence the classification of an animal according to its acetylator phenotype may in certain cases be difficult. On the other hand, the frequency of slow acetylator rabbits approximates 12% and simple phenotyping methods, using SDZ as substrate, will not avoid misclassifications (Souich et al 1978c) thus, it is relevant to dispose of accurate phenotyping methods. The aim of this study is to present the kinetics of sulphamethazine (SMZ) in the rabbit and to discuss the possible advantages as a phenotyping substrate.

Methods. Male and female New Zealand white rabbits (Canadian Breeding Farm and Laboratories Ltd), 2·6 to 3·5 kg, were maintained on Purina pellets with free access to water in individual well ventilated metabolic cages. Screens were placed below the animal living spaces to avoid faecal or debris contamination of urine specimens. Animals were kept at least 10 days before any experiment was undertaken. All animals (n = 26) were characterized in terms of SMZ plasma kinetics at a dose level of 20 mg kg^{-1} i.v. Blood samples (1 ml) were drawn at 0, 2, 4, 6, 8, 12, 16, 25, 40, 60, 90, 120, 150 and 180 min after drug administration, while urine was collected for 48 h after dose. Three rabbits received additional SMZ doses of 10 and 40 mg kg^{-1} at 10 day intervals. To assess the goodness of rabbit phenotyping using SMZ as a substrate, 5 animals received an additional i.v. 20 mg kg^{-1} dose of SDZ. SMZ or SDZ was assayed in plasma and urine by the Bratton-Marshall technique (Bratton & Marshall 1939). *N*-Acetyl sulphamethazine (NSMZ) or *N*-acetyl-sulphadiazine (NSDZ) were estimated by subtraction of free from total SMZ or SDZ after hydrolysis (6 M HCl at 80 °C for 5 min).

Graphical analysis of a plot of SMZ or SDZ plasma concentrations as a function of time depicted that SMZ or SDZ confer upon the body the characteristics of a two-compartment model. SMZ or SDZ concentration-time curves were adequately described by a biexponential equation, i.e.

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

As the metabolite (NSMZ or NSDZ) is more polar than the parent compound and no data concerning its body distribution are available, we have assumed a single compartment distribution. SMZ or SDZ pharmacokinetic parameters have been calculated by least squares non-linear regression analysis using a NONLIN program (Metzler 1969), fitting simultaneously parent compound and metabolite values in plasma and urine. Parameters calculated included: area under SMZ or SDZ plasma concentration time curve (AUC), peripheral and central volumes of distribution (Vp and Vc respectively), volume of distribution at steady state (Vss), volume of distribution ($V\beta$), total body clearance (Cl_{TB}), renal clearance (Cl_R), metabolic clearance

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Table 1. Sulphamethazine (SMZ) kinetic parameters of 4 slow and 22 fast acetylator rabbits after the i.v. administration of 20 mg kg^{-1} of SMZ.

	Slow		Fast	
AUC (μg min ml^{-1})	3754	± 1340**	1306	± 105*
Cl_M (ml min^{-1} kg^{-1})***	2·72	± 0·79	14·06	± 1·21*
km (min^{-1})	0·0178	± 0·0029	0·0943	± 0·0089*
Cl_R (ml min^{-1} kg^{-1})	5·69	± 2·69	3·20	± 0·66
ku (min^{-1})	0·0649	± 0·0351	0·0278	± 0·0064
Vc (ml kg^{-1})****	97·6	± 9·9	134·6	± 9·2
Vp (ml kg^{-1})	135·5	± 58·6	161·5	± 12·9
$V\beta$ (ml kg^{-1})	336·0	± 120·0	436·0	± 27·9
$t_{\frac{1}{2}}$ (min)	31·9	± 6·5	18·2	± 0·9*

* Significance of difference fast vs slow. Student's *t*-test (unpaired) $P < 0{\cdot}001$.
** Mean ± s.e.
*** Cl_{TB} has been omitted from the Table as $Cl_{TB} = Cl_M + Cl_R$.
**** Vss has been omitted from the Table as Vss = Vc + Vp.

(Cl_M), metabolic and urinary rate constants (km and ku respectively) and SMZ or SDZ plasma elimination half-life ($t_{\frac{1}{2}}$). Initial parameters were calculated graphically as described by Gibaldi & Perrier (1975). The Vc, Vp, Vss, $V\beta$, Cl_{TB}, Cl_M and Cl_R were all corrected for the weight of the animal.

Results. According to SMZ km values, our animals were segregated in 4 slow and 22 fast acetylator rabbits. SMZ pharmacokinetic parameters are shown in Table 1. Despite the great variability, there is a clear difference in SMZ Cl_M, km and $t_{\frac{1}{2}}$ between both groups. The AUC difference reflects clearance dissimilarities and the slightly higher SMZ volume of distribution observed in the fast acetylator rabbits. Changes in SMZ dose did not affect linearity (Table 2), as SMZ clearance and volume of distribution remained constant.

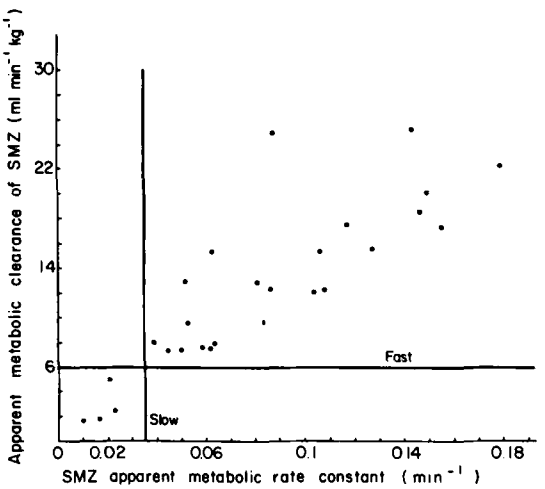

FIG. 1. Segregation of 4 slow acetylator rabbits from 22 fast acetylator rabbits according to the calculated SMZ metabolic rate constant (Km) and SMZ metabolic clearance (Cl_M).

There is a relatively good correlation between SMZ and SDZ kinetic parameters (Table 3). However, of the different phenotyping methods currently used ($t_{\frac{1}{2}}$, Cl_{TB}, percent of the acetylated metabolite in plasma at 1 h or in a 48 h urine collection) only SMZ Cl_M segregated both groups of rabbits without misclassification, when compared with the populations obtained according to SMZ km (Fig. 1).

Discussion. In the rabbit, sulphamethazine elimination kinetic parameters present the characteristic features of polymorphism. Gordon et al (1973) reported SMZ $t_{\frac{1}{2}}$ values of 38 ± 2 and 103 ± 5 min for fast and slow acetylators respectively, estimates very close to those observed by other authors (Weber personal communication; McMahon & O'Reilly 1972). The calculated values of SMZ $t_{\frac{1}{2}}$ in the present study differ from values described previously. The estimated SMZ km in the rabbits approaches the mean value observed by McMahon & O'Reilly (1972) in 4 slow acetylators, i.e. 0·0114 min^{-1}. However, the mean value of SMZ ku, in our animals and under our experimental conditions, is several times higher than the SMZ ku estimated by the latter investigators. These differences in renal elimination may explain the unexpected low SMZ $t_{\frac{1}{2}}$ found in our rabbits. Effectively, according to the relationship:

$$t_{\frac{1}{2}} = \frac{0{\cdot}693}{\beta} = \frac{0{\cdot}693\,\alpha}{k_{21}(km + ku)}$$

As α and k_{21} keep the same relative changes, if ku increases SMZ $t_{\frac{1}{2}}$ decreases. Presently, we have no explanation for the high ku observed in most of our animals. These data illustrate the hazards of using $t_{\frac{1}{2}}$ as the only parameter for determining the acetylator phenotype so, in the present study, none of the rabbits classified as slow acetylators according to their km, would be segregated by the $t_{\frac{1}{2}}$.

Despite SMZ kinetic parameters variability described previously, in five rabbits there is a rather good agreement between SDZ and SMZ kinetic parameters, at the

Table 2. Effect of dose on kinetics of sulphamethazine (SMZ) in the rabbit (n = 3).

SMZ dose (mg kg^{-1})	10	20	40
AUC (μg min ml^{-1})	606·0 ± 277·2*	640·8 ± 252·5**	608·1 ± 211·8**
Cl_{TB} (ml min^{-1} kg)	23·15 ± 7·26	20·34 ± 6·14	20·85 ± 6·70
Cl_M (ml min^{-1} kg^{-1})	17·66 ± 5·78	17·46 ± 5·22	18·84 ± 6·11
km (min^{-1})	0·0772 ± 0·0214	0·0933 ± 0·0302	0·0993 ± 0·0303
Vss (ml kg^{-1})	322·8 ± 48·8	370·0 ± 87·4	361·4 ± 55·9
Vβ (ml kg^{-1})	526·7 ± 96·1	556·7 ± 142·5	566·7 ± 145·2
$t_{\frac{1}{2}}$ (min)	18·6 ± 4·5	20·7 ± 3·7	20·3 ± 2·9

* Mean ± s.e.
** Values are corrected by the increase of dose.

Table 3. Pharmacokinetic parameters of sulphadiazine (SDZ) and sulphamethazine (SMZ), used to determine the acetylator phenotype of rabbits, following i.v. administration of 20 mg kg^{-1} of SDZ or SMZ.

Acetylator phenotype	km (min^{-1})		$t_{\frac{1}{2}}$ (min)		Cl_{TB} (ml min^{-1} kg^{-1})		Cl_M (ml min^{-1} kg^{-1})	
	SDZ	SMZ	SDZ	SMZ	SDZ	SMZ	SDZ	SMZ
Slow No 34	0·0018	0·0172	301·0	51·3	0·73	2·69	0·43	1·78
Fast No 37	0·0242	0·0639	43·3	17·3	3·82	9·39	3·06	7·93
39	0·0250	0·1042	49·5	20·0	3·64	13·60	3·08	11·99
40	0·0257	0·1170	43·9	19·4	3·66	19·48	3·17	17·53
42	0·0320	0·1430	34·8	13·2	5·02	27·56	4·14	25·27

dose level studied. The advantage of SMZ over SDZ as a phenotyping substrate is the fact that, at the range of SMZ doses studied, SMZ kinetics are first-order. Two factors may contribute to SMZ linearity, high Cl_{TB} (Cl_M and Cl_R) and small SMZ fraction bound to proteins when compared with SDZ binding (Anton & Boyle 1964). So, when SMZ doses are increased neither clearance nor protein binding capacity are saturated. However, when SDZ doses are increased, the unbound fraction increases and consequently the volume of distribution and SDZ $t_{\frac{1}{2}}$ (Souich et al 1978a,b).

It is concluded that as a substrate for phenotyping, SMZ presents a major advantage i.e. plasma kinetics are linear, when compared with SDZ. Minor advantages include: firstly, shorter period of blood sampling due to a short $t_{\frac{1}{2}}$, that is, the animal may stay shorter periods in a restraining cage and secondly, percent SMZ bound to proteins is lower, therefore the risk of distortioned kinetics under certain experimental conditions (i.e. renal insufficiency) is less important. On the other hand, with either substrate, to avoid mis-classifications it is relevant to segregate the rabbits according to their Clm or km due to the important variability observed in ku.

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