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Animal modelling of human polymorphic drug oxidation—the metabolism of debrisoquine and phenacetin in rat inbred strains

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The metabolism of debrisoquine (5mg kg^{-1} orally) was investigated in females of 7 strains of rat. Two major metabolic pathways, those of 4- and 6- hydroxylation were found to be polymorphic. The DA strain eliminated in urine only 7-10% of the dose as 4-hydroxy-debrisoquine together with 31-55% debrisoquine while the corresponding values for the Lewis strain were 44-55% and 11-17% respectively. Accordingly, DA and Lewis rats were proposed as models for the human PM (poor metabolizer) and EM (extensive metabolizer) drug oxidation phenotypes. To further test this model, DA and Lewis rats were given phenacetin (200 mg kg^{-1} orally). This underwent *O*-de-ethylation to paracetamol (52-55%) and aromatic 2-hydroxylation (7-8%) in Lewis rats. The corresponding findings in DA rats were 35-40% *O*-de-ethylation and 12-13% 2-hydroxylation. It is suggested that, with respect to both debrisoquine and phenacetin, Lewis and DA inbred rat strains afford a model of oxidative drug metabolism for the human EM and PM phenotypes respectively.

More than ten years have elapsed since the Committee on Problems of Drug Safety in the U.S.A. reported their deliberations on the application of metabolic data to the evaluation of drugs (Committee on Problems of Drug Safety 1969). This report raised a number of 'urgent needs' among which was the establishment of both quantitative and qualitative criteria of drug metabolism to indicate how different animal species resemble and differ from one another in these respects. It was felt that such criteria would help select improved animal models for toxicity testing of drugs for use in man. In addition, the committee never lost sight of the fact that man is a totally heterogeneous species and, as a further 'urgent need', asked for increased exploration of the underlying genetic factors determining metabolic variability in man.

Faced with wide interspecies variation in drug metabolism coupled with the observations of large intersubject variation in man, it has been difficult to envisage reliable animal metabolic models for man, considering that one man may be a poor metabolic model for another. However, the late R. T. Williams' perceptive question (Williams 1974) 'can those species which show defects in certain metabolic reactions of drugs be used for comparison with those groups of human beings which show similar defects in drug metabolism?' gives us a platform from which to amalgamate and investigate the two 'urgent needs' described above.

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Recently, we have had an insight into the magnitude of variability which exists in the population with respect to drug oxidation. The antihypertensive drug debrisoquine has proven useful in this respect, since it is metabolized almost exclusively to 4-hydroxy-debrisoquine, a reaction which shows genetic polymorphism in man (Mahgoub et al 1977; Idle & Smith 1979; Evans et al 1980). Some 9% of the British white population are phenotypically poor metabolizers (PM) of debrisoquine, characterized by a recessive autosomal inherited defective ability to 4-hydroxylate the drug (Evans et al 1980).

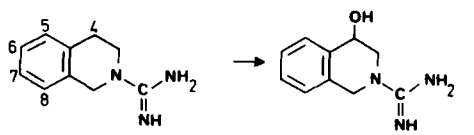

FIG. 1. Debrisoquine 4-hydroxylation.

The ratio between drug and metabolite excreted in 8 h after an oral 10 mg dose (the metabolic ratio) provides the basis of the phenotyping test and is bimodally distributed in the population over a range 0·01–200. The remaining 91% of the population comprises the extensive metabolizer (EM) phenotype, characterized by metabolic ratios $<12{\cdot}6$ (Evans et al 1980).

With the phenotyped panel approach described by Idle et al (1979a), it has been possible to demonstrate that many other drugs show polymorphic drug oxidation in man (see Ritchie et al 1980 for a review). Additionally, the phenotyping of patients

who have developed adverse drug reactions such as metiamide-induced agranulocytosis gives valuable information regarding background factors in the aetiology of the adverse reaction (Ritchie et al 1980).

Obviously, animal models for the EM and PM human oxidative phenotypes would go part way to help answer the urgent need for improved animal models for toxicity testing of drugs and other chemicals to which man may be exposed. In this paper, we describe the polymorphic 4-hydroxylation of debrisoquine in seven rat strains which provides us with two rat strain models, namely Lewis and DA, for the human EM and PM phenotypes respectively. Moreover, these two strains afford an acceptable predictive basis for the metabolism of phenacetin in EM and PM subjects.

MATERIALS AND METHODS

Materials

Debrisoquine, 4-, 5-, 6-, 7- and 8-hydroxy-debrisoquine, and [^{14}C]debrisoquine were all the gift of Roche Products Limited, U.K. and their properties have been previously described (Idle et al 1979b). 7-Methoxy-guanoxan was the gift of Pfizer Limited U.K. Phenacetin and paracetamol were commercially available. 2-Hydroxy-phenacetin was the gift of Dr R. M. Welch, Research Triangle Park, North Carolina, U.S.A. [G-^{3}H]phenacetin was synthesized from [G-^{3}H]paracetamol (Devonshire, H., Sloan, T. P., Idle, J. R., Smith, R. L., unpublished observation).

Animals

Female rats (150–200 g) of the following strains were used: Wistar, Lewis, Fischer, BN (all from St Mary's Hospital Medical School colony), A/GUS, PVG and DA (all from Bantin and Kingman, Grimstone, Hull, U.K.). With the exception of Wistar, all strains were inbred. Animals were maintained on Labshure 41B diet with free access to water.

Dosing of compounds

Various experiments were performed with debrisoquine (5 mg kg^{-1}), [^{14}C]debrisoquine (5 mg kg^{-1}) and phenacetin (200 mg kg^{-1}). In all cases, the drugs were administered as neutral solutions or suspensions in water directly into the stomach using a modified spinal needle. Animals were housed in either metal or plastic metabolism cages which separated faeces from the urine which were collected for 24 h. Both urine and faeces were stored at −20 °C until analysed.

Analytical procedures

Where [^{14}C]debrisoquine was administered to rats, aliquots of urine and faecal homogenates were counted for ^{14}C content in a toluene-triton scintillant using a Packard Model 3385 scintillation spectrometer operating in the external standard mode.

Debrisoquine, 4-, 5-, 6-, 7- and 8-hydroxy-debrisoquine were determined in urine and faecal homogenates as described by Idle et al (1979b) except that a Pye 204 gas chromatograph was used, fitted with a S8 Autojector and DP101 computing integrator. When suitably calibrated against known standards in rat urine, the equipment described gave concentrations (μg ml^{-1}) of debrisoquine and its various metabolites in the samples analysed.

When [^{3}H]phenacetin was administered to rats, paracetamol and 2-hydroxy-phenetidine were determined in urine by h.p.l.c. and colorimetry as described elsewhere (Devonshire et al unpublished). ^{3}H content of samples was determined by scintillation spectrometry (*vide supra*).

RESULTS

Six inbred strains of rat and one outbred strain (three females of each) were given debrisoquine orally (equivalent to 5 mg kg^{-1} free base) and the urinary excretion of drug and its various metabolites determined by gas chromatography. Of the known metabolites of debrisoquine, only the 4- and 6-hydroxy-metabolites were observed. In all the chromatograms, an unidentified drug-related peak was seen which eluted between 5- and 7-hydroxy-debrisoquine standards. An estimation of its quantitative importance has been made, based upon the calibration curves for the two metabolites it eluted between. The excretion of debrisoquine, 4- and 6-hydroxy-debrisoquine and the unidentified metabolite in 0–24 h rat urine for the seven strains is shown in Table 1.

The extent of metabolism of debrisoquine in these seven rat strains was variable, showing marked interstrain differences. In addition, the total urinary recovery of drug and metabolites was also variable, with two strains, Lewis (74·6 s.d. 7·0% of dose) and DA (56·0 s.d. 13·9%), showing reasonable recoveries as estimated by gas chromatography. When the metabolic ratio (% dose as debrisoquine/% dose as 4-hydroxy-debrisoquine) is plotted for each of the 21 animals studied as a frequency distribution, the 4-hydroxylation of debrisoquine in the rat shows polymorphism, as it does in man (see Fig. 2). DA rats had a metabolic ratio of 4·7 s.d. 0·6 compared with Lewis rats for example

Table 1. Metabolism of debrisoquine (5 mg kg^{-1} oral) in 7 rat strains.

Strain		% Dose excreted in 0–24 h urine as: Debrisoquine	40H	60H	U/K*	Total
Wistar	1	3	21	13	10	47
	2	3	26	14	10	53
	3	3	24	16	14	57
Lewis	1	11	44	5	8	68
	2	17	55	5	5	82
	3	14	46	6	8	74
Fischer	1	7	14	6	3	30
	2	2	7	2	1	12
	3	4	10	1	1	16
A/GUS	1	4	22	6	11	43
	2	4	6	5	3	18
	3	2	17	2	1	22
PVG	1	2	13	7	7	29
	2	4	10	8	6	28
	3	3	20	7	6	36
BN	1	3	15	6	10	34
	2	6	17	8	10	41
	3	4	9	4	7	24
DA	1	53	10	4	5	72
	2	34	8	2	5	49
	3	31	7	3	6	47

All animals were female.
40H = 4-hydroxy-debrisoquine.
60H = 6-hydroxy-debrisoquine.
*U/K = unidentified metabolite, phenolic in nature eluting between 5- and 7-hydroxy-debrisoquine.

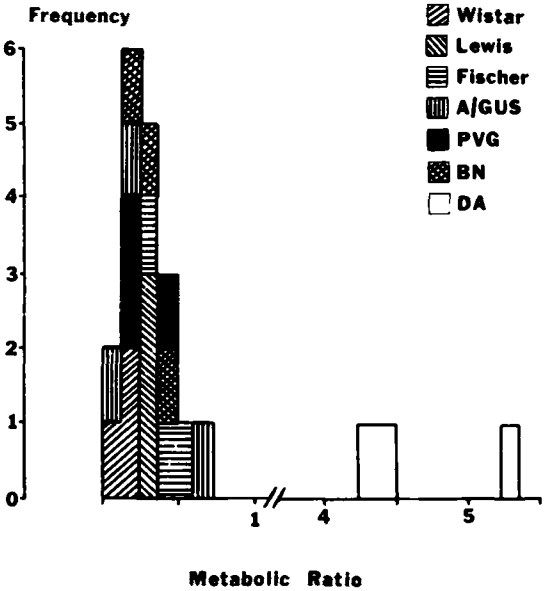

FIG. 2. Frequency distribution histogram of metabolic ratios (% debrisoquine/% 4-hydroxy-debrisoquine) for 7 rat strains.

which had a metabolic ratio of 0·29 s.d. 0·03. If Lewis and DA strains are compared with respect to 6-hydroxylation of debrisoquine, although the absolute amounts of 6-hydroxy-debrisoquine excreted in the two strains does not differ significantly, the ratio debrisoquine/6-hydroxy-debrisoquine also shows polymorphism. When experiments were repeated using [^{14}C]debrisoquine (2 μCi/animal), total recovery of drug and metabolite as measured by gas chromatography fell short of the ^{14}C recovery by between 10 and 30% of dose, indicating the presence of hitherto unidentified metabolites of debrisoquine in the urine.

To investigate the extent to which the Lewis and DA strains of rat behave metabolically like the human EM and PM phenotypes, these two strains were investigated with phenacetin, a substrate which shows large interphenotype metabolic differences in man. Phenacetin undergoes both *O*-de-ethylation and 2-hydroxylation in man and shows interphenotype differences in the former, but not the latter reaction (Sloan et al 1978; Ritchie et al 1980). PM subjects are slower excretors of paracetamol after an oral dose of phenacetin than EM subjects (Sloan et al 1978) and consequently have a higher body load of unchanged phenacetin available for 2-hydroxylation. Although the rate of formation of 2-hydroxylation products is not significantly faster in PM than in EM subjects, the absolute quantity excreted is larger because of the greater amount of the dose available for this pathway in PM subjects (Devonshire et al unpublished; Ritchie et al 1980). When Lewis and DA rats were given [^{3}H]phenacetin (200 mg kg^{-1} + 0·5 μCi) and urine analysed for total (free and conjugated) paracetamol and 2-hydroxylation products (2-hydroxy-phenacetin plus 2-hydroxy-phenetidine) an interstrain difference was observed in both the *O*-de-ethylation and 2-hydroxylation pathways (see Table 2). In Lewis rats, *O*-de-ethylation to paracetamol accounted for 54 s.d. 1·5% of the dose, whilst in DA rats there was a statistically significantly lower *O*-de-ethylation of 38 s.d. 2·2% ($2P < 0.001$). As a result of this, the alternative pathway of 2-hydroxylation was significantly higher ($2P < 0.001$) in DA rats (13 s.d. 0·6%) compared with the Lewis strain (7 s.d. 6%). No significant differences for either strain were observed in the estimation of total urinary recovery in 24 h based upon colorimetric and h.p.l.c. methods (Lewis, 61 s.d. 1·0; DA, 50 s.d. 2·5) and measurement of ^{3}H (Lewis, 59 s.d. 6·1; DA, 45 s.d. 6·4%) suggesting that *O*-de-ethylation and 2-hydroxylation of phenacetin are the only major pathways operable in these rats. Additionally, ^{3}H estimation in faeces and 24–48 h urine gave a total 0–48 h recovery of metabolites of 75–85% dose.

Table 2. Metabolism of phenacetin (200 mg kg^{-1} oral) in Lewis and DA inbred rat strains.

Strain		Paracetamol (A)	*2-hydroxy-phenacetin + 2-hydroxy-phenetidine (B)	Total (A + B)	^3H
Lewis	1	52	8	60	62
	2	55	7	62	52
	3	54	7	61	63
Mean (s.d.)		54 (1·5)	7 (0·6)	61 (1·0)	59 (6·1)
DA	1	35	12	47	54
	2	37	13	50	44
	3	39	12	51	39
	4	40	13	53	43
Mean (s.d.)		38 (2·2)	13 (0·6)	50 (2·5)	45 (6·4)

* Assay measures total 2-hydroxylation products by acid hydrolytic cleavage of conjugates and 2-hydroxy-phenacetin to 2-hydroxy-phenetidine.

DISCUSSION

This study has shown that the 4-hydroxylation of debrisoquine is polymorphic in the species rat. Of the seven strains studied, DA rats emerged as a poorer hydroxylating strain than other strains. DA rats were also poorer 6-hydroxylators of debrisoquine than the other rat strains. Only two strains gave good recoveries of drug and metabolites in 0–24 h urine, DA (56·0 s.d. 13·9%) and Lewis (74·6 s.d. 7·0%). Superficially, DA and Lewis rat strains appear to be metabolic models for the human PM and EM phenotpyes respectively. To test this more carefully, both strains were investigated with respect to phenacetin metabolism. The *O*-de-ethylation of phenacetin in man is polymorphic (Devonshire et al unpublished) and under the control of the same gene locus that determines the debrisoquine 4-hydroxylation polymorphism (Sloan et al 1978). Interestingly, DA rats excreted significantly less paracetamol (38 s.d. 2·2%) than Lewis rats (54 s.d. 1·5%) which compares well with the differences between the human PM and EM phenotypes with respect to the *O*-de-ethylation of phenacetin. Possibly of greater implication, is the observation that the alternative 2-hydroxylation pathway, known to be associated with haemotoxic effects in man (see Ritchie et al 1980), was significantly elevated in DA rats (13 s.d. 0·6%) above the Lewis strain (7 s.d. 0·6%). The DA strain of rat therefore appears not only to have a relatively defective ability to 4-hydroxylate debrisoquine, but also is partially deficient in its capacity to effect phenacetin *O*-de-ethylation, the sequela of which is a compensated increase in the toxic 2-hydroxylation pathway. Such findings are in excellent agreement, for both debrisoquine and phenacetin metabolism, with the interphenotype metabolic differences for these two drugs in man.

In man, the oxidative metabolism of a number of drugs is controlled to a greater or lesser degree by the same gene locus that determines debrisoquine 4-hydroxylation. Examples of such drugs include phenytoin (Idle et al 1979a), metiamide (Idle et al 1979c), phenacetin (Sloan et al 1978), guanoxan (Sloan et al 1978) and 4-methoxyamphetamine (Kitchen et al 1979). From the findings described in this paper, there is every reason to suspect that the metabolism of these and other drugs may be polymorphic in the rat, DA rats having a relative defective ability to oxidize drugs as has been shown for debrisoquine and phenacetin. Whether or not this polymorphism is genetic in origin remains to be established by breeding studies, but we feel it is likely to be so due to the close parallelism between Lewis/DA and EM/PM oxidative metabolism. Accordingly, we would propose that metabolic studies of drugs and other chemicals might more advantageously be carried out in Lewis and DA rat strains, in order to give a broader insight into the scope of variation of drug oxidation in man, where such a process may be under genetic control and show polymorphism.

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