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The Non-Human Primate: A Possible Model for Human Genetically Determined Polymorphisms in Oxidative Drug Metabolism

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SUMMARY

Genetic polymorphisms of drug oxidation are major determinants of interindividual variations in drug response and toxicity. Many animal models, including rats, have been used for clinical investigations of pharmacogenetics. However, because of large interspecies differences, these data are difficult to extrapolate to humans. We therefore phenotyped 64 non-human primates for debrisoquine and mephenytoin polymorphisms and identified poor metabolizers of both drugs. The frequency of poor metabolizers was 14% for debrisoquine (95% confidence limits, 6.5–25%) and 3% for mephenytoin (95% confidence limits, 0.5–10%). If family studies demonstrate a genetic basis for the two independent defects, this animal species could be used for *in vivo* and *in vitro* pharmacogenetic investigations.

The polymorphic oxidation of debrisoquine (1) and mephenytoin (2) is one of the most widely investigated models in the field of pharmacogenetics, i.e., study of genetic variations in drug metabolism (3, 4). *In vitro* models, including liver microsomes and hepatocyte cultures, have been used to predict drug metabolism pathways and cytotoxicity and to identify drugs linked to known polymorphisms. *In vivo* studies are, however, the only approach to investigate the pharmacokinetic consequences and the appearance of toxic effects related to individual phenotypes. Until now, because of the large interspecies differences in drug metabolism, *in vitro* and *in vivo* studies of pharmacogenetics have been mostly conducted in humans. This type of investigation is limited by the low frequency of poor metabolizers and by the difficulty of obtaining human liver samples. The present study has been undertaken in non-human primates in an attempt to define a suitable animal model for *in vitro* and *in vivo* investigations of polymorphic oxidative metabolism.

Methods

A total of 64 adult "crab-eater" monkeys (*Macaca fascicularis*), 20 males and 44 females, age range 4 to 19 years, were included. Renal and hepatic functions were normal within six months before the study. Anesthesia was performed using ketamine (5 to 10 mg/kg) intramuscularly to facilitate the simultaneous oral administration of 10 mg of debrisoquine (Declinax; Hoffmann-La Roche, Basel, Switzerland) and 100 mg of racemic mephenytoin (Mesantoin; Sandoz Bosle, Switzerland). Urine was collected over the following 8-hr period. The urine volume was determined and aliquots were kept frozen (−20°) until analysis.

Debrisoquine, 4-hydroxydebrisoquine, and 4-hydroxymephenytoin concentrations were determined using previously reported high performance liquid chromatography procedures (5). The two individual metabolic ratios, debrisoquine/4-hydroxydebrisoquine and (*S*)-mephenytoin/4-hydroxymephenytoin [(*S*)-mephenytoin being half the oral dose of racemic mephenytoin], were calculated.

The two frequency distribution histograms were constructed using individual metabolic ratios for debrisoquine and the logarithmic values of metabolic ratios for mephenytoin (because of the wide range of observed values). Statistical analysis was by Student's *t* test, with $p < 0.05$ as the minimal level of significance.

Results

Debrisoquine metabolism. Large variations in individual urinary debrisoquine and 4-hydroxydebrisoquine excretions were observed and individual metabolic ratios exhibited a bimodal distribution (Fig. 1). As only 64 primates were phenotyped, the exact value of the antimode could not be precisely determined. We arbitrarily separated two populations using the human antimode of 12.6 (2), which appeared compatible with the observed results in primates. Using the antimode of 12.6, two distinct types of primates were identified. A total of 55 primates were classified as extensive metabolizers; they eliminated large amounts of 4-hydroxydebrisoquine and had metabolic ratios under 11. In contrast, 9 primates eliminated large amounts of unmodified debrisoquine and had metabolic ratios over 15. The results are presented in Table 1.

Data from males and females were analyzed separately. No sex differences were found in extensive metabolizers for the output of debrisoquine and 4-hydroxydebrisoquine, and for the metabolic ratios. No sex differences were noted among poor metabolizers. The study was repeated after an interval of at least 2 weeks in seven primates. No differences were observed in debrisoquine metabolic ratios between the two experiments.

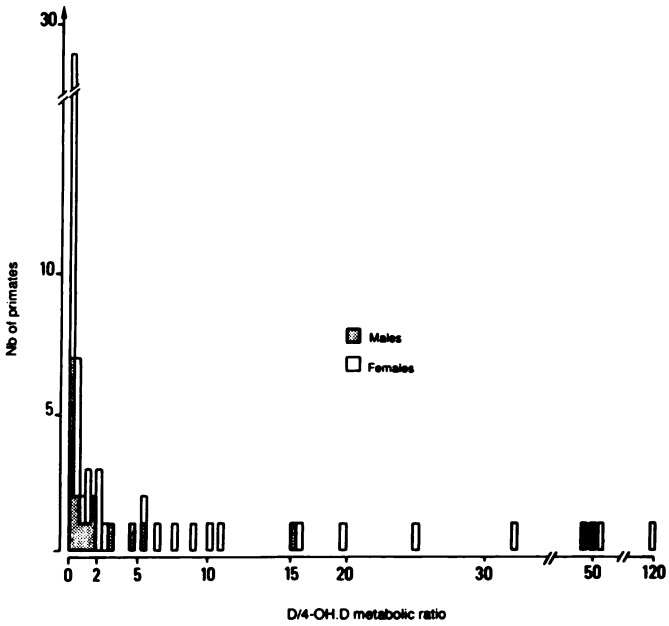

Fig. 1. Frequency distribution histogram of debrisoquine hydroxylation ratio in non-human primates. $n = 64$.

TABLE 1

Debrisoquine and 4-hydroxydebrisoquine contents of urine samples from 64 primates after an oral dose of 57 μmol of debrisoquine (mean ± standard error)

	Extensive metabolizers		Poor metabolizers	
	Male	Female	Male	Female
No. of animals	16	39	4	5
Debrisoquine output (μmol/8 hr)	8.3 ± 2.5	9.0 ± 1.7*	27.1 ± 7.4	29.0 ± 3.9
4-OH-Debrisoquine output (μmol/8 hr)	7.5 ± 0.9*	10.4 ± 0.9*	1.00 ± 0.39	0.98 ± 0.24
Metabolic ratio	1.3 ± 0.4*	1.7 ± 0.5*	36.7 ± 8.3	45.1 ± 20.0

* Significantly different ($p < 0.001$) from poor metabolizers irrespective of sex.

TABLE 2

Effects of anesthesia on debrisoquine metabolism in four extensive metabolizer primates

	Without anesthesia	With anesthesia
No. of animals	4	4
Debrisoquine output (μmol/8 hr)	5.2 ± 3.2	5.3 ± 3.1
4-OH-Debrisoquine output (μmol/8 hr)	25.7 ± 14.9	28.6 ± 16.5
Metabolic ratio	0.18 ± 0.1	0.17 ± 0.1

The influence of anesthesia was also eliminated by repeating the experiment without anesthesia in four primates (Table 2).

Mephenytoin metabolism. With mephenytoin, urinary hydroxylation indices showed bimodal distribution, with two discrete populations separated by a large antimode gap (Fig. 2). A total of 62 primates were extensive metabolizes and two were poor metabolizers. The results are presented in Table 3.

The output of 4-hydroxymephenytoin by male extensive metabolizers was higher than that of their female counterparts. The corresponding mephenytoin metabolic ratios were lower in the males than in the females.

TABLE 3

4-Hydroxymephenytoin content of urine samples from 64 primates after an oral dose of 229 μmol of (S)-mephenytoin (mean ± standard error)

	Extensive metabolizers		Poor metabolizers	
	Male	Female	Male	Female
No. of animals	19	43	1	1
4-OH-Mephenytoin output (μmol/8 hr)	147.9 ± 8.3*	111.0 ± 7.3	0.86	0.33
Hydroxylation index	1.68 ± 0.13*	2.65 ± 0.23	267	696

* Significantly different ($p < 0.01$) from female extensive metabolizers.

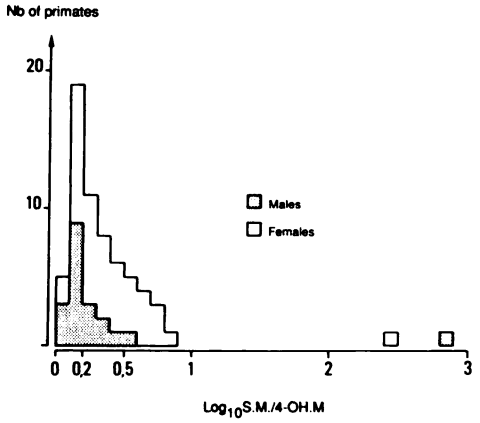

Fig. 2. Frequency distribution histogram of $\log_{10}$ mephenytoin metabolic index in non-human primates. $n = 64$.

Discussion

Debrisoquine and (S)-mephenytoin are known to exhibit independent and genetically determined polymorphisms in the formation of their respective metabolites, 4-hydroxydebrisoquine and 4-hydroxymephenytoin, and it is possible to characterize individual phenotypes by calculating the two metabolic ratios. The frequency of the poor metabolizer phenotype in the human population of Western Europe is 5–10% for debrisoquine and 3–6% for mephenytoin.

This study was undertaken to identify an animal model for human genetically determined drug oxidation polymorphisms. A total of 64 non-human primates were phenotyped with debrisoquine and mephenytoin. Poor metabolizers were identified for both drugs, but no primates carried the two enzymatic defects.

With mephenytoin, there was a clear-cut difference between extensive and poor metabolizers. The frequency of poor metabolizers was 3% (95% confidence limits, 0.5–10%). The significance of the male/female difference in 4-hydroxymephenytoin output is unknown. However this could be related to the relatively small number of males included in the study.

With debrisoquine, individual metabolic ratios were bimodally distributed, with a majority of primates being extensive metabolizers. Because of the small number of animals tested, the exact value of the antimode could not be determined. As it was not possible to increase the test sample, we selected the human antimode of 12.6, compatible with the bimodal distribution observed, to analyze the data. The estimated frequency of poor metabolizers was 14% for debrisoquine (95% confidence limits, 6.5–25%). Further studies would be necessary to verify

that the same antimodes apply for humans and non-human primates.

Debrisoquine and mephenytoin are specific marker substrates for two isoenzymes of cytochrome P450 (6). Multiple cytochrome P450 forms have been isolated in various species. They have different electrophoretic and immunological properties and high substrate specificities (7). The mechanism of enzyme specificity is not yet understood and it is not possible to identify, from their chemical structure, the drugs that exhibit altered metabolism linked with the already known polymorphisms. Neither is it possible to predict the eventual consequences of altered metabolism, except by *in vitro* and *in vivo* studies.

In vitro, information on the metabolism of a drug by hepatic microsomes can be provided by inhibition studies using either competitive substrates or antibodies that are specific inhibitors of individual forms of cytochrome P450. Using human microsomes, competition between sparteine and debrisoquine initially demonstrated that the two drugs were metabolized by the same enzyme (8). The recent development of human hepatocyte cultures now allows identification of drug metabolic pathways and hepatic cytotoxicity *in vitro* (9). *In vivo*, the "phenotype panel approach" (10) and "inhibition studies" (11) are used to identify new drugs the metabolism of which is dependent upon a known polymorphic enzyme. For a given drug, the pharmacokinetic consequences and the possible changes in pharmacological responses or the appearance of toxic effects related to individual phenotypes can only be evaluated *in vivo*.

Many animal species including rats have been examined for their potential as replacements for human volunteers in investigations of polymorphic drug metabolism. However, only mature female DA rats are poor metabolizers of debrisoquine, whereas male and female SD rats are extensive metabolizers. Furthermore, results obtained in rats can not be extrapolated to humans (12). The present study was performed in a large population of non-human primates and has identified rapid and slow metabolizers of debrisoquine and mephenytoin in both male and female animals. The preliminary results of *in vitro* enzymatic studies confirm a defective hepatic metabolism of debrisoquine or mephenytoin in corresponding poor metabolizers versus extensive metabolizers. Our results suggest that non-human primates could be an animal model for *in vivo* and *in vitro* pharmacogenetic studies and contradict a recent report, using a few animals (13), that suggested that primate monkeys are unsuitable for pharmacogenetic studies. However, complete validation of our model will depend on family studies to demonstrate a genetic basis for the two defects.

The cytochrome P450 system is involved, as well as drugs, in the metabolism of xenobiotics. Previous studies suggested that polymorphic hepatic metabolism could be implicated in the appearance of cancer (14) or major birth defects (15). Our animal model could open new ways for investigating variations in susceptibility to environmental factors. However, further studies are necessary to determine the exact molecular mechanism of the enzymatic defects found in non-human primates defined as poor metabolizers of debrisoquine and mephenytoin and to demonstrate the genetic origin of the two defects.

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