Biochemical Pharmacology, Vol. 31, No. 8, pp.1665-1668, 1982.
Printed in Great Britain.

0006-2952/82/081665-04$03.00/0

PRELIMINARY COMMUNICATION

ELEVATED SERUM CHOLESTEROL IN DRUG-OXIDATION-DEFICIENT RATS

Hekmat H. Nadir, Sabah G. Al-Dabbagh and Jeffrey R. Idle

Department of Pharmacology, St. Mary's Hospital Medical School, Paddington, London W2 1PG, UK.

(Received 14 January 1982; accepted 25 January 1982)

Genetic polymorphism of drug metabolic oxidation is now a well-established phenomenon in man (1-6) and has more recently been demonstrated in the rat (7,8). Thus, about one in every ten British white subjects is a Mendelian recessive for impaired hydroxylation of debrisoquine (1,2) and a number of other drugs (4-6). Various consequential aspects of this polymorphism have been investigated, but these have all been concerned with the pharmacology and toxicology of drugs and other foreign chemicals in the recessive poor metabolizers compared to extensive metabolizing persons (5,6,9,10).

The need for an animal model, having some of the metabolic and genetic characteristics of the human polymorphism, was soon apparent. Such a model meeting these criteria would permit screening of new substrates and allow pharmacological and toxicological testing of known substrates of the human polymorphism, which could not readily be achieved directly in man. Accordingly, females of the DA strain of rat have been shown to have a relative impairment of debrisoquine hydroxylation and phenacetin O-deethylation (7), similar to the human poor metabolizer phenotype. Breeding studies using hybrids between DA and Lewis (extensive hydroxylation character) strains, together with f_2 progeny, show that the defective metabolism observed in the maternal DA strain is, as in man, a simple Mendelian recessive trait (8).

The enzymic basis of the oxidative polymorphism in both man and rat almost certainly concerns a genetically variable form of cytochrome P-450. Davies and his coworkers have provided good evidence of this using hepatic microsomes from both species (11, 12). Our own studies of the spectral binding characteristics of hepatic-derived cytochrome P-450 with debrisoquine and other polymorphically metabolized substrates are strongly suggestive of the defect in the DA rat occurring at the cytochrome P-450-drug binding step in the catalytic cycle (13). This molecular defect, manifested as impaired oxidation in the DA rat, might have importance for the metabolism of those endogenous substrates, such as various lipids, which are normally hydroxylated by hepatic and adrenocortical cytochrome P-450. We have thus chosen to investigate certain aspects of cholesterol disposition in strains of rat including the drug-oxidation-deficient DA strain. In this preliminary communication, we report the serum cholesterol concentrations in these rats, in relation to their drug oxidative ability.

METHODS

The following strains (sex, number) of rat were used: DA (female, 9; male, 5), Lewis (female 5), Fischer F344 (female 5; male, 5) and PVG (female, 5; male 5). Details of animal suppliers and maintenance are reported elsewhere (7). Animals (150-200g) were sacrificed and blood withdrawn by cardiac puncture and allowed to clot. The resulting serum (0.1ml in duplicate) was analysed for total cholesterol content using a standard diagnostic colorimetric kit (Boehringer).

RESULTS

Serum total cholesterol concentrations were measured in drug-oxidation-deficient female DA rats, together with male DA rats and three other strains, Lewis, Fischer and PVG, all of which have been previously characterized as phenotypically extensive oxidizers (7). The results from individual animals of these strains are given in Table 1. Clearly, female DA rats have serum cholesterol concentrations (mean ± S.D.) considerably elevated (1100 ± 78μg ml^{-1}) above all of the extensive metabolizing strains studied, comprising female Lewis (746 ± 21), female Fischer (610 ± 84), male Fischer (542 ± 62), female PVG (738 ± 27) and male PVG (778 ± 57). Interestingly, male DA rats which are also phenotypically extensive oxidisers (8) had serum cholesterol levels considerably lower (769 ± 45) than their female counterparts. Statistical comparison of female DA and Lewis rats in this respect, the two strains which we have proposed as metabolic models for the two human oxidative phenotypes (7), shows a highly significant difference ($t = 9.8$, $P < 1ppm$) between the observed cholesterol concentrations. Female DA rat serum cholesterol was some 50% elevated above Lewis, PVG and male DA and about double that of Fischer rats.

DISCUSSION

Hypercholesterolemia in the rat was observed over 30 years ago by Kohn (14) whose data we believe, are consistent with two co-dominant serum cholesterol traits, Sprague-Dawley (1200 ± 60μg ml) and Osborne-Mendel (1320 ± 40) representative of a high cholesterol trait and Tumblebrook Hooded (685 ± 33) and Holtzman (653 ± 28) representative of a low cholesterol trait. Our data are in good agreement with these older findings but moreover possibly provide a basis for these genetic differences in rat serum cholesterol. A marked phenotypic difference exists between female DA rats and Lewis, Fischer and PVG rats with respect to certain drug oxidations (7). It is possible that this polymorphism influences the metabolic disposition of cholesterol, causing hypercholesterolemia in females of the metabolically-deficient DA strain. It has been determined that over 70% of total cholesterol synthesized is further hydroxylated to bile acids which are excreted as such (15, 16). Impaired bile acid formation in female DA rats might explain the elevated sterol levels we have observed in these animals. Alternatively, hypercholesterolemia in the rat may arise from a polymorphic gene locus closely linked to that which determines deficient drug oxidation in the rat. This latter hypothesis is thought to be unlikely for the following reason; only females of the DA strain exhibit the impairment of drug oxidation (7, 8), males appearing as phenotypically extensive oxidisers, even in the f_2 progeny of DA X Lewis crosses (8). The striking parallelism both between strains and within the DA strain (sex difference) in the metabolism of debrisoquine and the serum cholesterol levels, would suggest that cholesterol disposition in the rat is directly influenced by the drug oxidation polymorphism. Thus far, the DA/Lewis rat strain model has correlated well with the human poor and extensive oxidizer phenotypes with respect to drug oxidation. We are intrigued by the notion that cholesterol disposition in man may in some way, either genetically or biochemically, be linked to the human drug oxidation polymorphism as it appears to be in the rat.

Table 1. Serum cholesterol levels of rats of various strains

	* Serum cholesterol ($\mu g\ ml^{-1}$) of strain:						
Rat No.	DA female	DA male	Lewis female	Fischer female	Fischer male	PVG female	PVG male
Phenotype+	PM	EM	EM	EM	EM	EM	EM
1	1150	747	766	624	495	784	764
2	1160	720	734	475	653	744	693
3	1110	818	755	604	495	714	804
4	1060	827	723	644	525	724	844
5	950	732	745	713	525	744	804
6	1000						
7	1160						
8	1120						
9	1170						
Mean ± S.D.	1100± 78	769± 45	746± 21	610± 84	542± 62	738 27	778± 57

* mean of duplicates + PM = poor metabolizer; EM = extensive metabolizer

ACKNOWLEDGEMENTS

This work was supported by two scholarships from the University of Mosul, Iraq and by grants from the Wellcome Trust.

REFERENCES

1. A. Mahgoub, J.R. Idle, L.G. Dring, R. Lancaster and R.L. Smith, Lancet ii, 584 (1977).

2. D.A.P. Evans, A. Mahgoub, T.P. Sloan, J.R. Idle and R.L. Smith, J. med. Genet. 17, 102 (1980).

3. M. Eichelbaum, N. Spannbrucker, B. Steincke and H.J. Dengler, Eur. J. Clin. Pharmacol. 16, 183 (1979).

4. J.R. Idle and S.I. Islam, Br. J. Pharmac. 73, 177P.

5. J.C. Ritchie, T.P. Sloan, J.R. Idle and R.L. Smith, in Drug Metabolising Enzymes, Environmental Chemicals and Human Disease, Ciba Foundation Symposium, 76, pp. 219-235, (1980).

6. R.L. Smith and J.R. Idle, in Drug Reactions and the Liver (Eds. M. Davis, J.M. Tredger and R. Williams) pp. 95-104, Pitman Medical, London (1981).

7. S.G. Al-Dabbagh, J.R. Idle and R.L. Smith, J. Pharm. Pharmac. 33, 161 (1981).

8. S.G. Al-Dabbagh, J.R. Idle and R.L. Smith, Abstr. 8th World Congress of Pharmacology, (Tokyo), No. 1983 (1981)

9. R.R. Shah, N.S. Oates, J.R. Idle, R.L. Smith and J.D.F. Lockhart, Br. med. J. 284

10. J.R. Idle, A. Mahgoub, T.P. Sloan, R.L. Smith, C.O. Mbanefo and E.A. Bababunmi, Cancer Lett. 11, 331 (1981)

11. D.S. Davies, G.C. Kahn, S. Murray, M.J. Brodie and A.R. Boobis, Br. J. clin. Pharmac. 11, 89 (1981).

12. G.C. Kahn, A.R. Boobis, S. Murray, S. Plummer, M.J. Brodie and D.S. Davies, Br. J. Pharmac, in press.

13. A. Kupfer, S.G. Al-Dabbagh, J.C. Ritchie, J.R. Idle and R.L. Smith, Hepatology, in press.

14. H.I. Kohn, Am. J. Physiol. 163, 410 (1950).

15. A.C. Ivy, E. Karvinen, T.M. Lin and E.K. Ivy, J. appl. Physiol. 11, 1 (1957).

16. M.D. Siperstein and A.W. Murray, J. clin. Invest. 34, 1449 (1955).