J. Pharm. Pharmacol. 1988, 40: 694–670
Received February 4, 1988

Hydroxylation of Debrisoquine Using Perfused Liver Isolated from Sprague Dawley and DA Rats: Comparison With In-vivo Results

M. VINCENT-VIRY, S. DESHAYES, O. MOTHE*, G. SIEST* AND M. M. GALTEAU*

*Laboratoire du Centre de Médecine Préventive, 2 avenue du Doyen Jacques Parisot, 54501 Vandoeuvre-les-Nancy Cedex, France and * Centre du Médicament, UA CNRS no. 597, 30 rue Lionnois, 54000 Nancy, France.*

Abstract—The hydroxylation of debrisoquine was investigated in Sprague-Dawley (SD) and Dark-Agouti (DA) rats. Female and male rats were phenotyped in-vivo with debrisoquine six times during their growth. The ratios debrisoquine/4-hydroxydebrisoquine of the female DA rats increased until the 15th week and then decreased; but the values of the metabolic ratios never exceeded 2. Female DA rats cannot be considered as genetically deficient for hydroxylation of debrisoquine in regard to the metabolic ratio, but the percentage of debrisoquine excretion is up to ten fold higher than that in the other strains. Therefore SD and DA rat livers were perfused for 2 h when the clearance of debrisoquine was significantly lower in the female DA group than in the other groups. 4-Hydroxydebrisoquine in the perfusate increased with time, but the amount after 120 min was 12 fold lower in the female DA rat group than in the female SD rat group. The results of the male DA group fell between. This study confirms that female DA rats present a lower debrisoquine 4-hydroxylating capacity than other rats but shows that urinary metabolic ratio is not sufficient to assess the deficiency of debrisoquine hydroxylation.

The metabolism of debrisoquine, an antihypertensive drug, is under genetic control. The capacity of an individual to metabolize it is dependent upon both genetic and environmental factors. Its hydroxylation can be deficient in man (Mahgoub et al 1977; Dick et al 1982).

This polymorphism is associated with other drug metabolism patterns such as perhexiline and sparteine (Lennard et al 1982; Inaba et al 1983). In man, the optimal dosage and the toxic level of these drugs are dependent upon individual metabolic capabilities. The ability to metabolize these drugs is genetically transmitted as an autosomal recessive trait and hydroxylation capabilities are different from one ethnic group to another one. The clinical consequences of hydroxylation polymorphism might be important (Idle & Smith 1979).

Some years ago, Al-Dabbagh et al (1981) reported that debrisoquine hydroxylation was polymorphic in different female rat species. Female DA rats were found to be poor hydroxylators for debrisoquine as Sprague Dawley (SD) rats were extensive metabolizers. The lack of type I spectral binding in female DA rat liver microsomes was shown by Küpfer et al (1982).

In spite of the work performed on these species, some questions have not yet been solved, in particular the age at which this deficiency appears and whether it is concomitant with the puberty period. Before beginning work on these species of rats, they were phenotyped during their growth. The work was then continued using isolated perfused liver. Liver perfusion is adapted to study drug metabolism (Pang (1984) and has been extensively used in the past. It is especially interesting to study the clearance of a drug in a recirculating system (Shand et al 1975). In this study, female Sprague Dawley rat livers and male and female DA rat livers were perfused.

Correspondence to: M. M. Galteau, Laboratoire du Centre de Médecine Préventive, 2 avenue du Doyen Jacques Parisot, 54501 Vandoeuvre-les-Nancy Cedex, France.

Materials and Methods

Animals

Sprague Dawley rats were obtained from Iffa-Credo (St Germain sur l'Arbresle, France) and DA rats from the Institute of Biomedical Research S.A. (IBR) (Füllinsdorf, Switzerland). They were then bred in our laboratory. We also tested DA rats obtained from the Centre de sélection et d'élevage des animaux de laboratoire (CSEAL) (Orléans, France).

Analytical assays

Reagents. Debrisoquine sulphate and 4-hydroxydebrisoquine sulphate were a gift from Hoffman La Roche (Basel); guanoxan was a gift from Pfizer (Orsay, France).

Debrisoquine and its major metabolite, 4-hydroxydebrisoquine, levels were measured by gas chromatography as described by Lennard et al (1977) using guanoxan as internal standard. A Packard gas-chromatograph (model 429) equipped with a nitrogen-phosphorus flame ionization detector and a glass column packed with 3% OV-225 on gaschrom Q, 100–200 mesh were used. The flow rate of nitrogen was 40 mL min^{-1}.

In-vivo experiments

The protocol proposed by Al-Dabbagh et al (1981) was applied. Debrisoquine dissolved in a sucrose syrup was administered orally at a dose of 5 mg kg^{-1}.

Animals were housed separately in metabolic cages. Urine was collected over 24 h and stored at −20°C until analysed.

Liver perfusion study

The animals were anaesthetized with pentobarbitone (50 mg

kg^{-1}), then the bile duct, the vena portae and the inferior vena cava were cannulated. Liver was removed from the animal and connected to the perfusion apparatus. The total time of the surgical procedure was about 25 min and the period of ischaemia did not exceed 6–8 min.

The liver was perfused with a Krebs-Henseleit medium pH 7.4 containing bovine serum albumin (25 g L^{-1}). Debrisoquine was added to the perfusate at a concentration of 5 mg kg^{-1} and the perfusion was performed during 120 min. The system was oxygenated by carbogen O_2/CO_2 (95/5%) and the perfusion flow was adjusted to about 4 mL (g liver)$^{-1}$ min^{-1}. Samples of perfusate were withdrawn after 0, 5, 15, 30, 90 and 120 min.

Bile was collected at 30 min intervals for 2 h. The samples were stored at −20°C until analysed.

Liver viability

Two tests were used to assess liver viability, the rate of bile production, and the release of enzymes into the perfusion medium, i.e. lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Activities were measured using Bio-Mérieux kits adapted to a Cobas-Bio analyzer (Roche) (respectively Ref. 63451 and 63291).

Statistical analysis

Statistical analysis was done using the Fisher-Snedecor F-test and the Student's *t*-test.

Results

In-vivo experiments

Relation between metabolic ratio and age. Female and male Sprague-Dawley and DA rats were phenotyped for debrisoquine six times at five week intervals. Fig. 1 illustrates the distribution of metabolic ratios (% debrisoquine/% 4-hydroxydebrisoquine) for the four strains of rats as they grew older (mean ± s.e.m.). Except for female DA rats, the shift of the metabolic ratios was from 0 to 1 during the six experiments (except for one male SD rat at the 20th week which had a ratio of 1.75).

The ratios of the different strains of rats tend to increase until the 15th week and then decrease (except for one male SD rat at the 20th week). At the 11th and 15th weeks, the *t*-test shows a significant difference for the metabolic ratios of the female DA group and the other groups; at the 20th week, significant difference exists between the female DA rats and the other groups except with the male SD group. At the 6th, 25th and 30th weeks, no significant differences between the ratios of the four groups were shown. The greater differences in the ratio's values occur at the 15th week of the growth. Inter-individual variability in the excretion of both debrisoquine and 4-hydroxydebrisoquine was noted for all strains.

Relation between metabolic ratio, sex and strain. Our results confirm those of the literature for the difference between strain and sex. At the 25th week of the study the female DA rats have a metabolic ratios of 0·66 ± 0·04. The male DA rats show a mean ratio of only 0·11 ± 0·02; the female and male SD rats have, respectively, a mean ratio of 0·11 ± 0·02 and 0·39 ± 0·14. If we compare our results with those of Al-Dabbagh et al (1981) or Küpfer & Preisig (1983), the mean metabolic ratio observed in the female DA rats is up to six fold lower than those found by these authors. However, a *t*-test with regard to the female DA rat strain, gives results highly significant for female SD and male DA rats ($P < 0·001$) and a less significant value for male SD rats ($P < 0·05$).

Our strain of female DA rats was tested independently by Küpfer in his laboratory. He found the same results as ours i.e. 0.58 ± 0.06 for the mean metabolic ratio (personal communication). The age of the female DA rats was 24 weeks (Table 1). According to these results, we decided to phenotype another strain of female and male DA rats coming from CSEAL. They show a mean metabolic ratio respectively of 0·80 ± 0·24 and 0·12 ± 0·04 at 25 weeks. The two strains present a statistically significant difference ($t = 4·21$, $P < 0·01$). No actual deficiency was shown in regard to the metabolic ratio of the different strains, so we looked at the excretion of both debrisoquine and 4-hydroxydebrisoquine.

Rate of debrisoquine excretion (Fig. 2). The percentage of debrisoquine excretion in the female DA rats was up to ten fold higher than those of the other strains in our study. It

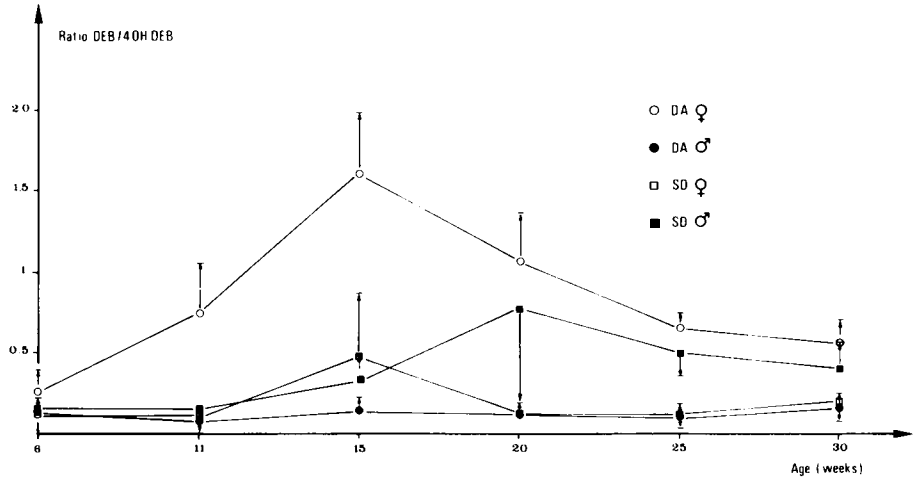

FIG. 1. Distribution of metabolic ratios as a function of time (each group included 5 rats)

Table 1. Comparison of the metabolic ratios in different strains of rats.

	Female DA		Female SD	Male DA	Male SD
	Küpfer study	This study		This study	
Age of the animals in weeks	24 (N = 6)	25 (N = 5)	25 (N = 5)	25 (N = 4)	25 (N = 5)
Metabolic ratios m ± s.e.m.	0·58 ± 0·06	0·66 ± 0·04	0·11 ± 0·02 ***	0·11 ± 0·02 ***	0·39 ± 0·14 *

t-test/female DA rats: *** $P < 0.001$.
* $P < 0.05$.

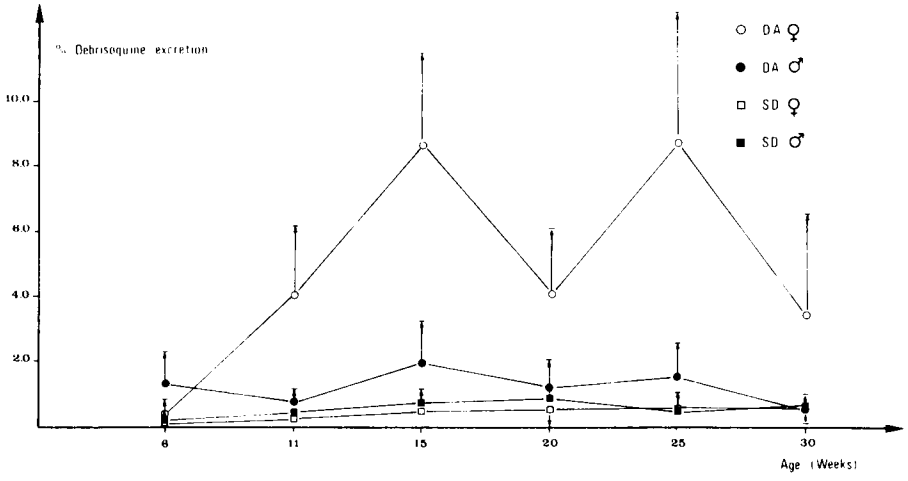

FIG. 2. Debrisoquine excretion rate in 0- 24 h-urine in four strains of rats during growth.

increased with age for female DA rats between 6 to 15 week-old. Here again the inter-individual variations for that rat strain were broad. Compared with the results of Al-Dabbagh et al (1981) and Küpfer & Preisig (1983), we observed that female DA rats used for our study excreted significantly less than those employed by those authors. On the contrary, debrisoquine excretion rate for our other groups was comparable. In our study it seemed that in female DA rats debrisoquine is rather better excreted than 4-hydroxylated compound.

Rate of 4-hydroxydebrisoquine excretion. Table 2 illustrates the 4-hydroxydebrisoquine excretion at the 25th week in the four strains studied. Inter-individual variations are important but our results are similar to those of Al-Dabbagh et al (1981) and Küpfer & Preisig (1983).

Excretion rates of both debrisoquine and 4-hydroxydebrisoquine were confirmed by Küpfer (personal communication). Female and male DA rats from CSEAL gave similar results.

For comparison with in-vivo experiments, we tested liver metabolism using an isolated perfused liver technique, to assess the 4-hydroxylation deficiency of female DA rats.

Isolated perfused liver

Rat and liver weights. Liver and body weights are given in Table 3. All the animals were adults of over 32 weeks. As previously indicated, the low body weight of the female DA rats is inherent to the species. They also have a low liver weight but the ratio liver weight/body weight was similar to those of the other rat strains.

Liver viability (Table 4). DA rats show a bile flow inferior to that of SD rats. This factor is independent of their body and

Table 2. Comparison of debrisoquine and 4-hydroxydebrisoquine excretion rates in four rat strains coming from CSEAL or from IBR at the 25th week of growth (mean ± S.E.M.).

	Age (weeks)	Metabolic ratio	Percentage dose excreted in 24 h urine	
			Debrisoquine	4-hydroxydebrisoquine
Female DA				
IBR	25 (N = 5)	0·66 ± 0·04	8·8 ± 4·0	13·6 ± 6·8
CSEAL	25 (N = 5)	0·80 ± 0·24	8·1 ± 4·0	9·8 ± 1·7
Küpfer (personal communication)	24 (N = 6)	0·58 ± 0·06	6·1 ± 1·2	10·7 ± 2·5
Male DA				
IBR	25 (N = 5)	0·11 ± 0·02	1·6 ± 1·0	11·0 ± 10·1
CSEAL	25 (N = 3)	0·12 ± 0·04	5·2 ± 3·6	40·0 ± 13·0
Female SD	25 (N = 5)	0·11 ± 0·02	0·7 ± 0·3	6·4 ± 3·9
Male SD	25 (N = 5)	0·39 ± 0·14	0·5 ± 0·5	1·5 ± 1·6

Table 3. Body and liver weights of the rats.

	Body weight (g)	Liver weight (g)	$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$
Female SD N = 11	275 ± 6	10·0 ± 0·4	3·7 ± 0·1
Female DA N = 11	205 ± 3	6·4 ± 0·2	3·1 ± 0·1
Male DA N = 6	342 ± 9	10·5 ± 0·5	3·1 ± 0·1

Data are given as the mean ± s.e.m.

Table 4. Bile flow of the perfused liver.

	Treatment		Bile flow: μL g^{-1} liver min^{-1} 1st hour	2nd hour
Female SD	None	5	0·82 ± 0·23	0·55 ± 0·18
	Debrisoquine	6	0·74 ± 0·14	0·72 ± 0·18
Female DA	None	6	0·53 ± 0·04	0·33 ± 0·05
	Debrisoquine	6	0·55 ± 0·09	0·32 ± 0·05

Data are given as the mean ± s.e.m.

liver weights. No difference in bile flow due to the addition of debrisoquine into the perfusate was observed either in the female DA rats or in the female SD rats. In the latter animals, debrisoquine seemed to maintain a better flow rate during the second hour.

The enzymes AST and LDH are usually used to test the viability of isolated cells (Aguiar et al 1985). Their activities in the perfusate showed no significant difference between the five groups of rats in the release of these two enzymes so debrisoquine had no effect on the viability of the model.

Decrease of debrisoquine. The decrease of the concentration of debrisoquine in the perfusate is seen from Fig 3.

On the basis of drug concentration data, the clearances of debrisoquine were calculated for each group: the clearances are given by $D_i/[AUC]^{120}$ where D_i = initial dose and AUC = area under curve. The estimation of AUC was made using the trapezoidal rule. At time 0 debrisoquine was not detectable in the perfusate as the samples were withdrawn after the medium passed through the liver. Therefore, a zero quantity was taken (at this time) in the estimation of the AUC between 0 and 5 min.

The Fisher-Snedecor F-test revealed a significant difference in the three groups of animals ($P < 0{\cdot}05$). The comparison using the t-test for the paired groups shows a clearance of debrisoquine significantly lower in the female DA rats than in the two other groups (compared with the male DA: $P < 0{\cdot}01$, compared with the female SD: $P < 0{\cdot}05$). No significant difference was found between the male DA and female SD rats. This latter group shows a great variability.

Appearance of 4-hydroxydebrisoquine. Fig. 4 represents the appearance of the main hydroxylated metabolite of debrisoquine i.e. 4-OH debrisoquine. The experiments were performed with groups of six animals but the time of the appearance of the metabolite was different for each animal. For example, in the female DA rat group, 4-OH debrisoquine was not detectable before 30 min; for one of the animals, 4-OH debrisoquine appeared only after 120 min of

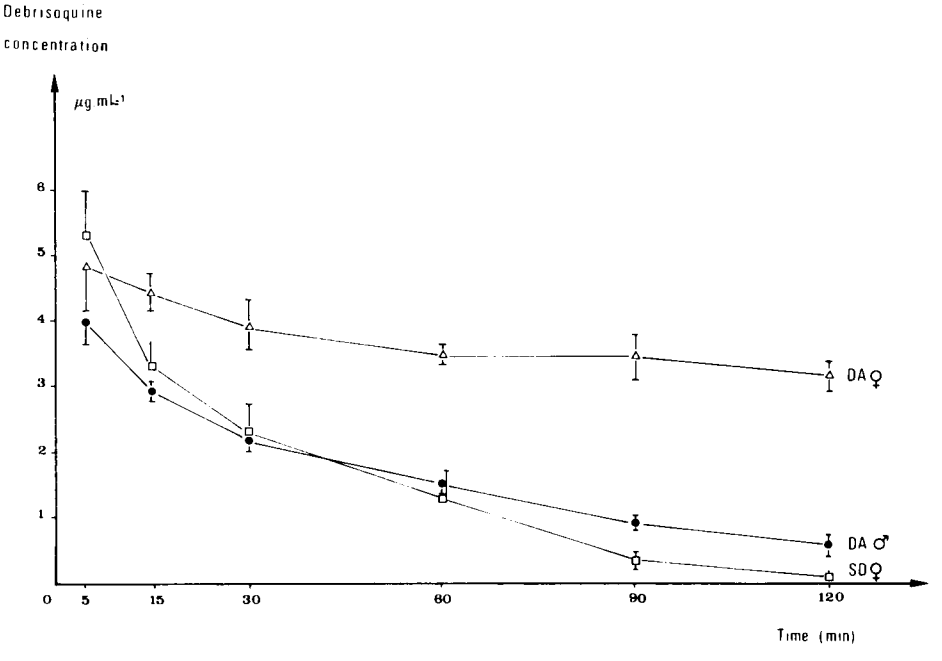

FIG. 3. Decrease of debrisoquine concentrations in the perfusate (each group included 6 rats) Clearance mL min^{-1}: female SD 9·95 ± 2·48, male DA 8·16 ± 0·39, female DA 2·47 ± 0·18.

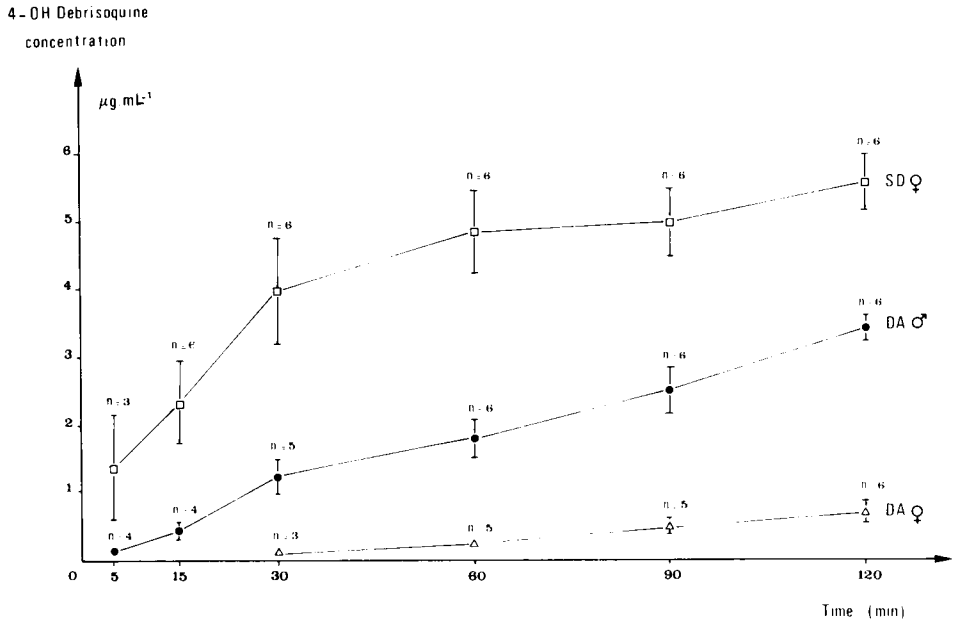

FIG. 4. Appearance of 4-hydroxydebrisoquine concentrations in the perfusate.

perfusion which explains the different numbers of animals included in each group.

The Fisher-Snedecor F-test shows a significant difference between the three groups ($P < 0{\cdot}001$). When the groups are compared in pairs with Student's t-test, the greatest amount of 4-OH debrisoquine is found in the female SD rat group, the least in female DA rats ($P < 0{\cdot}001$); the results of the DA male group fell between (comparison between male DA and female SD $P < 0{\cdot}01$, between male DA and female DA $P < 0{\cdot}001$). The experiments were not carried out for more than 2 h.

Fig. 4 shows that the increase of 4-OH debrisoquine follows approximately a straight line in the female DA rat group. The linear regression of this line gives the equation :

$$y = 0{\cdot}0064\,x - 0{\cdot}097 \text{ with } r = 0{\cdot}993$$

If the perfusion time is extrapolated at the time $x = 24$ h, the dose of 9·1 mg mL^{-1} of 4-OH debrisoquine is found.

Calculated for 150 mL of perfusion liquid, 1·3 mg of 4-OH debrisoquine would then be detectable.

Discussion

In-vivo, male DA rats and SD rats show a metabolic ratio less than 1; the female DA rats do not present ratios different from the other groups except at the 15th week of the study. These results do not correspond exactly with the data of Al-Dabbagh et al (1981) who found a metabolic ratio between 4 and 5 for the female DA rats. Those authors did not specify the age of the rats they tested; they only mentioned the weight of the animals i.e. 150–200 g. The female DA rats tested in our study weighed 165 g at the 15th week of the experiment and 180 g at the 20th, 25th and 30th weeks of the work. So, the range 150–200 g does not give a precise indication of age. The metabolic ratios of the female DA rats never exceeded 2 in our study. The increase in metabolic ratio seems to be parallel with puberty. McClellan-Green et al (1987) related phenotypic difference in expression of cytochrome P-450 g in outbred male SD rats. Some forms of cytochrome P-450 have been identified as female form (P-450 2d) or as male specific form (P-450 2c). So it is necessary to know that expression of phenotype may differ with age and sex. We can conclude that the female DA rats in our study were deficient for debrisoquine 4-hydroxylation but with metabolic ratios ranging around the value of 1·0. This might be due to the instability of the strain or to a change in the phenotypic expression with regard to age, sex and environmental factors. These female DA rats excreted more debrisoquine than the other strains of rats and equal quantity of 4-hydroxydebrisoquine than those used by Al-Dabbagh et al (1981) or Küpfer & Preisig (1983).

These differences in debrisoquine excretion rate in 24 h urine might be due to the mode of administration of the debrisoquine. Al-Dabbagh et al (1981) administered the drugs as neutral solutions or suspensions in water, while in our work, debrisoquine was dissolved in a sucrose syrup beforehand to be administered orally into the stomach of the rats. However, it is well known that the mode of administration can change the amount of debrisoquine or 4-hydroxydebrisoquine excreted (Küpfer & Preisig 1983).

To assess the metabolic function of isolated perfused liver, two criteria were chosen: bile production and release of enzymes.

The rate of bile production is important but fluctuates with many parameters, such as temperature (Brauer et al 1957), composition of the perfusion liquid (Rutishauser 1985) or perfusion flow rate. Perfusion flow rate has to be adjusted to a relatively rapid rate to keep a normal bile flow rate (Beaubien et al 1979) and to ensure a good oxygenation of the liver. If good oxygenation conditions are not ensured, metabolism (Jonen-Kern et al 1978) and clearance (Jones et al 1984) of drugs can be disturbed. The values of the bile flow rate found in our work were slightly less than those of Beaubien et al (1979), but they remain acceptable. A

difference between the DA and the SD rat strain in bile production was seen. This observation is not surprising as variability can be observed between species such as the rat and the guinea-pig (Rutishauser 1985).

The release of enzymes is a good test to assess liver integrity. After 2 h of liver perfusion, the activities of the enzymes released in the perfusate are less than 1 per cent of total organ values (Schimassek 1962). These data are confirmed by our results which are in the same order of magnitude as in other reports (Von Schmidt et al 1966). Debrisoquine does not influence the release of enzymes and therefore is not toxic for the liver cells. In our working conditions, it is therefore possible to presume that rat livers are in good condition during the 2 h perfusion time.

This study confirms earlier observations that the female DA rat has a lower debrisoquine 4-hydroxylating capacity than other rats (Kahn et al 1982).

The amount of 4-OH debrisoquine in the female DA rat liver perfusate after 120 min is 12 times lower than in the SD female rat liver perfusate. This result is in concordance with other recent data: debrisoquine 4-hydroxylase activity in microsomal fractions is up to 14 times lower in DA rats than in animals from other strains (same sex) (Kahn et al 1985). The fact that the female DA rats have a low debrisoquine 4-hydroxylase activity tends to prove that the responsible form of cytochrome P-450 for debrisoquine hydroxylation does exist in the DA rat strain, even if it is in a small quantity. This observation corresponds to the purification of a minor form of rat liver microsomal cytochrome P-450 in the SD rat and in the DA rat where the level of this form is only 5% of that in SD rat liver microsomes (Larrey et al 1984). In man, two forms of cytochrome P-450 involved in polymorphic oxidative activities were purified from liver microsomes (Distlerath et al 1985). Sex difference seems to have a major effect on debrisoquine 4-hydroxylase activity. This may be the expression of more than one form of cytochrome P-450 for debrisoquine hydroxylation. In man, sex difference does not affect the polymorphism of debrisoquine. In rat it is not rare that oxidation of other substrates is modified by sex difference but this is not the case with man (Kato & Kamataki 1982; Kremers et al 1981).

The discordance between the in-vivo and in-vitro results concerning debrisoquine hydroxylation deficiency in the female DA rats can be explained by the time gap between these two types of experiments.

When the rats were phenotyped in-vivo, debrisoquine was measured in the urine collected over 24 h. When livers were perfused, the experiments were not extended beyond 2 h, but by extrapolation an amount of some 1·3 mg of 4-OH debrisoquine was estimated to be detectable after a 24 h perfusion. As the mean body weight of the female DA rats was 200 g, they were given a dose of 1 mg of debrisoquine so an amount of 1·3 mg of 4-OH debrisoquine would signify that all the debrisoquine had been transformed—a result showing that the urinary metabolic ratio may not always reflect hepatic metabolism.

Other data show that, despite a good parallelism between the two types of experiments, differences between the in-vivo and the perfusion experiments exist. This type of observation is also true for other substances, for example, biliary excretion of indocyanine green (Noda et al 1975). On the contrary, substances like phenylbutazone can be studied for their drug elimination qualities in a perfused liver which reflects the in-vivo situation well (Von Bahr et al 1970).

Conclusion

This study shows the difficulty of working on a model without making the in-vivo/in-vitro distinction.

The metabolic ratios are dependent on age and are lower than those found by Al-Dabbagh et al (1981) and Küpfer & Preisig (1983). The urinary metabolic ratio may not reflect the hepatic function. The levels of urinary excretion of debrisoquine are clearly lower than those of the literature, but levels of 4-hydroxydebrisoquine excretion seem in agreement with them. Liver perfusion showed that the DA strain of female rats is deficient in 4-debrisoquine hydroxylase.

Acknowledgment

The authors wish to thank A. Küpfer for the complimentary study done in his Laboratory, and for helpful discussion.

References

Aguiar, T., Galteau, M. M., Siest, G., Fournel, S., Magdalou, J. (1985) Comparative effect of a series of hypolipidemic compounds on the release of enzymes form isolated hepatocytes. Biochem. Pharmacol. 34: 379–380

Al-Dabbagh, S. G., Idle, J. R., Smith, R. L. (1981) Animal modelling of human polymorphic drug oxidation. The metabolism of debrisoquine and phenacetin in rat inbred strains. J. Pharm. Pharmacol. 33: 161–164

Beaubien, A. R., Tryphonas, L., Pakuts, A.P., Mac Conaill, M., Combley, H. A. (1979) Liver perfusion technique using inexpensive nonbinding medium for first-pass metabolism studies of drugs. J. Pharmacol. Methods 2: 213–221

Brauer, R. W., Holloway, R. S., Leong, G. F. (1957) Temperature effects on radiocolloid uptake by the isolated rat liver. Am. J. Physiol. 189: 24–30.

Dick, B., Küpfer, A., Molnar, J., Braunschweig, S., Preisig, R. (1982) Hydroxylierungs defekt für Medikamente (Typus Debrisoquine) in einer stichprobe der Schweizer Bevölkerung. Schweiz med. Wschr. 112: 1061–1067

Distlerath, L. M., Reilly, P. E. B., Martin, M. V., Davis, G. G., Wilkinson, G. R., Guengerich, F. P. (1985) Purification and characterization of the human liver cytochrome P-450 involved in debrisoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. J. Biol. Chem. 260: 9057–9067

Idle, J. R., Smith, R. L. (1979) Polymorphisms of oxidation at carbon centers of drugs and their clinical significance. Drug Metab. Rev. 9: 301–317

Inaba, T., Winks, A., Otton, S. V., Kalow, W. (1983) Comparative pharmacogenetics of sparteine and debrisoquine. Clin. Pharmacol. Ther. 33: 394–399

Jonen-Kern, R., Jonen, H. G., Schupp, R. R., Minck, K. M., Kahl G. F., Netter, K. J. (1978) Reductive drug metabolism in isolated perfused rat liver under restricted oxygen supply. Xenobiotica 8: 271–280

Jones, D. B., Mihaly, G. W., Smalwood, R. A., Webster, L. K., Morgan, D. J., Madsen, N. D. (1984) Differential effects of hypoxia on the disposition of propranolol and sodium taurocholate by the isolated perfused rat liver. Hepatology 4: 461–466

Kahn, G. C., Boobis, A. R., Murray, S., Plummer, S., Brodie, M. J. Davies, D. S. (1982) Enzymatic basis for the defective 4-hydroxylation of debrisoquine. Br. J. Clin. Pharmacol. 13: 594 P

Kahn, G. C., Roubenfield, M., Davies, D. S., Murray, S., Boobis, A. R. (1985) Sex and strain differences in hepatic debrisoquine 4-hydroxylase activity of the rat. Drug Metab. Disp. 13: 510–516

Kato, R., Kamataki, T. (1982) Cytochrome P-450 as a determinant of sex difference of drug metabolism in the rat. Xenobiotica 12: 787–800

Kremers, P., Beaune, P., Cresteil, T., De Graeve, J., Columelli, S., Leroux, J. P., Gielen, J. E. (1981) Cytochrome P-450 monooxygenase activities in human and rat liver microsomes. Eur. J. Biochem. 118: 599–606

Küpfer, A., Al-Dabbagh, S. G., Ritchie, J. C., Idle, J. R., Smith, R. L. (1982) Spectral binding studies of the polymorphically metabolized drugs debrisoquine, sparteine and phenformin by cytochrome P-450 of normal and hydroxylation deficient rat strains. Biochem. Pharmacol. 31: 3193–3199

Küpfer, A., Preisig, R. (1983) Inherited defects of hepatic drug metabolism. Semin. Liver Dis. 3(4): 341–354

Larrey, D., Distlerath, L. M., Dannan, G. A., Wilkinson, G. R., Guengerich, F. P. (1984) Purification and characterization of the rat liver miscrosomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype for genetic variation in oxidative drug metabolism. Biochemistry 23: 2787–2795

Lennard, M. S., Silas, J. H., Smith, A. J., Tucker, G. T. (1977) Determination of debrisoquine and its 4-hydroxymetabolite in biological fluids by gas chromatography with flame-ionization and nitrogen-selective detection J. Chromatogr. 133: 161–166

Lennard, M. S., Silas, J. H., Freestone, S., Trevethick, J. (1982) Defective metabolism of metoprolol in poor hydroxylators of debrisoquine. Br. J. Clin. Pharmacol. 14: 301–303

Mahgoub, A., Dring, L. G., Idle, J. R., Lancaster, R., Smith, R. L. (1977) Polymorphic hydroxylation of debrisoquine in man. Lancet ii: 584–586

McClellan-Green, P., Waxman, D. J., Caveness, M., Golstein, J. A. (1987) Phenotypic differences in expression of cytochrome P-450g but not its mRNA in outbred male Sprague-Dawley rats. Biochem. Biophys. 253(1): 13–25

Noda, T., Araki, K., Koida, M., Kaneto, H. (1975) Drug metabolizing function of isolated perfused liver. Japan J. Pharmacol. 25: 325–333

Pang, S. K., (1984) Liver perfusion studies in drug metabolism and drug toxicity. In: Mitchell, J. R., Herning, M. G. (eds) Drug metabolism and drug toxicity, Raven Press, New-York, pp 331–352

Rutishauser, S. C. B. (1985) The sodium and bicarbonate dependence of bile secretion in the guinea-pig. Comp. Biochem. Physiol. 82A(2): 317–322

Schimassek, H. (1962) Perfusion of isolated rat liver with a semi-synthetic medium and control of liver function. Life Sciences 11: 629–634

Shand, D. G., Kornhauser, D. M., Wilkinson, G. R. (1975) Effects of route of administration and blood flow on hepatic drug elimination. J. Pharmacol. Exp. Ther. 195: 424–432

Von Bahr, C., Sjöquist, F., Levy, G. (1970) Relation between drug elimination kinetics in intact animals and isolated perfused liver systems: phenylbutazone. J. Pharm. Pharmacol. 22: 867–868

Von Schmidt, E., Schmidt, F. W., Herfarth, C., Dettmar, K. H., Fabel, H. (1966) Studien zum Austritt von Zell-Enzymen an Model der isolierten perfundierten Ratten-Leber. Enzym. Biol. Clin. 7: 167–184