Biochem. J. (1987) 243, 859–861 (Printed in Great Britain)

Development of multiple activities of UDP-glucuronyltransferase in human liver

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UDP-glucuronyltransferase activities towards eight substrates were assayed in samples of foetal, term and adult human liver. Activities towards bilirubin, androsterone, testosterone, 1-naphthol, 4-nitrophenol and 2-aminophenol were present in foetal and term liver samples at less than 14% of adult values, whereas activity towards 5-hydroxytryptamine was present in foetal and term liver at 109 and 121% of adult values respectively. Thus a 'foetal' form of UDP-glucuronyltransferase may exist in human liver that is more restricted in substrate specificity than are those of the rat or rhesus monkey.

INTRODUCTION

The development of UDP-glucuronyltransferase has been extensively studied in the laboratory rat (Lucier & McDaniel, 1977; Wishart, 1978; Wishart & Campbell, 1979; Matsui & Watanabe, 1982; Leakey, 1983, 1985), where its multiple activities can be divided into two developmental groups. Group 1 activities, which include those toward indoleamines and planar phenolic compounds develop prenatally during late gestation to reach adult values at birth, whereas group 2 activities, which include those towards compounds of more complex structure, such as bilirubin and steroid hormones, are low or absent at birth and develop postnatally.

Further evidence from purification studies, however, has now established that multiple isoenzymes of UDP-glucuronyltransferase exist in rat liver, so that at least five such isoenzymes develop postnatally and are responsible for the group 2 activities (Burchell, 1980; Weatherill & Burchell, 1980; Falany & Tephly, 1983; von Meyerinck *et al.*, 1985), whereas possibly only a single 'foetal' isoenzyme is responsible for all group 1 activities (Scragg *et al.*, 1985).

Hepatic UDP-glucuronyltransferase activities show differential development in the rhesus monkey that is analogous to that of the rat; activities towards the group 1 substrates 5-hydroxytryptamine, 1-naphthol, 2-aminophenol and 4-nitrophenol are all present in the foetal liver during late gestation at values approaching those of the adult, whereas activities towards the group 2 substrates bilirubin, oestradiol and testosterone are only present at less than 5% of adult values (Leakey *et al.*, 1983). However, it has been shown that in human liver UDP-glucuronyltransferase activities towards bilirubin and 2-aminophenol both develop postnatally (Onishi *et al.*, 1979). This suggests that either no 'foetal' form of UDP-glucuronyltransferase exists in man or that a form exists which lacks activity towards 2-aminophenol.

In order to establish whether or not a 'foetal' 'form of UDP-glucuronyltransferase does exist in man, we have compared, in the present paper, UDP-glucuronyltransferase activities towards eight substrates in post-mortem samples of foetal, term infant and adult human liver.

EXPERIMENTAL

Tissue samples

Post-mortem samples of human liver were removed from donors during autopsies which were performed within 15 h of death. Ethical approval was obtained from the Reproductive Medicine Ethics Committee of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh. Infants with multiple congenital abnormalities or chromosomal abnormalities were excluded.

The liver samples were immediately washed in cold 0.9% NaCl, weighed and homogenized in 3–4 vol. of Buffer A (250 mM-sucrose, 1 mM-dithiothreitol, 0.5 mM-EDTA, 25 mM-KCl, 0.1 mM-phenylmethanesulphonyl fluoride, 10% glycerol and 10 mM-sodium Hepes, pH 7.4), centrifuged at 8000 *g* for 20 min in a refrigerated centrifuge, and the resulting supernatants were rapidly frozen and stored at −70 °C until assay 2–6 weeks later.

On the day of assay, the supernatants were rapidly thawed and centrifuged at 100000 *g* for 30 min to sediment the microsomal fraction, which was washed in 154 mM-KCl, re-sedimented (100000 *g* for 20 min) and finally suspended in 50 mM-Tris/acetate buffer, pH 7.4, containing 20% glycerol at protein concentrations of 5–12 mg/ml.

Liver from Wistar rats was also used as positive controls for the transferase assays and to determine *post mortem* and storage effects on the UDP-glucuronyltransferase activities. Adult or neonatal rats were killed by cervical dislocation or decapitation respectively, and their livers either excised immediately or left *in situ* at room temperature for various times after death. In both cases, liver microsomal suspensions were prepared by the same methods used to prepare the human liver microsomal suspension.

Assay methods

UDP-glucuronyltransferase activities were assayed towards bilirubin, 5-hydroxytryptamine, 1-naphthol,

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2-aminophenol and 4-nitrophenol by adaptations of the methods of Van Roy & Heirwegh (1968), Leakey (1979), Otani *et al.* (1976), Dutton *et al.* (1981) and Burchell & Weatherill (1981) respectively, with respective substrate concentrations of 0.35, 1.0, 0.5, 0.5 and 0.5 mM. Optimum activation was achieved by using a range of digitonin concentrations (0.05–0.5% final concns.) as outlined previously (Leakey, 1979). UDP-glucuronyltransferase activities towards androsterone, testosterone and oestrone were assayed essentially by the method of Rao *et al.* (1976), with substrate concentrations of 125, 800 and 50 μM respectively, and with Brij 58 to achieve optimal activation as described previously (Leakey *et al.*, 1983). The UDP-glucuronic acid concentration used was 2 mM in all transferase assays except that for 5-hydroxytryptamine, where it was 4 mM. The amounts of microsomal suspension added and the incubation times were adjusted to achieve linear reaction rates. Microsomal protein was assayed by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

The samples of human liver used in this report were divided into three groups according to age. The foetal group consisted of either electively aborted foetuses or premature infants which were delivered between 16 and 25 weeks gestation and failed to survive for more than 2 h. The term group consisted of infants born between 37 and 41 weeks gestation and survived 0–4 days *post partum*. This group consisted of three females and four males; there were no observed sex differences in this group for any of the UDP-glucuronyltransferase activities. The adult group consisted of both male and female patients who died from cerebral haemorrhage and were aged between 33 and 76 years. UDP-glucuronyltransferase activities towards the eight substrates are shown in Table 1. Activities towards bilirubin, androsterone, testosterone, 1-naphthol, 2-aminophenol and 4-nitrophenol were all present in the samples from the foetal and term age groups at values less than 14% of those of adults. Activity towards oestrone was present in the foetal and term liver at 30% of adult values, and activity towards 5-hydroxytryptamine was present at 109 and 121% of adult values respectively.

These activities contrast markedly with those of the rat and rhesus monkey, where activities towards 1-naphthol, 2-aminophenol and 4-nitrophenol in addition to activity towards 5-hydroxytryptamine all approximate to adult values at term (Wishart, 1978; Leakey *et al.*, 1983).

The possibility exists that UDP-glucuronyltransferase activities towards 1-naphthol, 4-nitrophenol and 2-aminophenol are less stable in developing human liver than is activity towards 5-hydroxytryptamine. This appears to be unlikely, because these activities possess similar stabilities in neonatal-rat liver (Table 2). In this experiment less than 25% of all four activities were lost after a 5 h period *post mortem* at room temperature, when assayed over a range of digitonin concentrations to eliminate excessive membrane perturbation, and even after 12 h, when significant loss of activities had occurred, activity towards 5-hydroxytrytamine was no more resistant to degradation *post mortem* than were activities towards the phenolic substrates. Furthermore, two of the individual samples within the human foetal sample group had times *post mortem* of 1 and 2 h respectively, but did not exhibit activities towards 1-naphthol or 2-aminophenol that were greater than the sample mean. It therefore appears that a 'foetal' form of

Table 1. UDP-glucuronyltransferase activities in developing human liver

Microsomal suspensions were prepared and assayed for UDP-glucuronyltransferase activities as outlined in the Experimental section. Activities shown are those optimally activated with digitonin or Brij 58 and are expressed as means ± S.E.M. for the numbers of samples given in parentheses. Values are also shown as percentages of adult values of the corresponding activities.

		UDP-glucuronyltransferase activity (nmol/min per mg of microsomal protein)		
Substrate	Age group... / Mean time *post mortem*...	Foetal 7 h	Term 6 h	Adult 6 h
Bilirubin		0.001 ± 0.001 (7) < 1%	0.059 ± 0.015 (7) 6%	1.08 ± 0.13 (4)
Androsterone		0.023 ± 0.011 (4) 3%	0.120 ± 0.035 (5) 13%	0.89 ± 0.23 (4)
Testosterone		0.069 ± 0.023 (7) 8%	0.096 ± 0.022 (7) 11%	0.62, 1.18†
Oestrone		0.030 ± 0.003 (4) 30%	0.031 ± 0.009 (5) 31%	0.101 ± 0.060 (4)
5-Hydroxytryptamine		0.61 ± 0.21 (6) 109%	0.68 ± 0.17 (7) 121%	0.56 ± 0.05 (4)
1-Naphthol		0.34 ± 0.07 (7) 5%	0.64 ± 0.07 (7) 9%	7.46 ± 0.96 (4)
2-Aminophenol		0.114 ± 0.03 (7) 6%	0.110 ± 0.05 (7) 6%	1.84 ± 0.43 (4)
4-Nitrophenol		1.61 ± 0.77 (6) 10%	1.80 ± 0.62 (7) 11%	16.08 ± 2.75 (4)

* Mean time *post mortem* was 6 h for $n = 4$.
† Liver samples from the two female donors only; samples from male donors had less activity and were not included here.

Table 2. Decreases *post mortem* in microsomal UDP-glucuronyltransferase activities in neonatal-rat liver

Six litters of 2-day-old rat pups were randomized, killed by decapitation, and their livers removed and processed either immediately or after the carcasses had been let for 5 or 12 h at room temperature. Sufficient livers were pooled at each time point to provide duplicate 3 g samples. Liver microsomal fractions were prepared and microsomal UDP-glucuronyltransferase activities assayed as outlined in the Experimental section.

	Mean percentage of initial activity remaining after:	
Substrate	5 h	12 h
Bilirubin	72	61
Oestrone	84	61
Testosterone	80	75
5-Hydroxytryptamine	77	56
1-Naphthol	76	62
2-Aminophenol	88	81
4-Nitrophenol	79	58

UDP-glucuronyltransferase occurs in human liver that is active towards 5-hydroxytryptamine, but is much less active towards the three phenolic substrates. Our findings are thus in agreement with Onishi *et al.* (1979), who found that activity towards both 2-aminophenol and bilirubin develops postnatally in man. Previous studies have demonstrated that moderate amounts of UDP-glucuronyltransferase activities towards oestriol (Burchell, 1974) and morphine (Pacifici *et al.*, 1982) are present in human foetal liver. We demonstrate here that a moderate amount of UDP-glucuronyltransferase activity towards oestrone is also present in human foetal and neonatal liver.

The absence of significant UDP-glucuronyltransferase activities towards 1-naphthol, 2-aminophenol and 4-nitrophenol is of particular relevance to perinatal toxicology testing. It appears unlikely that conjugation with sulphate can fully compensate for deficient glucuronidation of such phenols in man; hepatic sulphotransferase activity towards 1-naphthol was found to be present in human foetal liver cytosol, but only at activities less than 3% of those found in foetal rhesus-monkey liver cytosol (J. E. A. Leakey, unpublished work). The human perinate therefore appears to be relatively inefficient at conjugating phenolic compounds with either glucuronic acid or sulphate. The testing of drugs or xenobiotics with planar phenolic structures in perinatal rats or rhesus monkeys may result in serious underestimations of their toxicity to the human perinate if conjugation is the major route of detoxication of these compunds in the test animal species.

We thank Mr. Donald Gardiner and Mrs. Pat Fotheringham for their skilled technical assistance and Ms. Barbara Jacks for her excellent secretarial assistance. This work was supported by a grant from the Scottish Hospital Endowments Research Trust.

REFERENCES

Burchell, B. (1974) J. Steroid Biochem. **5**, 261–267
Burchell, B. (1980) FEBS Lett. **111**, 131–135
Burchell, B. & Weatherill, P. J. (1981) Methods Enzymol. **77**, 169–176
Dutton, G. J., Leakey, J. E. A. & Pollard, M. R. (1981) Methods Enzymol. **77**, 383–391
Falany, C. N. & Tephly, T. R. (1983) Arch. Biochem. Biophys. **227**, 248–258
Leakey, J. E. A. (1979) Biochem. J. **175**, 1119–1124
Leakey, J. E. A. (1983) in Biochemical Basis of Detoxication (Jakoby, W. B. & Caldwell, J., eds.), pp. 77–103, Academic Press, New York
Leakey, J. E A. (1985) in Advances in Glucuronide Conjugation (Matern, S., Bock, K. W. & Gerok, W., eds.), pp. 111–117, MTP Press, Lancaster
Leakey, J. E. A., Althaus, Z. R., Bailey, J. R. & Slikker, W., Jr. (1983) Biochem. J. **214**, 1007–1009
Lowry, O. H., Rosebrough, N. J., Farr, N. J. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Lucier, G. W. & McDaniel, O. S. (1977) J. Steroid Biochem. **8**, 867–872
Matsui, M. & Watanabe, H. K. (1982) Biochem. J. **204**, 441–447
Onishi, S., Kawade, N., Itoh, S., Isobe, K. & Sugiyama, S. (1979) Biochem. J. **184**, 705–707
Otani, G. Abou-El-Markarem, M. M. & Bock, K. W. (1976) Biochem. Pharmacol. **25**, 1293–1297
Pacifici, G. M., Säwe, J., Kager, L. & Rane, A. (1982) Eur. J. Clin. Pharmacol. **22**, 553–558
Rao, G. S., Haueter, G., Rao, M. L. & Breuer, M. (1976) Anal. Biochem. **74**, 35–40
Scragg, I., Celier, C. & Burchell, B. (1985) FEBS Lett. **183**, 37–42
Van Roy, F. P. & Heirwegh, K. P. M. (1968) Biochem. J. **107**, 507–518
von Meyerinck, L., Coffman, B. L., Green, M. D., Kirkpatrick, R. B., Schmoldt, A. & Tephly, T. R. (1985) Drug Metab. Dispos. **13**, 700–704
Weatherill, P. J. & Burchell, B. (1980) Biochem. J. **189**, 377–380
Wishart, G. J. (1978) Biochem. J. **174**, 485–489
Wishart, G. J. & Campbell, M. T. (1979) Biochem. J. **178**, 279–291

Received 31 December 1986/18 February 1987; accepted 25 February 1987