UDP-glucuronyltransferase activity exhibits two developmental groups in liver from foetal rhesus monkeys*

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UDP-glucuronyltransferase was assayed in liver from adult rhesus monkeys and fetuses during late gestation. Activities toward 2-aminophenol, 5-hydroxytryptamine, 1-naphthol and 4-nitrophenol in the foetal liver ranged from 46 to 114% of adult values, whereas activities toward bilirubin, oestradiol and testosterone were less than 5% of adult values. This suggests that in primates UDP-glucuronyltransferase develops differentially in two clusters analogous to that in the rat.

The development of UDP-glucuronyltransferase (EC 2.4.1.17) has been extensively studied in rodents by Wishart and co-workers (Wishart, 1978; see Dutton & Leakey, 1981; Leakey, 1983). In rat liver, two clusters of UDP-glucuronyltransferase activities exist which differ in substrate specificity: Cluster 1 surges towards adult values during late gestation, whereas Cluster 2 remains low until after birth and then surges neonatally (Wishart, 1978). Thus the newborn rat could be more susceptible to toxic effects of substrates glucuronidated by the latter 'neonatal' cluster than by the former 'late foetal' cluster.

The available clinical evidence in man, mostly from autopsied postnatal liver and with the use of two substrates, suggests that UDP-glucuronyltransferase activities belonging to both clusters in the rat may develop together postnatally in man (Onishi *et al.*, 1979). However, no systematic search for late-foetal activities of UDP-glucuronyltransferase in primate liver has been reported.

Non-human primates such as the rhesus monkey (*Macaca mulatta*) are becoming increasingly attractive as experimental animals for perinatal drug-metabolism studies, since their hepatic cytochrome P-450-dependent mono-oxygenase activities develop similarly to those of man (Nau & Neubert, 1978) and not like those of non-primates such as rodents. The present study compared UDP-glucuronyltransferase activities towards seven substrates in liver microsomal preparations from rhesus monkey fetuses during late gestation with those

from adult female monkeys. Differential development was observed in two substrate groups; these groups have a composition comparable with that described in the rat. The possible reasons for the differences between these results and those of Onishi *et al.* (1979) are discussed.

Experimental

Fetuses from time-mated rhesus monkeys were used at gestational ages between 143 and 145 days. Term in this species is 165 ± 5 days. The adult non-pregnant female monkeys were between 7 and 10 years old. The animals were anaesthetized as described previously (Slikker *et al.*, 1982). The fetuses were removed by Caesarian section, killed by exsanguination, and their livers and kidneys were collected. When adult animals were used, 2–4 g of liver was surgically removed from the central lobe and the animal was allowed to recover.

Duplicate samples (1.5–2.5 g) of liver from each animal were homogenized at 0–4°C in 8.5 ml of 10 mM-Tris/HCl buffer, pH 7.6, containing 0.25 M-sucrose, 1 mM-dithiothreitol, 0.5 mM-EDTA, 25 mM-KCl, 0.1% (w/v) phenylmethanesulphonyl fluoride and 5% (v/v) glycerol. The homogenates were centrifuged at 8000g for 15 min; the supernatants were centrifuged at 100 000g for 30 min and the resulting microsomal pellets washed in 154 mM-KCl containing 10 mM-Tris/HCl, pH 7.4, and finally resuspended in 0.1 M-potassium phosphate buffer, pH 7.4, containing 20% glycerol, at a concentration of approx. 1 ml of buffer/g liver wet wt. Microsomal preparations were similarly prepared from foetal kidneys.

* Dedicated to Professor Geoffrey J. Dutton on the occasion of his retirement.

The microsomal suspensions were stored initially at 0–4°C on wet ice for 36 h and then at –20°C for several weeks. UDP-glucuronyltransferase activity was assayed in both fresh and stored samples. Microsomal protein was measured by the method of Lowry *et al.* (1951).

Activities towards bilirubin, 5-hydroxytryptamine, 4-nitrophenol and oestradiol were assayed by the methods outlined by Van Roy & Heirwegh (1968), Leakey (1979), Burchell & Weatherill (1981) and Rao *et al.* (1976) respectively. Activities towards 2-aminophenol, 1-naphthol and testosterone were assayed as outlined by Dutton *et al.* (1981). Assay incubation times and amount of microsomal suspension added were adjusted to give maximum sensitivity while retaining linear rates of UDP-glucuronyltransferase activity towards all sever substrates. Optimally activated activities were obtained by assaying over a range of digitonin concentrations (0.05–0.5% in the assay mixture) or, for the steroid substrates, over a range of Brij-58 (polyoxyethylene 20-cetyl ether) concentrations (0.005–0.05%).

The recovery of UDP-glucuronyltransferase in the microsomal fraction and its subsequent stability on storage was examined in detail by using the activity towards 2-aminophenol. At least 70% of total activity towards this substrate was recovered in the microsomal fraction of both adult and foetal liver samples; low activity was also present in the 8000 g pellet. No activity towards bilirubin was detected in either the 8000 g pellet or the 100 000 g supernatant from the foetal liver.

Maximum UDP-glucuronyltransferase activity towards 2-aminophenol did not change significantly on storage of the liver microsomal preparations at either 0–4°C for 36 h alone or additionally at

–20°C for 6 weeks, when assayed under optimally activating conditions. However, the native (un-activated) activity increased during both these stages of storage, and the concentration of digitonin required to achieve maximum activation decreased. This occurred with microsomal preparations from both foetal and adult liver.

Results

The hepatic microsomal UDP-glucuronyltransferase activities of 143–145-day rhesus monkey foetuses are compared with those of adult female monkeys in Table 1. Relatively high activities towards 2-aminophenol, 5-hydroxytryptamine, 1-naphthol and 4-nitrophenol are present at this stage of gestation, whereas activities towards bilirubin, oestradiol and testosterone remain at <5% of adult activities. Significant activities towards the former four substrates were also present in unactivated microsomal preparations from foetal monkey liver (results not shown), and activities towards 2-aminophenol, 1-naphthol and 4-nitrophenol (but not toward bilirubin) were also present in foetal-kidney microsomal preparations (Table 2).

Discussion

In rat liver, UDP-glucuronyltransferase activities towards 2-aminophenol, 5-hydroxytryptamine, 1-naphthol and 4-nitrophenol develop as a late-foetal cluster, whereas those towards bilirubin, oestradiol and testosterone develop neonatally (Wishart, 1978). Our findings indicate that two differentially developing clusters also occur in liver from the rhesus monkey and, moreover, that the constituent activities of these respective clusters are similar in

Table 1. *UDP-glucuronyltransferase activities in rhesus monkey liver microsomal preparations*

Duplicate liver samples were assayed from three (143–145 days gestation) rhesus monkey foetuses and from three adult non-pregnant females. Microsomal preparations were prepared as outlined in the Experimental section and assayed for UDP-glucuronyltransferase activity. The specific activities (means $\pm$ S.E.M.) are those optimally activated with digitonin or with Brij-58. Substrates indicated as group (1) or (2) correspond, in rat liver, to cluster (1) (late foetal) and cluster (2) (neonatal) activities respectively.

Substrate	UDP-glucuronyltransferase activity		
	Adult female (nmol/min per mg of protein)	Foetus	
		(nmol/min per mg of protein)	(% of adult value)
2-Aminophenol (1)	8.14 $\pm$ 0.89	3.71 $\pm$ 0.63	46
5-Hydroxytryptamine (1)	0.162 $\pm$ 0.051	0.144 $\pm$ 0.022	89
4-Nitrophenol (1)	25.18 $\pm$ 3.40	28.07 $\pm$ 2.29	114
1-Naphthol (1)	18.57 $\pm$ 1.82	12.91 $\pm$ 1.77	70
Bilirubin (2)	0.589 $\pm$ 0.142	0.003 $\pm$ 0.001	0.5
Oestradiol (2)	1.94 $\pm$ 0.57	0.083 $\pm$ 0.007	4.3
Testosterone (2)	2.92 $\pm$ 0.32	0.094 $\pm$ 0.026	3.2

Table 2. *UDP-glucuronyltransferase activities in foetal rhesus monkey renal microsomal preparations*

Both kidneys from each foetus used in the liver experiment were pooled and microsomal preparations made. The UDP-glucuronyltransferase activities are those optimally activated as in Table 1 and are expressed as either means $\pm$ S.E.M., or *mean $\pm$ range. Numbers of samples are in parentheses.

Substrate	UDP-glucuronyltransferase activity (nmol/min per mg of protein)
2-Aminophenol	0.967 ± 0.146 (3)
1-Naphthol	7.57 ± 0.77 (3)
4-Nitrophenol	16.09 ± 1.42 (3)
Bilirubin	0.001 ± 0.001 (2)*

monkey and rat. Primates may therefore resemble non-primates in this respect.

In addition, we find that UDP-glucuronyltransferase activities towards 2-aminophenol, 1-naphthol and 4-nitrophenol are all present in kidney microsomal preparations from rhesus monkey foetuses, whereas activity towards bilirubin is absent. It is therefore probable that the hepatic and renal transferase activities develop here in analogous clusters, though possibly the monkey kidney transferase never develops activity towards bilirubin; in adult human kidney, UDP-glucuronyltransferase is reported to be inactive towards bilirubin (Fevry *et al.*, 1977).

Our conclusions appear to conflict with the clinical findings of Onishi *et al.* (1979), where hepatic UDP-glucuronyltransferase activity towards 2-aminophenol developed neonatally like that towards bilirubin, and not late-foetally. Possible reasons for this disagreement are: (1) the human liver transferase may indeed possess no late-foetal activities; (2) the human liver transferase may indeed possess late-foetal activities, but that towards 2-aminophenol is not one of them; (3) the human liver transferase may possess late-foetal activities, but they are lost on storage or between death and autopsy. Further work will decide, but consistent with reason (3) is the known susceptibility to membrane perturbation of the late-foetal transferase activity (see Dutton & Leakey, 1981; Leakey, 1983) and loss of UDP-glucuronyltransferase activity on storage of pieces and homogenates of mouse liver at either 0 or 37°C, when assayed in the presence of 0.2% digitonin (Burchell

et al., 1975). When UDP-glucuronyltransferase is assayed in fresh or stored samples of perinatal liver it is therefore advisable to use a range of detergent concentrations, as was used in the present study.

Clearly, now that differential development, or 'functional heterogeneity' (see Wishart, 1978), of UDP-glucuronyltransferase has been demonstrated in a primate, it is important to extend such systematic studies to man. Until it is established whether any, and if so which, UDP-glucuronyltransferase activities do develop in the human foetus, one cannot profitably extrapolate animal studies on the perinatal toxicology of potential group 1 UDP-glucuronyltransferase substrates to man or predictably design less-toxic drugs for the perinatal human.

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