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The Inadequacy of Perinatal Glucuronidation: Immunoblot Analysis of the Developmental Expression of Individual UDP-Glucuronosyltransferase Isoenzymes in Rat and Human Liver Microsomes

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SUMMARY

Two anti-rat UDP-glucuronosyltransferase (UDPGT) antibody preparations, exhibiting different specificity of recognition of UDPGT isoenzymes on immunoblot analysis, were used to investigate the molecular basis of the perinatal inadequacy of glucuronidation in rats and humans. Immunoblot analysis of microsomes from developing rat liver demonstrated that the deficiency in bilirubin and testosterone glucuronidation in the fetus was due to the absence of the UDPGT isoenzyme proteins responsible for these conjugations. In contrast, phenol UDPGT enzyme activity and protein was detectable in significant amounts in fetal rat liver (>30% of adult levels). In human liver, only one major immunoreactive polypeptide was observed in fetal microsomes. The remaining UDPGTs present in adult human liver developed postnatally, in parallel with the appearance of enzyme activities. Therefore, there was a correlation between the development of enzyme activity and enzyme protein. The possible consequences of developmental inadequacy of conjugation reactions for the fetus is discussed.

Conjugation with glucuronic acid is a major pathway in the biotransformation and elimination of a wide variety of lipophilic endogenous compunds and xenobiotics (1). The reactions are catalysed by a family of closely related UDPGT isoenzymes (2). The existence of multiple forms of UDPGT has been demonstrated by purification (2, 3), differential induction (4), ontogenic studies (5–7), and cloning of cDNAs (8–10).

The ontogeny of UDPGT enzyme activities in the rat has been extensively studied and several distinct developmental periods demonstrated, based on the appearance of UDPGT catalytic activity towards various substrates. Activity toward simple planar phenols (e.g., 1-naphthol, 2-aminophenol) surges before birth and attains greater than adult levels 1–2 days post partum. These activities then fall during infancy to a minimum at 13–16 days postnatal age, before increasing again to adult levels (5, 11, 12). Immediately after birth, UDPGT activities towards a second group of substrates (e.g., bilirubin, morphine, testosterone) surge and do not exhibit a temporary postnatal decline; maximum values are obtained at 25–30 days. (11, 13, 14). A third distinct period occurs after weaning (approximately 25 days), when activities towards androsterone (6), pregnanediol (14), and digitoxigenin monodigitoxoside (7) appear and rise quickly to adult levels.

During the perinatal period, the individual is sensitive to the deleterious effects of many drugs, e.g., chloramphenicol (15, 16) and novobiocin (17). It is important, therefore, in therapeutics, to understand the substrate specificity of drug glucuronidation, and the mechanisms controlling the expression of distinct UDPGTs at different ages. In humans, 5-hydroxytryptamine, 1-naphthol (18), and morphine (19) are the only substrates so far found to be glucuronidated to any significant extent by fetal liver microsomes. Activity towards all other substrates measured developed soon after birth (18, 20, 21). The boundaries of substrate specificity of human liver UDPGTs have not yet been described; however, the existence of multiple forms has been demonstrated by cDNA cloning (22), purification (23), enzyme activity measurements (24), and immunochemical analysis (22).

To date, none of the developmental data obtained from enzyme activity measurements have been correlated with re-

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ABBREVIATIONS: UDPGT, uridine 5′-diphosphoglucuronosyltransferase; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulphonic acid; RAL1, sheep anti-rat liver UDPGT testosterone/4-nitrophenol UDPGT; RAK1, goat anti-rat kidney UDPGT bilirubin/phenol UDPGT; NBT, nitro blue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl phosphate, *p*-toluidine salt; SDS, sodium dodecyl sulfate.

sponse at the molecular level and, therefore, activation of the enzymes during development cannot be ruled out as a mechanism for ontogenic variation. Therefore, in the present study we have employed immunoblot analysis, with two polyclonal anti-rat UDPGT antibody preparations exhibiting different isoenzyme recognition patterns, in an attempt to correlate measured UDPGT enzyme activities (using isoenzyme-specific substrates) during development with the expression of individual UDPGT isoenzymes in both rat and human liver microsomes. A preliminary report of this work has appeared (25).

Experimental Procedures

Chemicals. [4-^{14}C]Testosterone (57 mCi/mmol) was purchased from Amersham (Bucks, England) and was determined by the manufacturer to be of >97% radiochemical purity; testosterone, bilirubin, UDP-glucuronic acid, and Lubrol PX were obtained from Sigma Ltd. (Poole, England); 2-aminophenol was from Aldrich Chemical Co. (Milwaukee, WI) and was twice resublimed before use; ethylanthranilate was from Kodak (Rochester, NY); electrophoresis chemicals were obtained from BDH Ltd. (Poole, England). Donkey anti-sheep/goat IgG and peroxidase anti-peroxidase complex (sheep) were generously provided by the Scottish Antibody Production Unit (Carluke, Scotland). Alkaline phosphatase-conjugated rabbit anti-goat IgG, NBT, and BCIP were purchased from Sigma. All other chemicals were of analytical grade, purchased from commonly used suppliers.

Treatment of animals and preparation of microsomal fractions. Adult male and female WAG rats (Harlan-Olac Ltd., Blackthorn, England) were time mated. At the appropriate development age (17 days or 21 days fetal; 2, 5, 10, 16, 20, 26, 30, 40, and 75 days post partum), offspring were killed by decapitation and their livers were removed and immediately frozen in liquid nitrogen. From fetuses, 8 to 10 livers were pooled; from 2, 5, 10, 16, and 20 day post partum animals, five livers were pooled, and individual livers were used for all other ages. Livers were stored at −70° until use (within 2 months). Only male offspring were used, with the exception of fetal animals, for which sexing was not possible. Microsomes were prepared by differential centrifugation at 4°. Livers were thawed and a 20% (w/v) homogenate was prepared in 250 mM sucrose, 5 mM HEPES, pH 7.4 (buffer A). Homogenates were centrifuged at 7000 × *g* for 10 min and the resulting supernatants were centrifuged at 105,000 × *g* for 50 min. Microsomal pellets were resuspended in buffer A to give a protein content of approximately 20 mg/ml. Microsomes were stored at −70° until assay (within 1 month).

Human tissue samples. Samples of human neonatal liver were removed at autopsy, usually performed within 12 hr post mortem. (The majority of samples originated from cases of sudden infant death syndrome.) Ethical approval was obtained from the Reproductive Medicine Ethical Sub-Committee, The Royal Infirmary of Edinburgh. The liver samples were washed in ice-cold buffer A and homogenized in 4 volumes of buffer A. After centrifugation at 7000 × *g* for 10 min at 4°, the supernatants were rapidly frozen and stored at −70°. Microsomes were harvested by centrifugation of thawed 7000 × *g* supernatants at 105,000 × *g* for 50 min and the pellets were suspended in buffer A at a protein content of 5–20 mg/ml. Microsomes were stored at −70° until analysis. For adult human liver, samples obtained at autopsy were frozen in liquid nitrogen and stored at −70° until preparation of microsomal fractions (as for rat liver).

Enzymes assays. All UDPGT assays were performed using established assay procedures in the presence of optimally activating concentrations (determined for each developmental age) of the nonionic detergent Lubrol PX, in order to counteract any effects of variable latency during development (15) and to allow the measurement of the maximum glucuronidation potential of the microsomal sample. Microsomes that had been frozen and thawed once only were used. The following concentrations of aglycone were used: 500 μM 2-aminophenol (26), 500 μM 1-naphthol (27), 600 μM testosterone (28), and 150 μM bilirubin (29).

Anti-UDPGT antibodies. The production and characterization of anti-rat liver testosterone/4-nitrophenol UDPGT (RAL 1) and anti-rat kidney 1-naphthol/bilirubin UDPGT (RAK 1) have been described elsewhere (Refs. 30 and 31, respectively).

Electrophoresis and immunoblot analysis. Microsomal samples were resolved on 7.5% polyacrylamide gels in the presence of 0.1% SDS, essentially as described by Laemmli (32). For immunoblot analysis of resolved microsomal UDPGTs, proteins were transferred after electrophoresis to nitrocellulose by the method of Towbin *et al.* (33), and chromogenic detection of immunoreactive UDPGT polypeptides was performed using the peroxidase-linked method, with 4-chloro-1-naphthol as peroxidase cosubstrate (34). For immunostaining of Western blots of human liver microsomes, the more sensitive alkaline phosphatase-linked detection system was used, with a mixture of NBT and BCIP as chromogenic substrate (35). This method gave a 10–20-fold increase in sensitivity compared with the peroxidase/anti-peroxidase system (not shown). Briefly, nitrocellulose filters were blocked for 30 min in 1% bovine serum albumin dissolved in 10 mM Tris, 154 mM NaCl, 0.05% Tween 20, pH 7.4 (TBSTw). Filters were then exposed for 1 hr to primary antibody (as IgG fraction) diluted to 20 μg/ml in 50 mM Tris, 154 mM NaCl, 0.05% Tween 20, pH 10.2. After five washes (6 min each) in TBSTw, alkaline phosphatase-conjugated rabbit anti-goat IgG (0.5 μg/ml in TBSTw/1% bovine serum albumin) was added to the dishes. After 30-min incubation, filters were again washed five times (6 min each) in TBSTw. For color development, filters were incubated in 30 ml of 100 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5, containing 200 μl of NBT (50 mg/ml in 70% aqueous dimethylformamide) and 100 μl of BCIP (50 mg/ml in 100% dimethylformamide). The reaction was stopped, when the desired staining intensity relative to background was obtained (usually 10–20 min), by washing the filters extensively in distilled water.

Protein determination. Protein in microsomal samples was determined by the method of Lowry *et al.* (36) on microsomal preparations that had been frozen and thawed once only. Bovine serum albumin was used to construct standard curves.

Results

WAG rats are an inbred variant of the Wistar strain, exhibiting the LA (low androsterone) phenotype; that is, they are deficient in androsterone UDPGT activity (37). This has been shown to result from a deletion in the androsterone UDPGT gene (38). These animals were chosen for this study in order to eliminate the possibility of interference, in the immunoblot analysis of UDPGT isoenzymes, from randomly distributed low and high androsterone UDPGT phenotype rats present in the colony of Wistar rats maintained in this Institute.

Development of UDPGT enzyme activities. Microsomes prepared from livers of male WAG rats of various ages were assayed for UDPGT activity with testosterone, 2-aminophenol, and bilirubin as acceptor substrates (Fig. 1). Glucuronidation of testosterone was not detected in fetal liver microsomes to any significant extent but rose steadily after birth to reach maximum values at about 30 days postnatal age (Fig. 1A). A brief temporary decline in enzyme activity was observed at 40 days post partum with this substrate. With bilirubin, UDPGT enzyme activity surged dramatically after birth and reached a peak at 30 days post partum. As with testosterone, a temporary decline in activity at 40 days was observed (Fig. 1B). Conversely, with 2-aminophenol as substrate (Fig. 1C), UDPGT activity was detectable in fetal samples 5 days before birth, and peaked sharply immediately after birth (about 2 days) at greater than adult levels. The activity then declined rapidly to reach

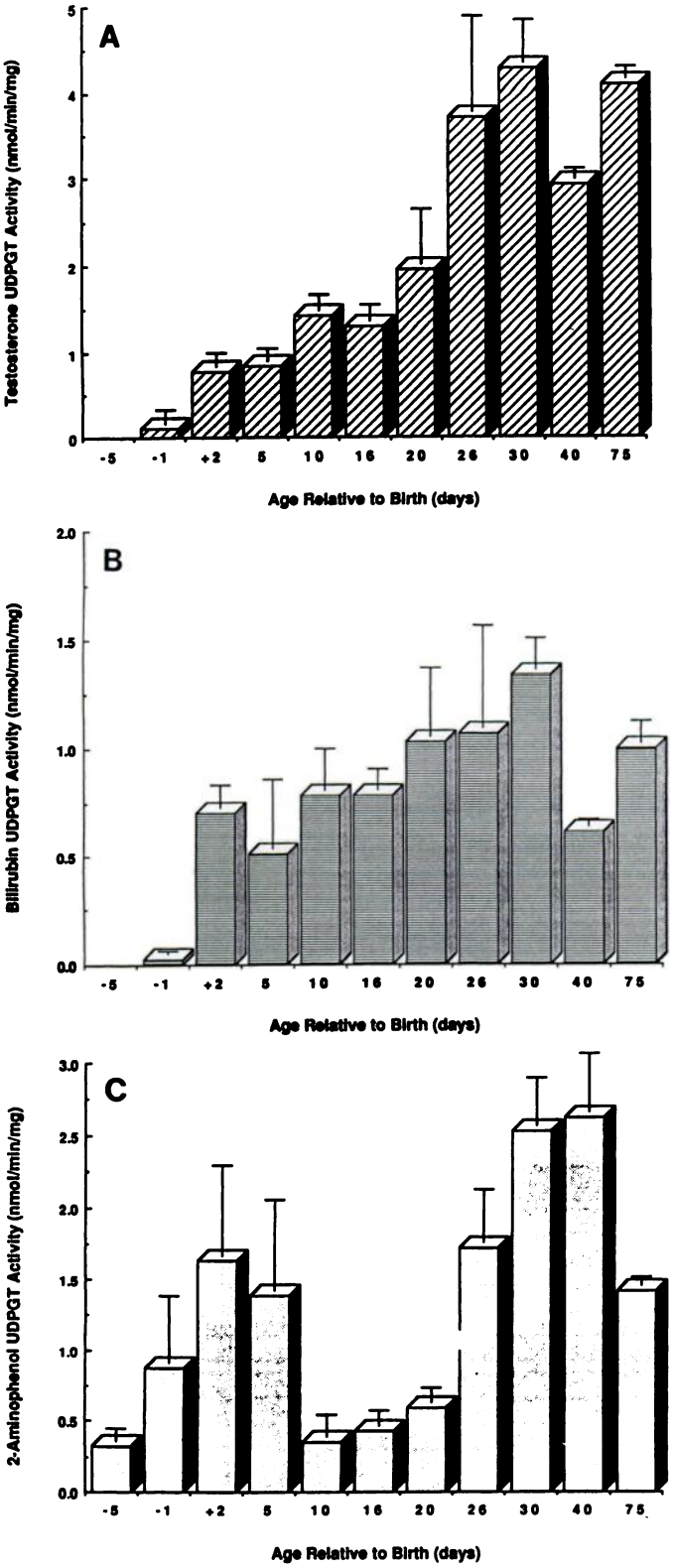

Fig. 1. Development of UDPGT enzyme activities in WAG rat liver microsomes. Liver microsomes prepared from WAG rats of different developmental ages were assayed for UDPGT activity towards testosterone (A), bilirubin (B), and 2-aminophenol (C). Enzyme assays were performed in the presence of optimally activating concentrations of Lubrol PX. Results are expressed as mean ± standard deviation for determinations on three separate pools of liver for each age.

approximately 15% of maximum at 10 days post partum. A second surge of 2-aminophenol UDPGT activity resulted in maximum activity at 40 days postnatal age, with activity once again falling off into adulthood. On examination of the ontogeny of UDPGT with these isoenzyme-specific substrates, the enzyme activity data confirm the existence of at least two developmentally regulated groups of UDPGT isoenzymes; one appears before birth, catalyzing the glucuronidation of 2-aminophenol (and other simple planar phenols; see Refs. 11 and 12), and the other group surges immediately after birth, consisting of more than one isoenzyme, including those responsible for the glucurondiation of bilirubin and testosterone (13, 14). [Analysis of substrates representing the third developmental group (i.e., androsterone, digitoxigenin monodigitoxoside) appearing after weaning was not carried out in this study.] The possibility exists of other, as yet unrecognized, isoenzymes belonging to one or other of these groups.

UDPGT enzyme activity towards three substrates (bilirubin, testosterone, and 1-naphthol) was also measured in microsomal preparations from human liver samples obtained at various developmental ages (Table 1). Activity towards all substrates was very low in a sample from a fetus of 30 weeks gestation that failed to survive for more than 2 hr, as was activity in liver microsomes from full term infants (37–41 weeks of gestation). However, after 8–15 weeks post partum, activity towards all three substrates tested increased up to 50% of adult values, in both infants born prematurely and those born at term. Bilirubin glucuronidation was detected at adult levels by 1 year postnatal age; however testosterone UDPGT activity appears to take longer to develop than either bilirubin or 1-naphthol activity, indicating differential regulation of UDPGT gene expression during development in humans.

Immunoblot analysis of UDPGT during development. We have previously reported the isolation of two anti-rat UDPGT antibody preparations, one raised against purified rat liver testosterone/4 nitrophenol UDPGTs [RAL 1] (30) and one raised against purified rat kidney bilirubin/1-naphthol UDPGTs [RAK 1] (31). These two preparations exhibit different specificity of recognition of UDPGT isoenzymes when used to probe Western blots of microsomes from rat liver and other sources; the antibody RAK1 recognizes only the bilirubin and phenol isoenzymes in rat liver, whereas the antibody RAL 1 is

TABLE 1

UDPGT activities in human (postmortem) liver microsomes

Data are presented as either individual results or means ± standard deviations. Figures in parentheses are the number of samples assayed. All assays were performed in the presence of optimal amounts of the nonionic detergent Lubrol PX, determined for each microsomal preparation.

Specimen age	UDPGT Activity		
	Bilirubin	Testosterone	1-Naphthol
	nmol/min/mg of protein		
Premature			
30 weeks	0.05	0.0	0.56
30 weeks (+ 10 week survival)	0.4, 1.0	0.14, 0.85	3.0, 1.8
Full term			
Survival 1–10 days (N = 7)	0.07 ± 0.04	0.10 ± 0.06	0.75 ± 0.68
Survival 8–15 weeks (N = 6)	0.64 ± 0.32	0.12 ± 0.05	2.4 ± 1.1
Survival 22–55 weeks (N = 5)	0.99 ± 1.1	0.09 ± 0.06	3.6 ± 2.1
Adult (male) (N = 3)	0.76 ± 0.43	0.46 ± 0.61	7.2 ± 2.2

less specific, recognizing at least five immunoreactive polypeptides in rat (31) and human liver (20). A schematic representation of the pattern of recognition of UDPGT isoenzymes by RAL1 and and RAK1 on Western blot analysis is shown in Fig. 2.

When these two antibodies were used to probe Western blots of WAG rat liver microsomes from each of the developmental ages under investigation (Fig. 3), the different specificity of these preparations enabled analysis of variations in the relative amounts of different UDPGT isoenzymes during development. The appearance of immunoreactive polypeptides at 53,000 Da (phenol UDPGT), 54,000 Da (bilirubin UDPGT), and 50,000 Da (testosterone [17β-hydroxysteroid] UDPGT) correlated with the enzyme activity data displayed in Fig. 1. The presence of phenol UDPGT in fetal liver was clearly visible (Fig. 3 a and b; *lanes −5* and *−1*), whereas neither the testosterone nor the bilirubin form were detectable by this immunological analysis in fetal WAG rat liver microsomes. The use of the more specific antibody RAK1 clearly demonstrated the differential development of phenol and bilbirubin UDPGTs. The postnatal dip in phenol UDPGT activity between 10 and 20 days and the peak at 40 days were visible at the level of immunoreactive protein, as was the peak of bilirubin UDPGT enzyme protein at 20–30 days post partum (Fig. 3b). The broad spectrum anti-UDPGT antibody RAL1 demonstrated the development of testosterone (17β-hydroxysteroid) UDPGT, with the characteristic absence from the fetus and the peak of immunoreactive enzyme protein at 20–30 days post partum (Fig. 3a). The RAL1 antibody also recognized several other immunoreactive polypeptides on Western blot analysis of developing WAG liver microsomes; however, catalytic activities have as yet not been assigned to these proteins. A second minor protein (M_r = 51,000) was present in fetal WAG rat liver microsomes; a polyeptide of 52,000 Da developed after 30 days, and two polypeptides of about 56,000 and 57,000 Da, which show peak intensity of staining at 20 days post partum, were detected by this antibody preparation.

When microsomes prepared from perinatal human liver microsomes were analyzed by immunoblot analysis, a similar result was obtained (Fig. 4). As early as 18 weeks gestation, an immunoreactive polypeptide of approximately 53,000 Da was visible with antibody RAL1. A number of other low molecular weight bands are detectable in this sample. We have performed control incubations using nonimmune IgG as primary antibody and determined that these are the result of nonspecific binding of IgG to these proteins, which are then recognized by the alkaline phosphatase conjugated secondary atibody. It is not clear why these proteins should show up so strongly in the lane containing the 18-week gestation liver sample, but we have observed this phenomenon with other liver samples of similar gestational age (not shown). This 53,000-Da protein was also the only major UDPGT present in liver microsomes prepared from a fetus of 27 weeks gestation. However, at term at least three immunoreactive bands were visible and, within 3 months post partum, the majority of the UDPGT isoenzymes present in adult liver microsomes had developed, although not to adult

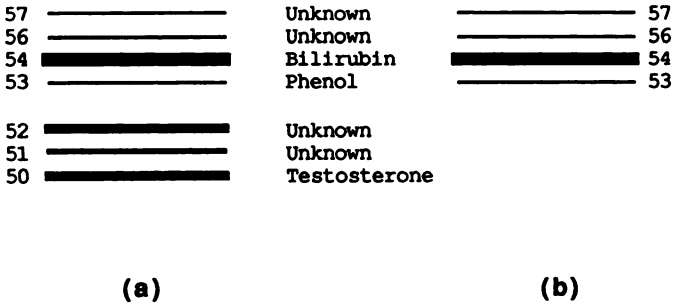

Fig. 2. Schematic representation of the recognition of UDPGT isoenzymes by antibodies RAL1 and RAK1 on immunoblot analysis of adult WAG rat liver microsomes. Assignment of UDPGT enzyme activities to immunoreactive polypeptides was based on analysis of microsomes from developing, genetically deficient and xenobiotic-treated rats in comparison with purified UDPGTs (21, 30). Representations for RAL1 (a) and RAK1 (b) are shown. Molecular weights were estimated by parallel electrophoresis and Coomassie blue staining of the marker proteins albumin (68,000), pyruvate kinase (57,000), fumarase (49,000), and aldolase (40,000).

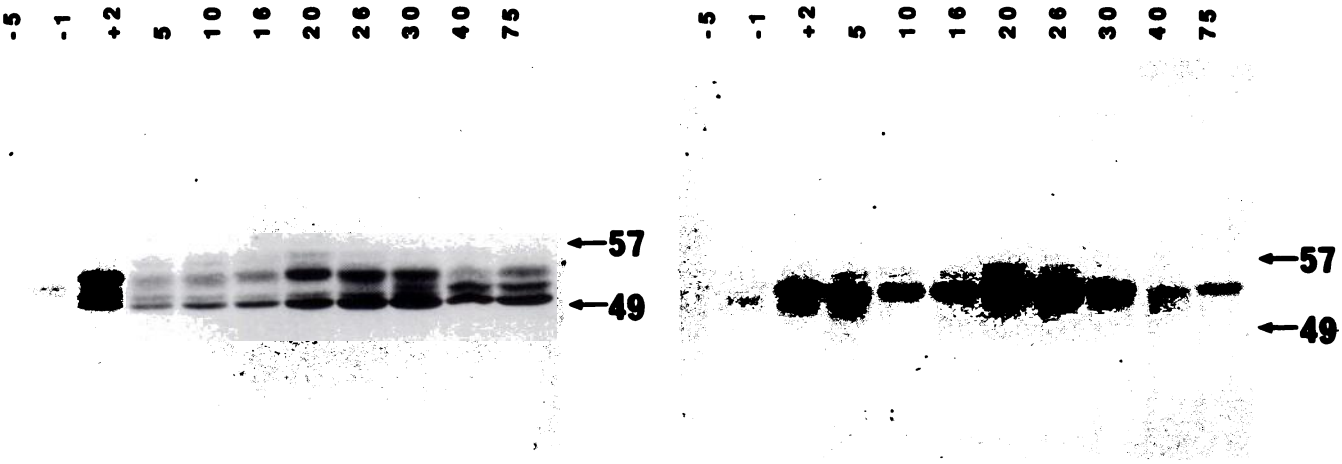

Fig. 3. Immunoblot analysis of UDPGT in developing WAG rat liver microsomes. Microsomes were subjected to electrophoresis on 7.5% polyacrylamide gels in the presence of 0.1% SDS and transferred to nitrocellulose sheets. Immunostaining with anti-UDPGT antibodies RAL 1 (a) and RAK 1 (b) was performed using the immunoperoxidase method, with 4-chloro-1-naphthol as chromogenic substrate. Liver microsomes (25 μg) from the ages indicated in the figure were appled to each gel, and analyses were performed at the same time under the same conditions. The relative mobilities of the molecular weight marker proteins pyruvate kinase (57 kDa) and fumarase (49 kDa) are indicated in the margins.

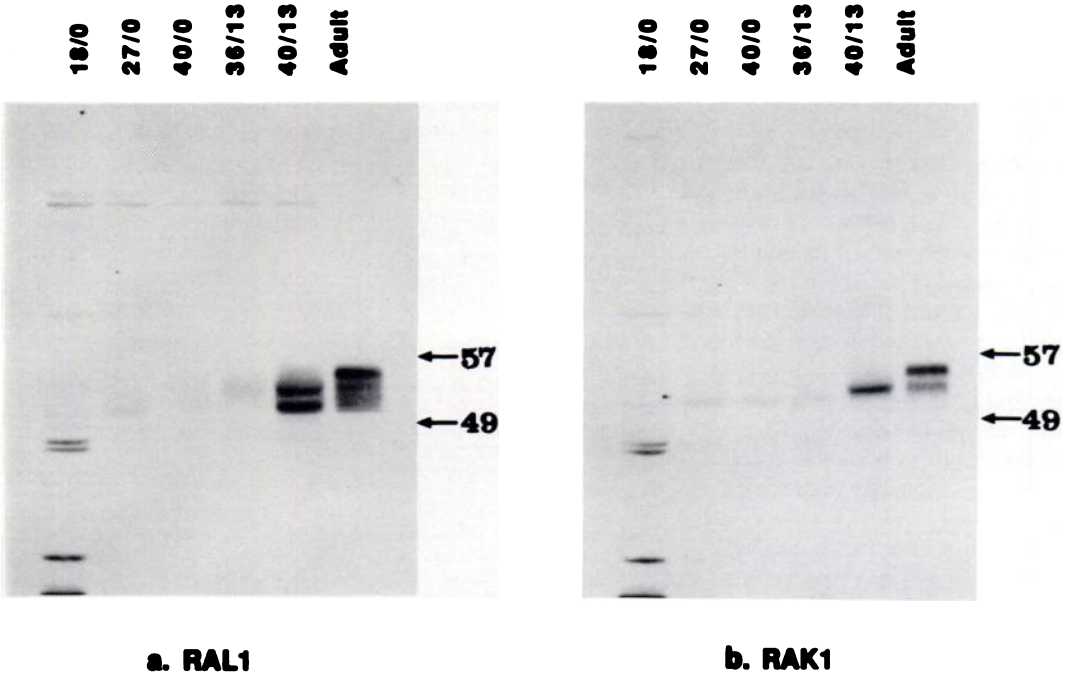

Fig. 4. Immunoblot analysis of developing human liver microsomes. Liver microsomes, prepared from human liver samples as indicated, were resolved on 7.5% polyacrylamide gels in the presence of SDS (0.1%). After transfer to nitrocellulose, proteins immunoreactive with RAL1 (a) and RAK1 (b) were detected by alkaline phosphatase immunostaining, with NBT/BCIP as substrate. Age is described as, for example, 18/0, 18 weeks gestation/0 weeks post partum survival. The following amounts of microsomal protein were loaded: 20 μg (18/0; 27/0; 40/0 36/13; 40/13) and 5 μg (adult). The relative mobilities of the standard proteins pyruvate kinase (57 kDa) and fumarase (49 kDa), as determined on identical Coomasie blue-stained gels run at the same time, are shown.

levels. As with the rat, the antibody preparation RAK1 demonstrated increased specificity of recognition of UDPGT isoenzymes on Western blot analysis, showing selectivity for only two or three polypeptides.

Discussion

It has been known for a number of years that UDPGT enzyme activities are subject to differential development, such that several "clusters" of enzyme activity can be identified (5–7, 15–17). The present report provides the first correlation of this differential development of enzyme activity with changes in the levels of individual UDPGT isoenzyme proteins. By using anti-UDPGT antibody preparations to probe Western blots of liver microsomes prepared from rats of different developmental ages, the surges and troughs in the enzyme activities towards three isoenzyme-specific substrates (2-aminophenol, testosterone, and bilirubin)[3] were shown to reflect changes in levels of the immunoreactive polypeptides corresponding to these enzyme activities. The developmental profiles of 2-aminophenol UDPGT demonstrated this particularly well. The 53,000-Da polypeptide appeared late-fetally, peaked immediately after birth, and declined in infancy before rising again after weaning (Fig. 3b), following the profile of enzyme activity (Fig. 1C). Similarly, for testosterone (17β-hydroxysteroid) and bilirubin UDPGTs, little or no enzyme activity or protein was detectable in the late fetal stage but, after birth, enzyme activity developed in parallel with immunoreactive protein. It is not clear whether these changes in levels of protein result from regulation at the level of transcription or translation. These possibilities are currently being investigated.

The substrates chosen for this analysis demonstrate a high degree of isoenzyme specificity for UDPGT. However, the involvement of other UDPGT isoenzymes in the glucuronidation of these compounds in the developmental period under study cannot be entirely ruled out, but we consider it to be negligible for the reasons stated.[3]

At least four unidentified immunostaining proteins in rat liver microsomes are recognized by the broad spectrum antibody preparation RAL1, of molecular weight 51,000, 52,000, 56,000, and 57,000, respectively. To date, no UDPGT enzyme activity has been assigned to these proteins. The band of subunit M_r = 51,000 present in 21 day fetal liver microsomes, is the only RAL1-immunoreactive UDPGT isoenzyme present in Gunn rat kidney microsomes, in which the phenol and bilirubin isoenzymes are deficient (39). Therefore, it appears that there are two UDPGT isoenzymes belonging to the "late fetal" cluster. For the 52,000-Da polypeptide, immunoreactive protein appeared at 30 days post partum (Fig. 3b), and the 56,000- and 57,000-Da proteins developed postnatally, peaking at 20 days post partum. Recently, morphine UDPGT has been purified from rat liver, and its subunit molecular weight determined to be 56,000 (40). However, it is unlikely that either of these two proteins (56,000 and 57,000 Da) recognized by our antibodies represents morphine UDPGT, inasmuch as we have previously determined that neither was induced by treatment of rats with phenobarbitone (31) [morphine UDPGT activity is induced at least 4-fold by phenobarbitone (4)] and both unidentified proteins were absent from Gunn rat liver microsomes (39) [morphine UDPGT activity is normal in the Gunn rat (41)]. We are currently investigating numerous compounds as potential substrates for these proteins.

The ontogeny of human liver microsomal UDPGT enzyme activity using eight substrates has been studied previously (18, 19), and only enzyme activity towards 5-hydroxytryptamine, 1-naphthol, and morphine was observed to any significant extent

[3] We have determined 2-aminophenol to be a more specific substrate for rat phenol UDPGT than, for example, 1-naphthol, inasmuch as the contribution of other UDPGT isoenzyme(s) to the conjugation of this substrate, as determined by measurements in liver microsomes prepared from the defective Gunn rat, is approximately 5% compared with approximately 20% for 1-naphthol (31). Testosterone is primarily conjugated by 17β-hydroxysteroid UDPGT as determined with the purified enzymes described by Falany *et al.* (3). There is no evidence in the literature that bilirubin is conjugated by any UDPGT isoenzyme other than bilirubin UDPGT, e.g., bilirubin UDPGT activity is completely absent in the Gunn rat (see Ref. 31 for references).

in fetal liver microsomes. When the anti-rat UDPGT antibodies RAL1 and RAK1 were used to probe immunoblots of perinatal human liver microsomes, the peroxidase/anti-peroxidase method was not sufficiently sensitive to allow a satisfactory interpretation of the data. However, when an alkaline phosphatase-linked system was employed, there was a significant improvement in the sensitivity of detection of the UDPGT polypeptides (up to 20-fold). By direct comparison with the peroxidase/anti-peroxidase method, we determined the limit of detection to be < 500 pg of adult human liver microsomes (not shown). This method could therefore find application in the immunoblot analysis of UDPGTs in very small samples of human liver, e.g., from needle biopsy samples. When Western blots of human liver microsomes prepared from human fetuses of 18 and 27 weeks of gestation were probed with RAL1 and RAK1, only one major immunoreactive polypeptide was visible, strongly suggesting that this protein corresponds to 5-hydroxytryptamine and/or morphine UDPGT. The majority of UDPGT isoenzymes did not appear until after birth, and by 3 months of age a full complement of isoenzymes was present, although at reduced levels (approximately 25%) compared with adult. Although this is similar to the rat, in which only two UDPGTs appear to be present in fetal liver, the substrate specificity of the major UDPGT isoenzyme present in the fetus appears to be different in the two species. In the rat, the main form present in the fetus (phenol UDPGT) conjugates planar phenols, including many lipophilic compounds present as environmental pollutants, e.g., benzo(*a*)pyrene and dibenz(*a,h*)anthracene metabolites (42, 43). This is likely to afford some protection to the developing rat from exposure to such potentially toxic and/or carcinogenic compounds. However, in human fetal liver, planar phenols such as 1-naphthol and 4-nitrophenol (substrates for rat phenol UDPGT) were not extensively glucuronidated, compared with the adult. This may have profound implications for the human fetus when exposed to toxic xenobiotics. However, the exact substrate specificities of human UDPGTs and of Phase I enzymes (cytochromes P-450, epoxide hydrolase) present in the human fetus, which might possibly be involved in the bioactivation of procarcinogens, remain to be determined. Purification of individual isoenzymes and the cloning and expression of human UDPGT cDNAs will allow the identification of human UDPGTs involved in the detoxification of foreign compounds, and we may then be in a position to identify particular developmental periods during which the fetus or newborn is at risk from the toxic effects of specific drugs or xenobiotics.

The studies reported here represent work performed on human tissue obtained at autopsy, sometimes up to 12 hr post mortem. There are obviously possible variations in the "quality" of human tissue obtained in such a manner, as well as the expected variability arising out of genetic and/or environmental factors. Previous work from this laboratory has demonstrated that such post mortem tissue should still be viable for measurement of UDPGT enzyme activity (18), and we have also determined that storage of microsomes frozen at −70° for periods of 3–4 months does not apparently change the pattern of polypeptides observed on immunoblot analysis (not shown). As with all work performed on human tissue, these possible sources of variation must be considered in the interpretation of experimental data. The results presented here demonstrate a correlation between UDPGT enzyme activity and UDPGT isoenzyme protein over the perinatal period, as determined by immunoblot analysis, and show that the inadequacy of certain glucuronidation reactions in the neonate is the result of the presence of low levels of various UDPGT isoenzymes in liver microsomes prepared from both rats and humans.

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