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Immunocharacterization of UDP-glucuronyltransferase isoenzymes in human liver, intestine and kidney

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UDP-glucuronyltransferases are enzymes located in the endoplasmic reticulum of cells from organs like lung, stomach, liver, kidney and intestine. The function of these phase II enzymes is the detoxification of various endogenous and exogenous compounds, by transfer of glucuronic acid from UDP-glucuronic acid to an acceptor molecule [1]. These activities are mediated by a family of isoenzymes probably composed of 1 to 4 subunits [2–5].

UDP-glucuronyltransferase multiplicity has been studied by analyzing enzymatic profiles during development [6], after induction [7] or by radiation inactivation analysis [3–5]. Purification of different isoforms also has yielded important information on the UDP-glucuronyltransferase enzyme system [2, 8–14].

Now, immunological methods have become a valuable tool for the study of UDP-glucuronyltransferase heterogeneity [15–19]. These methods are particularly helpful in characterizing and quantifying individual isoenzymes. Monoclonal antibodies, being specific for a single epitope, constitute a precise and powerful tool which may give important information on the structure, homology or heterogeneity of several UDP-glucuronyltransferases. A monoclonal antibody was developed which recognizes a common epitope of UDP-glucuronyltransferases, mediating the glucuronidation of bilirubin, 4-nitrophenol and 4-methylumbelliferone in human liver [20]. In the present study, this antibody was used to compare the isoenzyme patterns in different organs and species.

Materials and methods

Tissue. Human tissue was obtained by surgical biopsy or autopsy (liver and kidney) and biopsy or surgical resections (intestine). Most liver tissue was from kidney transplant donors with no or unknown drug treatment. Tissue was washed in ice-cold 0.9% NaCl solution as soon as possible and stored at −80° or used immediately. Tissue homogenate or microsomes were prepared as described before [21].

Rat liver microsomes from 3-methylcholanthrene or phenobarbital treated animals were obtained as described [5].

Enzyme assays. UDP-glucuronyltransferase assays for bilirubin, 4-nitrophenol and 4-methylumbelliferone were determined under optimally activated conditions, by published methods [3, 5, 21].

Immunoblotting. Proteins were separated by SDS–polyacrylamide gel electrophoresis (7% w/v) and then transferred to nitrocellulose (0.45 μm, Schleicher and Schüll, F.R.G.). The nitrocellulose sheet was blocked with 1% gelatin in phosphate buffered saline (PBS) for 1 hr at room temperature, washed 5 times with PBS/Tween 20 (0.05% v/v) and incubated for 1 hr at room temperature with monoclonal antibody against UDP-glucuronyltransferase, diluted in PBS containing 1% gelatin. After washing five times with PBS/Tween the sheet was incubated with 1:700 dilution of peroxidase conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Denmark) for 1 hr. After washing five times with PBS/Tween the sheet was immersed in the substrate solution: 60 mg 4-chloro-1-naphthol in 20 ml cold methanol, added to 100 ml PBS, containing 60 μl of 30% hydrogen peroxide. The colour developing reaction was terminated by washing the sheet in water, containing 20% methanol.

Results and discussion

Electrophoretic separation of microsomal proteins, followed by electroblotting and immunodetection via a monoclonal antibody (MAb) against UDP-glucuronyltransferase, and a peroxidase labeled second antibody, gives the results presented in Fig. 1. UDP-glucuronyltransferase subunits can be detected in human liver, kidney, small and large intestine. In human liver two or three bands are visualized, depending on the preparation used. The liver specimens in lane 4, 6 and 8 contain three protein bands with molecular masses of 57,000, 54,000 and 53,000 Da. The other liver samples apparently give only two UDP-glucuronyltransferase protein bands of 57,000 and 54,000 Da. Thus, interindividual variations in UDP-glucuronyltransferase composition are present. Lanes 3 and 5 show liver samples from patients with Gilbert's syndrome, which is characterized by a significant reduction of hepatic bilirubin UDP-glucuronyltransferase activity [21]. These preparations, like the samples from normal liver in the lanes 1, 2, 7 and 11 apparently completely lack or may contain only reduced amounts of 53,000 Da protein.

In rat liver microsomes from untreated, 3-methylcholanthrene- or phenobarbital-treated animals, no UDP-glucuronyltransferase subunits could be detected. When using pig liver microsomes the result was also negative. These results are in accordance with immunofluorescence studies (see Ref. 20) on pig or rat liver slices of treated and untreated animals (see above) where no fluorescense was detectable. Also no inhibition of UDP-glucuronyltransferase activity was observed with this MAb in rat liver microsomes [20]. Clearly this MAb gives no cross-reactivity with non-human tissue.

In lanes 10 and 12 of Fig. 1 human small and large intestine UDP-glucuronyltransferase subunits are detected. Only one band with an apparent molecular mass of 54,000 Da is visualized. Human kidney (lane 9) shows one broad band of molecular mass 57,000 Da.

Table 1 shows the results of UDP-glucuronyltransferase activity measurements in human tissue using bilirubin, 4-nitrophenol and 4-methylumbelliferone as substrates. Activity towards bilirubin was detectable in all liver specimens, in 50% of intestinal tissue but not in kidney.

Since little information on bilirubin glucuronidation in human intestinal epithelium is available [22, 23], the chromatographical separation patterns of bilirubin conjugates, formed by UDP-glucuronyltransferase from human intestine is shown (Fig. 2). The activities we find (41 and 62 nmoles/hr.g tissue), however, are considerably lower as compared to the literature values. Anand *et al.* [22] reported values of 200–300 nmoles/hr.g tissue in duodenum and Hartmann and Bissell [23] found that the activities in intestine were about equal to that in human liver.

UDP-glucuronyltransferase activity towards 4-nitrophenol is higher in liver as compared to kidney or intestine (see Table 1). Similar results were found in the rat [15].

The finding that 4-nitrophenol conjugating activity in liver of patients with Gilbert's syndrome is normal is in accordance with the results of Auclair *et al.* who reported that this activity is diminished in a patient with Crigler-Najjar syndrome but not in patients with Gilbert's syndrome [24].

In liver, the UDP glucuronyltransferase activities with 4-

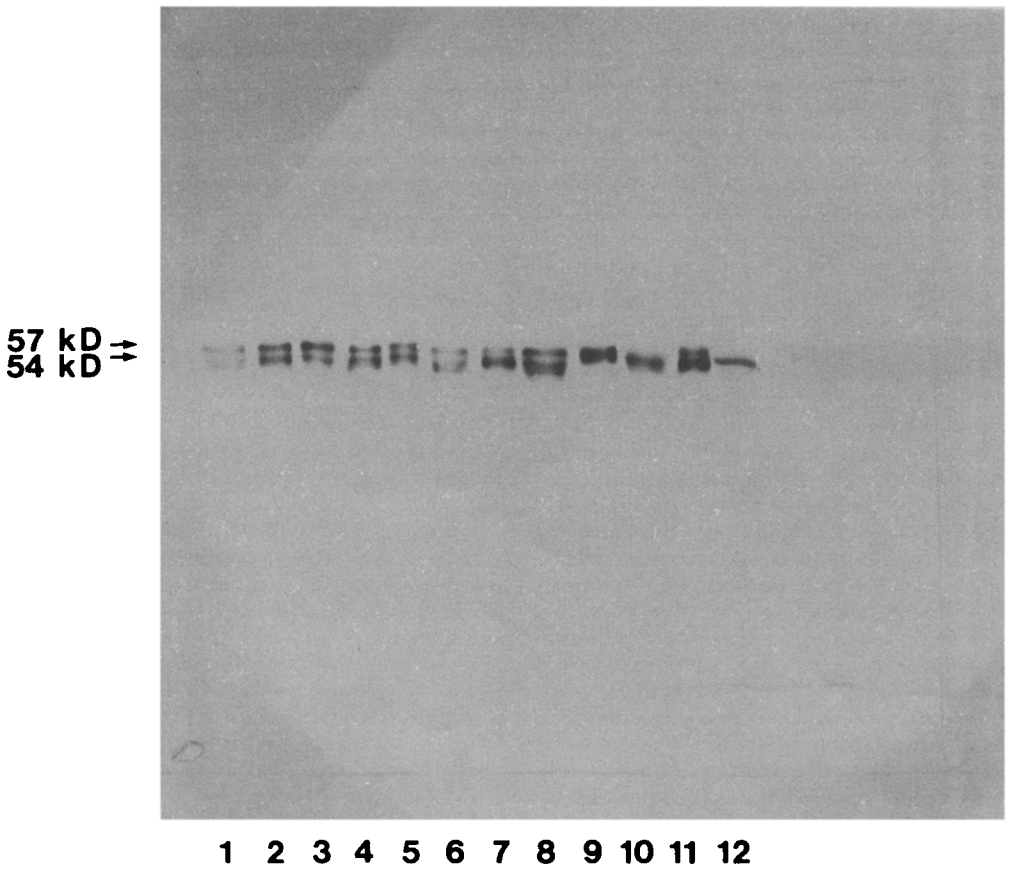

Fig. 1. Detection of UDP-glucuronyltransferase subunits by immuno staining. Polyacrylamide gel electrophoresis of human microsomal preparations, followed by electroblotting and immunodetection, was performed as described under Materials and Methods. Lanes contain human microsomal protein of: (1) liver 1 (2 μg); (2) liver 2 (2.6 μg); (3) liver 3 (3.5 μg); (4) liver 4 (2.9 μg); (5) liver 5 (2.9 μg); (6) liver 6 (2.3 μg); (7) liver 7 (2.5 μg); (8) liver 8 (3.1 μg); (9) kidney (4.4 μg); (10) duodenum (14 μg); (11) liver 9 (3.3 μg); and (12) colon (64 μg). Samples from liver 3 and 5 (lanes 3 and 5) are from patients with Gilbert's syndrome, other liver samples are from patients with no known liver dysfunction. Marker proteins used were bovine albumin (68,000), egg albumin (45,000) carbonic anhydrase (29,000) and trypsinogen (24,000).

methylumbelliferone and 4-nitrophenol as substrates are about equal. Similar results were reported before by Bock *et al.* [25].

The data of the 4-methylumbelliferone activity measurements may furthermore indicate that this activity is higher in intestinal mucosa, as compared to liver and kidney. However, definite conclusions are not possible because of the restricted number of specimens investigated.

In the kidney, bands representing 54,000 or 53,000 Da proteins are lacking (Fig. 1, lane 9), and so is bilirubin UDP-glucuronyltransferase activity (Table 1). This could mean that this activity is catalyzed by the 54,000 or 53,000 Da protein(s). Since all tissues tested contain 4-nitrophenol and 4-methylumbelliferone activity (see Table 1), most probably these activities are catalyzed by both high (57,000) and low (53,000 and/or 54,000) molecular mass subunits.

This hypothesis fits very well with data obtained recently with purified rat liver UDP-glucuronyltransferase isoforms. Activity for bilirubin was catalyzed by 53,000 subunit(s) while activity for 4-nitrophenol was catalyzed by 56,000 and 52,000 Da subunits [2]. Also by immunochemical analysis of UDP-glucuronyltransferases from humans and rats, 54,000 and 56,000 Da proteins were detectable, which most probably are correlated with 1-naphthol conjugating activity [17], while a low molecular mass subunit (53,000 Da) most probably is involved in bilirubin UDP-glucuronyltransferase activity [19].

A monoclonal antibody against human liver UDP-glucuronyltransferase was used for immunodetection of this enzyme in human and animal tissue. UDP-glucuronyltransferase subunits could be detected in human liver, kidney, small and large intestine, but not in liver of pig and rat. In liver, subunits with molecular masses of 57,000, 54,000 and 53,000 Da were detectable. In kidney, only the high molecular subunit(s), and in intestine only the low molecular mass subunit(s) were present. By correlation with enzyme activity assays, we postulate that the low molecular mass subunit(s) may be responsible for bilirubin UDP-glucuronyltransferase activity, whereas both high and low molecular mass subunits catalyze the 4-nitrophenol and 4-methylumbelliferone conjugation.

Table 1. UDP-glucuronyltransferase activities towards various substrates in human liver, kidney and intestinal tissue

Tissue	UDP-glucuronyltransferase activity towards:		
	bilirubin(a)	4-nitrophenol(b)	4-methylumbelliferone(b)
Liver 1	1503	18.4	9.2
2	3690	21.8	10.6
3	170	25.6	11.8
4	2406	21.9	14.4
5	300	41.5	29.0
6	2073	24.9	11.8
7	1545	15.5	12.6
8	2679	35.9	14.2
Kidney 1	n.d.	9.5	15.8
2	n.d.	6.9	15.4
Ileum 1	62	4.2	20.4
2	n.d.	6.4	25.6
Colon 1	n.d.	14.4	38.4
2	41	4.0	17.2

UDP-glucuronyltransferase activities towards various substrates was determined as described under Materials and Methods. Activity is expressed as: (a) nmol/hr.g tissue; (b) nmol/min.mg protein.

Kidney sample 1 and colon sample 1 corresponds to the material in Fig. 1, lanes 9 and 12 respectively.

n.d.: not detectable; detection limit for bilirubin UDP-glucuronyltransferase assay is about 20 nmoles/hr.g tissue.

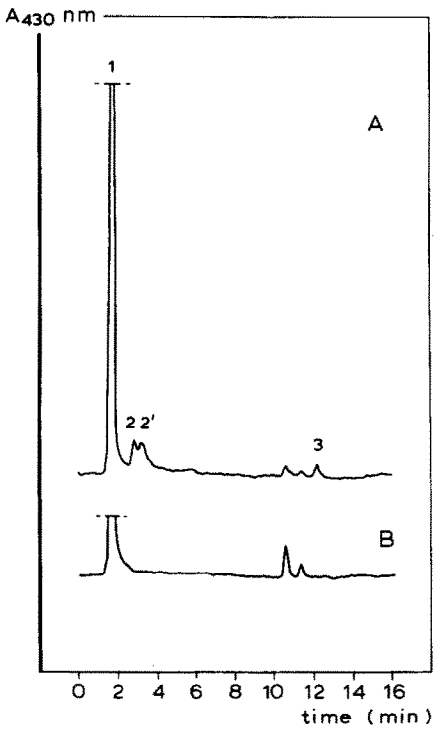

Fig. 2. Chromatogram of bilirubin and its glucuronidation products formed by human intestinal microsomes. Panel A: human colon microsomes (1 mg protein) were incubated with bilirubin and UDP-glucuronic acid and products were quantified by HPLC as described under Materials and Methods. Panel B: same conditions as panel A, but microsomes were inactivated by an overdose detergent. Peak 1, unconjugated bilirubin; peak 2 and 2', bilirubin monoconjugates (C8 and C12 isomers, respectively); peak 3, bilirubin diconjugates. Other peaks were unidentified.

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