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DIFFERENTIAL INDUCTION OF HUMAN LIVER UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES BY PHENOBARBITAL-TYPE INDUCERS

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Abstract—(1) UDP-glucuronosyltranferase (UDP-GT) activities and their inducibility were investigated in human liver microsomes from a "liver bank". (2) UDP-GT activities were differentially induced in liver microsomes from patients treated with the phenobarbital-type inducers phenytoin or pentobarbital. UDP-GT activity towards bilirubin was induced 3-fold. Enzyme activities towards paracetamol, benzo(*a*)pyrene-3,6-quinol, 4-methylumbelliferone and 1-naphthol were moderately induced and to similar extents (2-fold). In contrast, morphine and 4-hydroxybiphenyl glucuronidation were not significantly affected. Cytochrome P-450 dependent 7-ethoxycoumarin *O*-deethylase was increased 5-fold. (3) A human hepatoma cell line (Hep G2) was studied to obtain information on the inducibility of human UDP-GT activities by 3-methylcholanthrene-type inducers. UDP-GT activities towards benzo(*a*)pyrene-3,6-quinol and 1-naphthol were moderately but significantly induced by 3-methylcholanthrene-treatment of the cells (2-fold), whereas 7-ethoxyresorufin *O*-deethylase and 7-ethoxycoumarin *O*-deethylase were increased over 100- and 10-fold, respectively. (4) The results suggest the existence of differentially inducible UDP-GT isoenzymes in human liver. The findings may be useful as a guide to characterize human liver UDP-GT isoenzymes.

Conjugation with glucuronic acid represents a major pathway in the inactivation, detoxication and elimination of a variety of drugs, environmental pollutants and endogenous compounds such as steroid hormones and bilirubin [1]. The reaction has been shown to be catalyzed in experimental animals by a family of isoenzymes with different but overlapping substrate specificity [2–4]. However, in human liver information on UDP-GT* isoenzymes and their inducibility is scarce although recently two isoenzymes have been purified [5].

One of the major functions of UDP-GTs, shared by sulfotransferases, may be detoxication of phenolic and polyphenolic metabolites. In rat liver BP-3,6-quinol glucuronidation appears to be a particular probe for a MC-inducible UDP-GT [6, 7]. Conjugation of the quinol is of toxicological interest since this reaction prevents toxic quinone/semiquinone/quinol redox-cycles [6, 8]. Thereby the toxicity of aromatic hydrocarbons may be reduced. In human liver, paracetamol glucuronidation may turn out to become a valuable probe to compare regulation of phenol glucuronidation *in vivo* and *in vitro*. In contrast to most model substrates for *in vitro* studies paracetamol belongs to the "intermediate clearance drugs" [9] for which glucuronide formation *in vivo* is not only determined by liver blood flow but also by the liver UDP-GT activity. Paracetamol is a widely used analgesic which is safe at low doses. Its pharmacokinetics has been extensively studied (for references; see ref. 10). Recently a simple non-invasive "paracetamol test" has been developed to monitor paracetamol glucuronidation in patients [11]. Using this test it has been shown that paracetamol glucuronidation is increased both in patients treated with phenytoin or rifampicin (confirming the results of detailed pharmacokinetic studies [12, 13]) and in heavy smokers [11]. These findings suggest that paracetamol glucuronidation is induced by both phenobarbital- and MC-type inducers. Moreover, paracetamol glucuronidation appears to be under hormonal control since it is increased in women taking oral contraceptives [14–16].

Availability of a number of well-characterized human liver microsomal samples from a liver bank, including samples from patients treated with phenobarbital-type inducers, prompted an extension of previous studies on the inducibility of UDP-GT activities [17, 18]. Paracetamol and BP-3,6-quinol have now been included as substrates of UDP-GT. Moreover induction by MC-type inducers was studied in a human hepatoma cell line, Hep G2 [19]. It is shown that groups of UDP-GT activities are differentially inducible in human liver and that considerable species differences exist in comparison with rats.

MATERIALS AND METHODS

Liver donors. Human liver was obtained shortly after death from kidney transplant donors as previously described [17, 18]. Characteristics of patients are summarized in Table 1. Patients 8, 13, 20 and 26 were repeatedly treated with high doses of phenytoin or pentobarbital. Sample number 29 was obtained from a smoker. Liver microsomes were prepared as previously described and stored at −80°.

* Abbreviations used: UDP-GT, UDP-glucuronosyltransferase or UDP-glucuronyltransferase (EC 2.4.1.17); MC, 3-methylcholanthrene; BP, benzo(*a*)pyrene.

Table 1. Characteristics of patients and livers

	Sample	Sex	Age	Cause of death	Drug intake in the last week	Other relevant information
(a)	11	F	62	Samples taken at partial liver resection		Extirpatation of gall bladder 2 months earlier. Liver morphology: fatty change (+) and cholestasis (+)
	12	M	25	Suicide, multiple trauma	Repeated doses of furosemide and diazepam; single doses of droperidol, hydromorphone, penicillin G, and dexamethasone	7 days at hospital; mental depression. Liver morphology: fatty change (++), inflammation (+), cholestasis (+), and injured liver cells on electron microscopy
	15	F	40	Cerebral aneurysm, subarachnoidal hemorrhage	Few doses of betamethasone	Earlier essentially healthy
	17	M	24	Head injury, subdural hematoma	Few doses of betamethasone	Earlier essentially healthy
	18	F	59	Cerebral aneurysm	Few doses of betamethasone	Earlier essentially healthy,
	19	M	13	Head injury		No known regular drug intake
	29	F	62	Cerebral aneurysm	Smoker, 10 cigarettes/day*. Few doses of betamethasone	Parkinson's disease
	37	F	59	Cerebral aneurysm	No information	–
(b)	9	M	43	Cerebral aneurysm	At hospital repeated doses of phenytoin and pentazocine	10 days at hospital
	13	F	53	Cerebral aneurysm	Repeated doses of phenytoin diazepam, and pentazocine; few doses of aminocaproic acid, dexamethasone, and paracetamol	1 week at hospital after sudden headache; mild hypotonia
	20	F	57	Cerebral aneurysm	Repeated doses of pentobarbital	Earlier essentially healthy
	26	F	59	Cerebral aneurysm	Repeated doses of pentobarbital	Earlier essentially healthy

* Not last 4 days.

Assay of UDP-GT. Enzyme activities towards various substrates were assayed at the following aglycone concentrations and by methods already described: 0.5 mM 1-naphthol [20], 0.5 mM 4-methylumbelliferone [21], 1.5 mM morphine [22], 0.5 mM 4-hydroxybiphenyl [21], 0.2 mM bilirubin [23], 0.025 mM BP-3,6-quinol [6], 5 mM paracetamol. For reasons of standardization and comparison, the assays were performed at 37° in the presence of 0.1 M Tris–HCl, pH 7.4 and 5 mM $MgCl_2$. Microsomes were fully activated by addition of Brij 58 (0.5 mg/mg protein), except for bilirubin UDP-GT which was fully activated by addition of digitonin (3 mg/mg protein). Reactions were started by addition of 3 mM UDP-glucuronic acid (1 mM UDP-glucuronic acid in the case of paracetamol UDP-GT). Fluorescence assays were carried out in a Perkin-Elmer model 650-10S fluorescence spectrophotometer. Fluorescence intensity was calibrated with quinine sulfate.

Paracetamol glucuronidation was measured by a modification of published HPLC methods [24, 25]. In essence paracetamol was incubated for 30 and 60 min in 0.1 ml of the incubation mixture described above containing 0.2 mg microsomal protein. The reaction was stopped by the addition of 5% perchloric acid. The precipitated protein was centrifuged and 20 μl of the supernatant was injected into a Waters Associates RAD PAK reversed phase column (5 μm). The mobile phase consisted of 3% acetonitrile in 1% acetic acid. Flow was approximately 1.5 ml/min and pressure 800 psi. Paracetamol glucuronide was quantitated by UV-absorption at 254 nm. Retention times were: paracetamol glucuronide 5.2 min, paracetamol 11 min. Calibration was carried out with paracetamol since UV-absorption remains unchanged by glucuronidation [24]. Zero-time blanks were subtracted. Initially 4-fluorophenol (retention time 25 min) was used as internal standard. It was omitted subsequently since the recovery of added 4-fluorophenol was very reproducible and virtually complete.

Cytochrome P-450 dependent monooxygenase assays. 7-Ethoxycoumarin *O*-deethylase was determined according to Ullrich and Weber [26] as modified [27], 7-ethoxyresorufin *O*-deethylase according to Burke *et al.* [28, 29] and BP monooxygenase or arylhydrocarbon hydroxylase according to Nebert and Gelboin [30]. Protein was determined according to the method of Lowry *et al.* [31] using bovine serum albumin as protein standard.

Culture and MC-treatment of Hep G2 cells. Hep G2 cells were grown in 60 mm plastic culture dishes at 37° in a humidified atmosphere of 5% CO_2 in air, and maintained in Eagles minimal essential medium, supplemented with 10% fetal calf serum, penicillin G (100 units/ml) and streptomycine (100 μg/ml). MC (5 μM) was added in dimethylsulfoxide to a final solvent concentration of 0.1%. Sodium phenobarbital was included at a concentration of 1 mM. Control plates received 0.1% DMSO. Cells were harvested 48 hr after addition of inducers and washed with phosphate-buffered saline. They were homogenized by sonication in 0.25 M sucrose containing 0.1 M Tris–HCl, pH 7.4, and stored at −80°.

RESULTS

UDP-GT activity towards paracetamol was clearly detectable in human liver microsomes (Table 2). For isolation of paracetamol glucuronide the HPLC system was applied which has been previously used to analyze paracetamol metabolites in urine [11].

Table 2. Drug metabolizing enzymes in human liver microsomes*

		UDP-glucuronyltransferase activities				Monooxygenase activities
	Sample	Paracetamol	Benzo(*a*)pyrene-3,6-quinol (nmol/min/mg protein)	4-Methyl umbelliferone	Bilirubin	7-Ethoxycoumarin
(A)	11	0.36	0.7	11	—	0.11
	12	0.15	0.8	12	—	0.03
	15	0.19	1.5	20	—	0.06
	17	0.19 (±0.02)	1.9 (±0.1)	24 (±6)	0.47	0.14
	18	0.32	1.9	23	—	0.22
	19	0.17	0.8	18	0.2 (±0.1)	0.04
	29	0.37	2.4	23	1.2	0.14
	37	0.17 (±0.09)	1.6 (±0.1)	14	0.50	0.07
(B)	9	0.60	2.0	32	—	0.53
	13	0.37	3.2 (±0.1)	31 (±5)	2.4	0.58
	20	0.44	3.4	38 (+6)	1.5 (±0.4)	0.43 (±0.05)
	26	0.31 (±0.01)	2.7 (±0.2)	33	1.0	0.27

* Groups A and B represent liver microsomal samples from patients not treated with phenobarbital-type inducers and those treated with phenytoin or pentobarbital, respectively. When more than 4 determinations were available means ± SD are listed.

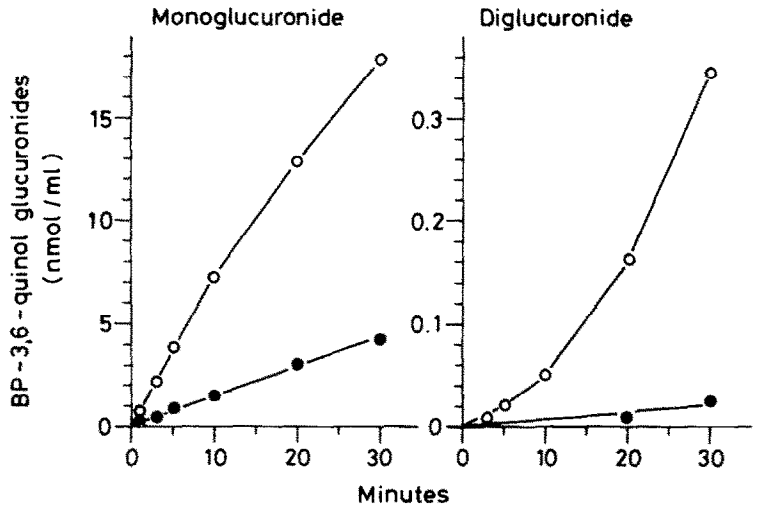

Fig. 1. Formation of benzo(*a*)pyrene-3,6-quinol mono- and diglucuronide by human liver microsomes. ●, Sample 11 (no inducer), ○, sample 20 (pentobarbital-treatment).

The glucuronidation reaction proceeded linearly up to 30 min. Apparent K_m-values of 5 and 0.3 mM were determined for paracetamol and UDP-glucuronic acid, respectively. The reaction showed kinetic properties similar to those described with rat liver microsomes [32]. Enzyme activity towards paracetamol could be activated by 3 mM UDP-N-acetylglucosamine or the detergent Brij 58, but activation was found to be low compared with other substrates (1.3-fold and 1.7-fold, respectively: not shown).

The time course of BP-3,6-quinol mono- and diglucuronide formation in human liver microsomes is illustrated in Fig. 1. Enzyme activity of liver microsomes from a patient not exposed to inducing agents (sample 11) was compared with that of a patient treated with high doses of pentobarbital (sample 20). Initial rates of BP-3,6-quinol monoglucuronide formation could be clearly measured. However, conversion of the monoglucuronide to the diglucuronide appears to be very low in human liver, in contrast to findings in rats [6, 7].

Despite large interindividual differences UDP-GT activities from patients treated with phenobarbital-type inducers can be distinguished from those of patients not receiving these inducing drugs (Table 2). Liver microsomal enzyme activities of a moderate smoker (sample 29) are included in group A. UDP-GT activities of this sample were in the upper range of group A and (in contrast to 7-ethoxycoumarin *O*-deethylase which was lower) 7-ethoxyresorufin *O*-deethylase activity (V_{max} = 0.29 nmol/min/mg protein) was higher than the means of enzyme activities detected in other samples of groups A and B (Table 3). Therefore sample 29 was excluded in calculation of the means in Table 3.

A close correlation was found between glucuronidation of 4-methylumbelliferone and BP-3,6-quinol (r = 0.91; Fig. 2). The correlation was weaker

Table 3. Induction of human liver UDP-GT activities by phenobarbital-type inducers*

Enzyme substrate	Enzyme activities: No inducers (A) (nmol/min/mg protein)	Enzyme activities: Phenobarbital-type inducers (B) (nmol/min/mg protein)	Induction factor
UDP-GT			
(1) Paracetamol	0.22 ± 0.08 (7)	0.32 ± 0.13 (4)	2.0
Benzo(*a*)pyrene,3,6-quinol	1.33 ± 0.52 (7)	2.87 ± 0.68 (4)	2.2
4-Methylumbelliferone	17 ± 5 (7)	33 ± 3 (4)	1.9
1-Naphthol	16 ± 3 (5)	29 ± 5 (3)	1.8
(2) Morphine	0.54 ± 0.21 (5)	0.73 ± 0.05 (3)	1.4
4-Hydroxybiphenyl	2.0 ± 0.2 (5)	2.1 ± 0.6 (3)	1.1
(3) Bilirubin	0.4 ± 0.2 (3)	1.3 ± 0.7 (3)	3.3
Cytochrome P-450			
7-Ethoxyresorufin	0.17 ± 0.06 (3)	0.24 ± 0.1 (3)	1.4
7-Ethoxycoumarin	0.10 ± 0.07 (7)	0.45 ± 0.1 (4)	4.5

* Means ± SD of the enzyme activities of groups A and B (Table 2) are compared. In addition means of UDP-GT activities towards 1-naphthol, morphine and 4-hydroxybiphenyl are shown. The number of samples from different individuals is listed in parentheses.

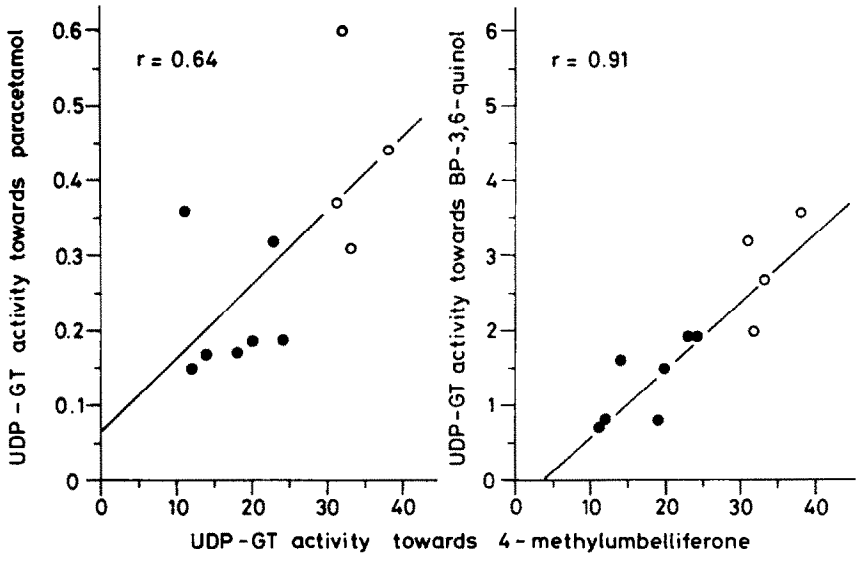

Fig. 2. Correlations between various human liver UDP-GT activities. ●, Samples from patients receiving no inducers; ○, samples from patients treated with phenytoin or pentobarbital.

between glucuronidation of 4-methylumbelliferone and paracetamol ($r = 0.64$). To be able to distinguish between interindividual variability and the effects of phenobarbital-type inducers a comparison was made between the means of UDP-GT activities in samples of patients who were unexposed or treated with these inducers (Table 3). As expected 7-ethoxycoumarin *O*-deethylase activity was much higher in samples from patients treated with phenobarbital-type inducers. UDP-GT activities were differentially affected by phenobarbital-type inducers. As is well known bilirubin UDP-GT activity was markedly induced by phenobarbital-type inducers (3.3-fold). UDP-GT activities towards paracetamol, BP-3,6-quinol, 4-methylumbelliferone and 1-naphthol were also significantly induced and to similar extents (2-fold), whereas enzyme activities towards morphine and 4-hydroxybiphenyl were not affected.

To study the inducibility of human drug metabolizing enzymes by MC-type inducers a widely used hepatoma cell line (Hep G2 cells) was investigated (Table 4). UDP-GT activities towards BP-3,6-quinol and 1-naphthol were moderately but significantly increased by MC-treatment of the cells. However, MC-induction of UDP-GT activities was much weaker than that of cytochrome P-450 dependent monooxygenase activities. UDP-GT activity towards paracetamol could not be determined in Hep G2 cells probably since UDP-GT activities in general are low in this cell line.

DISCUSSION

Our findings extend earlier studies with human liver samples from a "liver bank" [17, 18], including additional samples and paracetamol and BP-3,6-quinol as substrates which are important in pharmacology and toxicology. Correlations between

Table 4. Induction of drug-metabolizing enzymes in a human hepatoma cell line (Hep G2)*

Enzyme Substrate	Enzyme activities Untreated controls (pmol/min/mg protein)	Phenobarbital-treatment	MC-treatment
UDP-GT			
1-Naphthol	27 ± 7	36 ± 6 (1.3)	56 ± 7 (2.1)
Benzo(*a*)pyrene-3,6-quinol	5.7 ± 1.0	6.6 (1.2)	11.3 ± 2.2 (2.0)
Cytochrome P-450			
7-Ethoxyresorufin	1.9 ± 0.5	12 ± 4 (6)	245 ± 64 (129)
7-Ethoxycoumarin	1.7 ± 0.5	2.2 ± 0.6 (1.3)	17.8 ± 6.1 (10)
Benzo(*a*)pyrene	0.9 ± 0.4	1.1 (1.2)	8.9 ± 2.5 (10)

* Hep G2 cells were treated with 1 mM phenobarbital or 5 μM MC for 48 hr. Enzyme activities were measured in cell homogenates. Means ± SD of 4 experiments are listed. Induction factors are given in parenthesis.

various groups of human liver UDP-GT activities and differential responses to phenobarbital-type inducers were found: (a) Bilirubin UDP-GT activity was increased 3-fold. (b) UDP-GT activities towards phenolic substrates (including paracetamol, BP-3,6-quinol, 4-methylumbelliferone and 1-naphthol) were moderately induced (2-fold). (c) Glucuronidation of morphine and 4-hydroxybiphenyl was not significantly affected.

Marked species differences in the response to phenobarbital are obvious. In liver microsomes from phenobarbital-treated rats UDP-GT activities towards paracetamol [33] and BP-3,6-quinol [6] are not increased. In addition morphine and 4-hydroxybiphenyl are prototype substrates for phenobarbital-inducible UDP-GTs [2]. In human liver conversion of BP-3,6-quinol mono- to its diglucuronide appears to be very low compared with rats (Fig. 1). Marked species differences have also been described for the cytochrome P-450 dependent monooxygenases. 7-Ethoxycoumarin appears to be an overlapping substrate for both phenobarbital and MC-inducible isoenzymes of cytochrome P-450 in rat liver [34]. In human liver 7-ethoxycoumarin *O*-deethylase seems to be increased by phenobarbital-treatment but not in smokers. Only 7-ethoxyresorufin appears to be a selective substrate for the isoenzyme inducible by cigarette smoking [35].

Increased UDP-GT activity towards paracetamol in liver microsomes from patients treated with phenytoin or pentobarbital (Table 3) correlates well with increased paracetamol glucuronidation in patients treated with phenobarbital-type inducers [12, 13]. The findings in microsomes support the contention that increased paracetamol glucuronidation *in vivo* is mainly due to enzyme induction in liver.

It could be demonstrated recently that paracetamol glucuronidation *in vivo* is also increased in heavy smokers which are presumably exposed to MC-type inducers [11] and in humans ingesting Brussels sprouts and cabbage [36], foods which are thought to contain MC-type inducers [37]. It is very difficult to obtain adequate MC-induced liver samples from smokers since the inducing effect may be at least partially reversed at the time when the liver sample is available (as in sample 29). Therefore MC-induction of UDP-GT activity was studied in an inducible human hepatoma cell line [19]. UDP-GT activities towards BP-3,6-quinol and 1-naphthol could be significantly, albeit moderately induced (2-fold). In contrast, cytochrome P-450-dependent reactions were strongly inducible. It is understood that regulation of drug metabolizing enzymes in hepatomas does not necessarily reflect regulation in normal liver. Nevertheless our finding of increased UDP-GT activities in human MC-treated hepatoma cells suggests that some UDP-GT isoenzymes are potentially inducible by MC-type inducers.

Some liver samples were obtained from patients who received betamethasone or dexamethasone (Table 1), and glucucorticoids are suspected inducers of some human liver UDP-GT isoenzymes [5, 38]. An inducing effect of glucocorticoids is not excluded from our data (Table 2). However, the inducing effects appear to be weaker than those of phenobarbital-type inducers. Nevertheless, further studies are warranted to explore direct inducing or "permissive" effects of glucocorticoids on human liver UDP-GT activities.

Differential induction of UDP-GT activities by xenobiotics and hormones is probably useful as a guide to characterize different UDP-GT isoenzymes. Preliminary results (Bock and Schirmer, unpublished) suggest that paracetamol appears to be an overlapping substrate of the two human liver UDP-GT isoenzymes characterized so far [5].

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