Pergamon

Biochemical Pharmacology, Vol. 49, No. 11, pp. 1601–1606, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00095-X

FORMATION OF MONO- AND DIGLUCURONIDES AND OTHER GLYCOSIDES OF BENZO(*a*)PYRENE-3,6-QUINOL BY V79 CELL-EXPRESSED HUMAN PHENOL UDP-GLUCURONOSYLTRANSFERASES OF THE UGT1 GENE COMPLEX

HARALD GSCHAIDMEIER,* ALBRECHT SEIDEL,† BRIAN BURCHELL‡ and KARL WALTER BOCK*§

*Institute of Toxicology, University of Tübingen, D-72074 Tübingen, Germany; †Institute of Toxicology, University of Mainz, D-55131 Mainz, Germany; and ‡Department of Biochemical Medicine, Ninewells Hospital, Dundee DD1 9SY, U.K.

(*Received* 24 *October* 1994; *accepted* 5 *February* 1995)

Abstract—Glucuronidation of quinols of polycyclic aromatic hydrocarbons (PAHs) represents an important detoxication pathway preventing toxic quinone/quinol redox cycles. Therefore, mono- and diglucuronide formation of benzo(*a*)pyrene-3,6-quinol was investigated and compared to that of structurally related 3,6-dihydroxychrysene and simple phenols (1-naphthol and 4-methylumbelliferone) using V79 cell-expressed human UGT1.6 (=P1) and human UGT1.7 (=P4). Properties of human UGT1.6 were compared to those of the rat ortholog. Cofactors related to UDP-glucuronic acid such as UDP-galacturonic acid and UDP-glucose were also studied. It was found that rat and human UGT1.6 and human UGT1.7 catalyse monoglucuronide formation of planar PAH quinols. Diglucuronide formation was only detectable with human UGT1.7. The UGT isozymes studied also formed galacturonides and, although only to a minor extent, glucosides. Rat UGT1.6 (but not the human ortholog) catalysed digalacturonide formation of benzo(*a*)pyrene-3,6-quinol; the *in vivo* significance of galacturonide formation remains to be established. The results suggest that planar PAH phenols and quinols are conjugated more efficiently by human UGT1.7 than by UGT1.6, which preferentially conjugates simple planar phenols.

Key words: benzo(*a*)pyrene-3,6-quinol; human UDP-glucuronosyltransferases (UGTs); V79 cell-expressed UGTs; UGT1.6; UGT1.7; UGT1 gene complex

PAHs‖ are well known carcinogens both in humans and experimental animals. The mechanism of chemical carcinogenesis has been extensively studied with benzo(*a*)pyrene, the prototypic member of this class of carcinogens. Many studies have shown that benzo(*a*)pyrene is metabolized to an array of phenols, dihydrodiols, quinones and diolepoxides, with benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide as one of the ultimate carcinogens [1, 2]. However, phenols and polyphenols of benzo(*a*)pyrene can be converted to radicals, semiquinones and quinones, the latter undergoing quinone/quinol redox cycles which generate semiquinones and reactive oxygen species [3–5]. The extent of detoxification of PAH quinones is largely determined by the balance between phase I and phase II drug metabolism. Previous studies have shown that mutagenicity of benzo(*a*)pyrene and benzo(*a*)pyrene-3,6-quinone in the Ames test was markedly reduced upon addition of UDP-glucuronic acid, thereby emphasizing the inactivating role of glucuronidation [6]. After reduction of quinones by quinone reductase glucuronidation of the resulting quinols appears to be mainly catalysed by 3-methylcholanthrene-inducible UGTs [7–9]. Similar results have been obtained with structurally related 3,6-dihydroxychrysene as substrate [9]. Diglucuronides of benzo(*a*)pyrene-3,6-quinol have been found as major biliary metabolites after intra-tracheal instillation of benzo[*a*]pyrene [10].

UGTs represent members of a superfamily of isozymes firmly bound to endoplasmic reticulum and nuclear membranes [11–13]. Two families of UGTs have been defined based on their amino acid sequence identity [14]. Family 1 consists of various phenol and bilirubin UGTs formed by differential splicing of a single UGT1 gene complex. This complex is located on human chromosome 2 whereas the genes of family 2 members (encoding various steroid UGTs) appear to be clustered on human chromosome 4. The nomenclature used to designate the cloned expressed UGT isozymes of the UGT1 complex follows a modified format [15] to that already outlined [14]. The asterisk notation has been replaced by a full stop. The "O" of rat UGT1.06 has been dropped since the rat and human isozymes are indeed orthologs and are transcribed from the sixth unique exon (located in front of five other first

§ Corresponding author. Tel. (49) 07071-29-2274; FAX (49) 07071-29-2273.

‖ Abbreviations: UGT, UDP-glucuronosyltransferase; PAHs, polycyclic aromatic hydrocarbons; MG, monoglucuronide; DG, diglucuronide; MGal, monogalacturonide; DGal, digalacturonide, HPLC, high-performance liquid chromatography.

unique exons) in addition to the conserved sequences from exons 2 to 5. Operational terms used in previous publications are P1 for human UGT1.6 [16] and UGT1A1 for rat UGT1.6 [9]. P4 [17] has been designated UGT1.7 although it is not certain whether it contains the distal exon next to that of UGT1.6.

Cell lines with stably transfected UGT isozymes represent important tools to investigate their substrate specificity [18]. In the present study conjugation of planar PAH phenols and quinols in comparison with simple phenols was investigated using two human phenol UGTs (UGT1.6 and UGT1.7) previously shown to conjugate planar and bulky phenols, respectively [19]. In addition to aglycone specificity acceptance of UDP-galacturonic acid and UDP-glucose as cofactors was also studied.

MATERIALS AND METHODS

Chemicals. Benzo(*a*)pyrene-3,6-quinone was synthesized and chromatographically separated from the isomeric 1,6- and 6,12-quinones as described [20]. Benzo(*a*)pyrene-3,6-quinol was formed from the corresponding quinone immediately before use by addition of 0.25 M ascorbic acid. The synthesis of 6-hydroxychrysene and 3,6-dihydroxychrysene was accomplished according to the previously published methodology [9, 21]. Brij 58 was obtained from Serva (Heidelberg, Germany), 4-methylumbelliferone from EGA-Chemie (Steinheim, Germany) and [^{14}C]1-naphthol (20 mCi/mmol) from Amersham Buchler (Braunschweig, Germany). β-Glucuronidase of *Escherichia coli* and UDP-glucuronic acid were obtained from Boehringer (Mannheim, Germany), UDP-galacturonic acid and UDP-glucose from Sigma (St. Louis, MO, U.S.A.) and β-glucosidase of sweet almonds from Serva (Heidelberg, Germany).

Recombinant UGT isozymes. Stably transfected rat UGT1.6 [9], human UGT1.6 [22] and human UGT1.7 [17] were cultured and cell homogenates prepared as described. The cell lines stably expressed UGT activities over 10 passages, except where indicated.

Human liver microsomes. Human livers were obtained shortly after death from kidney transplant donors. HL17 was a sample obtained from a 70-year-old male smoker (ethanol abusus). HL18 was obtained from the normal liver of a 25-year-old woman. Tissue homogenates and microsomes were prepared as described previously and stored at −80° [23].

Rat liver microsomes. Male Wistar rats were treated once i.p. with 3-methylcholanthrene (40 mg/kg, dissolved in corn oil) 3 days before sacrifice, and untreated animals were used as controls. Liver microsomes were prepared as described [24].

UGT assays. Published procedures were used to determine UGT activities towards 1-naphthol [24, 25], 4-methylumbelliferone [26], benzo(*a*)-pyrene-3,6-quinol [9], 6-hydroxychrysene and 3,6-dihydroxychrysene [9]. In the case of benzo(*a*)-pyrene-3,6-quinol mono- and diglucuronides can be determined by their differential fluorescence spectra [7]. Usually, however, mono- and diglucuronides of the two PAH quinols as well as the corresponding glycosides (described subsequently) were separated by HPLC [9]. In brief, the incubation mixture (total volume 500 μL) consisted of benzo(*a*)pyrene-3,6-quinol (0.05 mM), $MgCl_2$ (5 mM), 0.5 M Tris/0.25 M ascorbic acid, pH 7.4, V79 cell homogenate (0.4 mg protein) and Brij 58 (0.5 mg/mg protein). The reaction was started by the addition of UDP-glucuronic acid (3 mM). After 10 min incubation at 37° the reaction was stopped by cooling on ice, extraction with 2 mL chloroform and centrifugation. The supernatant (200 μL) was injected into the HPLC system. Reversed-phase HPLC consisted of two 501/510 pumps (Waters, Milford, U.S.A.), a Spherisorb ODS2 (5 μm) pre-column (20 × 4 mm), a Spherisorb ODS2 (5 μm) separation column (250 × 4.6 mm) and a fluorescence detector (Model S 3400, Sykam, Gilding, Germany). The mobile phase was composed of two solvents. Solvent A consisted of 0.05 M ammonium acetate, pH 6.4, and solvent B of 0.05 M ammonium acetate, pH 6.4/acetonitrile (70/30, v/v). A linear gradient of 100% solvent A to 100% solvent B was used up to 15 min followed by isocratic elution with 100% solvent B. Fluorescence of the glucuronides was measured at excitation/emission wavelengths of 385/433 nm. Note that in the HPLC system both mono- and diconjugates are recorded at the same wavelength. Different excitation/emission wavelengths were used for the direct fluorescence assay [7]. Protein was determined according to Lowry *et al.* [27].

Formation of galacturonides and glucosides. Enzyme assays were identical to those described for UGT activities towards 1-naphthol and benzo(*a*)-pyrene-3,6-quinol, except that 3 mM UDP-galacturonic acid or 3 mM UDP-glucose was used instead of 3 mM UDP-glucuronic acid. Higher concentrations of UDP-nucleotides (up to 9 mM) did not enhance glycoside formation, suggesting that saturation had been reached. All incubations contained Brij 58 (0.5 mg/mg protein). Under these conditions glucuronide and galacturonide formation was activated two- to three-fold by the detergent. However, glucoside formation could not be activated. With homogenates from untransfected or "mock" V79 cells no UDP-glycosyltransferase activities towards PAH phenols and quinols were detectable. The detection limit of glycoside formation was determined in 60 min incubations. Glycosides were identified by selective hydrolysis with β-glucuronidase for glucuronides and galacturonides and β-glucosidase for glucosides. HPLC retention times of the glucuronides, galacturonides and glucosides of benzo(*a*)pyrene-3,6-quinol were similar: 18 and 13 min for mono- and diconjugates, respectively.

Quantitation of glycosides. 1-Naphthol glucoside formation was calibrated as follows: incubations with labelled 1-naphthol contained 10 nmol [^{14}C]1-naphthol (0.4 μCi) in addition to 250 nmol unlabelled 1-naphthol. Hence, total radioactivity present in 0.5 mL incubation mixture at 0 time was 260 nmol 1-naphthol. On this basis the amount of radiolabelled glycosides could be calculated and their fluorescence calibrated. The fluorescence spectra and the relative fluorescence of 1-naphthol glucuronide, galacturonide and glucoside were found to be identical. Identical fluorescence spectra were also

Table 1. Mono- and diglucuronide formation of benzo(*a*)pyrene-3,6-quinol by human UGT1.6 and UGT1.7 in comparison with other substrates

Substrate	UGT1.6 (nmol/min/mg protein)	UGT1.7 (nmol/min/mg protein)	Liver microsomes (nmol/min/mg protein)	
			HL17	HL18
Benzo(*a*)pyrene-3,6-quinol				
(6-O-MG formation)	0.11 ± 0.08	6.6 ± 0.5	10.9	6.4
(DG formation)	ND	0.04 ± 0.01	0.6	0.2
3,6-Dihydroxychrysene				
(6-O-MG formation)	0.001	0.015	0.13	0.10
(3-O-MG formation)	0.002	ND	0.14	0.09
(DG formation)	ND	0.0001	0.01	0.01
4-Methylumbelliferone	1.2 ± 0.4	1.5 ± 0.6	51.3	10.9
6-Hydroxychrysene	0.012 ± 0.001	1.8 ± 0.2	1.9	1.1

Data represent means ± SD of three experiments or means of two experiments (in the absence of SD). ND, not detectable. The low rate of 3,6-dihydroxychrysene DG formation (0.0001 nmol/min/mg protein) is approx. 5-fold above the detection limit (see the Methods section).

found for benzo(*a*)pyrene-3,6-quinol glycosides. Therefore, the same relative fluorescence values were assumed for PAH quinol galacturonides and glucosides, which were then calibrated according to the methods used to calibrate the corresponding glucuronides [7, 9].

RESULTS

Mono- and diglucuronide formation of benzo(a)*-pyrene-3,6-quinol*

Human UGT1.6 and UGT1.7 catalysed the formation of monoglucuronides of benzo(*a*)pyrene-3,6-quinol, 3,6-dihydroxychrysene and 6-hydroxychrysene (Table 1). Diglucuronide formation was only detectable with UGT1.7. Compared to 4-methylumbelliferone UGT activity (comparable with the two UGT-expressing clones) monoglucuronide formation of PAH phenols appeared to be much lower with UGT1.6. The ratio of diglucuronide to monoglucuronide formation was found to be higher with human microsomes than with UGT1.7.

Formation of galacturonides and glucosides

Human UGT1.6, UGT1.7 and rat UGT1.6 were found to catalyse the formation of benzo(*a*)pyrene-3,6-quinol and 1-naphthol galacturonides, whereas glucoside formation was very low (Tables 2 and 3). Rat UGT1.6 (but not human UGT1.6 and UGT1.7) catalysed the formation of benzo(*a*)pyrene-3,6-quinol digalacturonide. Interestingly, the formation of glycosides with rat liver microsomes was clearly inducible by 3-methylcholanthrene treatment (Table 3). The resulting high rates of glucoside formation are in contrast to the low capacity of UGT1.6 to carry out this reaction.

DISCUSSION

The present investigation demonstrates that two human UGTs of the UGT1 gene complex (UGT1.6 and UGT1.7) are able to form mono- and diglucuronides of planar PAH phenols and quinols. In comparison with simple phenols, UGT1.7 appeared to be more efficient than UGT1.6 in conjugating the larger PAH phenols, confirming the previous notion that UGT1.6 primarily conjugates small planar phenols [16, 19]. The UGT1.6 level in the cell lines used was probably lower than that of UGT1.7 [28], and therefore UGT activities of the two cell lines do not permit absolute comparisons. Nevertheless, some conclusions can be drawn by comparing K_m values (which are independent of the expressed enzyme levels) and relative activities toward various substrates, as has been done in previous studies on the glucuronidation of paracetamol [23] and arylamines [29]. Efficient conjugation of 6-hydroxychrysene is suggested by its seven-fold lower K_m with UGT1.7 (0.02 mM) than with UGT1.6 (0.14 mM; Ref. 23). In the two UGT-expressing clones 4-methylumbelliferone UGT activity was similar. However, the relative activity towards PAH phenols and quinols was much lower with the UGT1.6-expressing clone. The role of UGT1.7 in clearing PAH quinols is underlined by its ability to form diglucuronides of PAH quinols. However, the enzymology of diglucuronide formation may be complex since evidence has recently been obtained by radiation inactivation analysis that tetrameric UGT oligomers (in contrast to monoglucuronide forming dimers) may be involved in this reaction in microsomes *in situ* [30].

Substrate specificity of one UGT only (UGT1.6) has been studied in the rat UGT1 gene complex [9, 29]. cDNA cloning of a rat UGT corresponding to human UGT1.7 has been achieved, but the cDNA clone isolated was found to contain a stop codon in the first exon (Ref. 31 and C. Brierley and H. Gschaidmeier, unpublished). Hence, it probably represents a pseudoexon. However, several UGT1.7-like phenol UGTs have been identified in rats (T. Iyanagi, unpublished) and humans (Brierley *et al.*, unpublished).

Glycosylations other than glucuronidation are well known; for example, bilirubin's glucoside and xyloside conjugates as well as its glucuronides have

Table 2. Glucuronidation, galacturonidation and glucosidation of benzo(*a*)pyrene-3,6-quinol and 1-naphthol by cell-expressed human UGT1.6 and UGT1.7

Substrate	UGT1.6 (nmol/min/mg protein)	UGT1.7* (nmol/min/mg protein)	Liver microsomes (nmol/min/mg protein)	
			HL17	HL18
Benzo(*a*)pyrene-3,6-quinol				
6-*O*-MG formation	0.23	0.38	10.0	—
DG formation	ND	0.1	0.6	—
6-*O*-MGal formation	0.013	0.13	4.0	—
DGal formation	ND	ND	ND	—
6-*O*-glucoside formation	0.007	0.01	0.72	—
Diglucoside formation	ND	ND	ND	—
1-Naphthol				
Glucuronide formation	13.3 ± 0.6	0.33 ± 0.15	17.7	16.8
Galacturonide formation	1.0 ± 0.2	0.08 ± 0.02	9.2	6.7
Glucoside formation	ND	ND	0.8	0.6

Data represent means ± SD of three experiments or means of two experiments (in the absence of SD). ND, not detectable; —, not determined.

* Enzyme activities were lower than those given in Table 1 since a high passage cell line was used and passaged in the absence of G418 selection [22].

Table 3. Glucuronidation, galacturonidation and glucosidation of benzo(*a*)pyrene-3,6-quinol and 1-naphthol by cell-expressed rat UGT1.6

Substrate	UGT1.6 (nmol/min/mg protein)	Liver microsomes (nmol/min/mg protein)	
		Untreated controls	Methylcholanthrene treatment
Benzo(*a*)pyrene-3,6-quinol			
6-*O*-MG formation	1.33	6.4	85.4
DG formation	0.25	0.4	9.0
6-*O*-MGal formation	0.078	1.5	13.8
DGal formation	0.004	0.2	1.2
6-*O*-glucoside formation	0.004	1.5	13.3
Diglucoside formation	ND	0.2	1.5
1-Naphthol			
Glucuronide formation	13.6 ± 0.5	43.8 ± 1.3	111.0 ± 0.1
Galacturonide formation	1.1 ± 0.1	7.5 ± 0.7	17.2 ± 0.2
Glucoside formation	ND	0.3 ± 0.01	0.6 ± 0.01

Data represent means ± SD of three experiments or means of two experiments (in the absence of SD). ND, not detectable

been described [32, 33]. Galacturonidation in particular resembles glucuronidation. Recently, the presence of different human UDP-glucuronosyl- and UDP-glucosyltransferases has been suggested by studies using active site-directed photo-affinity analogues [34]. In addition, it has been shown that UGT2B4 (catalysing 6-O-glucuronidation of hyodeoxycholic acid) was not involved in its 6-O-glucosidation, which, however, was detected in human liver microsomes. It was therefore of interest to study the specificity of the cell-expressed enzymes for related UDP-sugars, for example UDP-galacturonic acid and UDP-glucose. It was shown that rat and human UGT1.6 and UGT1.7 formed galacturonides. The formation of glucosides was detectable but very low. With the rat enzyme (but not the human enzymes) a digalacturonide of benzo(*a*)pyrene-3,6-quinol could also be detected. However, UDP-galacturonic acid levels were not detected in cells. Hence, galacturonide formation may be of questionable significance *in vivo*. Interestingly, the formation of glucosides is clearly 3-methylcholanthrene-inducible in rat liver microsomes. The high rate of glucoside formation in microsomes contrasts with the low capacity of rat UGT1.6 to form glucosides, suggesting the existence of additional methylcholanthrene-inducible enzymes responsible for this reaction.

In conclusion, the present study adds PAH quinols to the list of substrates (paracetamol [23] and arylamines [29]) studied with cell-expressed human UGT1.6 and UGT1.7 and rat UGT1.6. Recently, it

has been shown that UGTs of family 2 also possess high UGT activities toward benzo(*a*)pyrene phenols [35, 36]. Therefore, to evaluate the role of particular UGT isozymes in detoxifying PAH phenols and quinols it may be necessary to understand the substrate specificity of all major family 1 and 2 UGTs. Moreover, knowledge of their tissue distribution under basal and xenobiotic-induced conditions may be required.

Acknowledgements—The authors wish to thank Mrs Simone Beck-Gschaidmeier for her expert technical assistance and the Deutsche Forschungsgemeinschaft for its financial support.

REFERENCES

1. Conney AH, Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial lecture. *Cancer Res* **42:** 4875–4917, 1982.
2. Hall M and Grover PL, Polycyclic aromatic hydrocarbons; Metabolism, activation and tumour initiation. In: *Handbook of Experimental Pharmacology* (Vol. 94/I) (Eds. Cooper CS and Grover PL), pp. 327–372. Springer, Berlin, Germany, 1990.
3. Lorentzen RJ and Ts'o POP, Benzo(*a*)pyrenedione/benzo(*a*)-pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen. *Biochemistry* **16:** 1467–1473, 1977.
4. Chesis PL, Levin DE, Smith MT, Ernster L and Ames BN, Mutagenicity of quinones: Pathways of metabolic activation and detoxification, *Proc Natl Acad Sci USA* **81:** 1696–1700, 1984.
5. Bock KW, Roles of UDP-glucuronosyltransferases in chemical carcinogenesis. *Crit Rev Biochem Mol Biol* **26:** 129–150, 1991.
6. Bock KW, Lipp H-P and Bock-Hennig BS, Induction of drug-metabolizing enzymes by xenobiotics. *Xenobiotica* **20:** 1101–1111, 1990.
7. Lilienblum W, Bock-Hennig BS and Bock KW, Protection against toxic redox cycles between benzo(*a*)pyrene-3,6-quinone and its quinol by 3-methylcholanthrene-inducible formation of the quinol mono- and diglucuronide. *Mol Pharmacol* **27:** 451–458, 1985.
8. Bock KW, Schirmer G, Green MD and Tephly TR, Properties of a 3-methylcholanthrene-inducible phenol UDP-glucuronyltransferase from rat liver. *Biochem Pharmacol* **37:** 1439–1443, 1988.
9. Bock KW, Gschaidmeier H, Seidel A, Baird S and Burchell B, Mono- and diglucuronide formation from chrysene and benzo(*a*)pyrene phenols by 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase. *Mol Pharmacol* **42:** 613–618, 1992.
10. Bevan DR and Sadler VM, Quinol diglucuronides are predominant conjugated metabolites found in bile of rats following intratracheal instillation of benzo(*a*)-pyrene. *Carcinogenesis* **13:** 403–407, 1992.
11. Burchell B and Coughtrie MWH, UDP-glucuronosyltransferases. *Pharmacol Ther* **43:** 261–289, 1989.
12. Mackenzie PI, Rodbourn L and Stranks S, Steroid UDP glucuronosyltransferases. *J Steroid Biochem Mol Biol* **8:** 1099–1105, 1992.
13. Jansen PLM, Mulder GJ, Burchell B and Bock KW, New developments in glucuronidation research: report of a workshop on "Glucuronidation, its role in health and disease". *Hepatology* **15:** 532–544, 1992.
14. Burchell B, Nebert DW, Nelson DR, Bock KW, Iyanagi T, Jansen PLM, Lancet D, Mulder GJ, Chowdhury JR, Siest G, Tephly TS and Mackenzie PI, The UDP glucuronosyltransferase gene superfamily: suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* **10:** 487–494, 1991.
15. Lamb JG, Straub P and Tukey RH, Cloning and characterization of cDNAs encoding mouse UGT1.6 and rabbit UGT1.6: Differential induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochemistry* **39:** 10513–10520, 1994.
16. Harding D, Fournel-Gigleux S, Jackson MR and Burchell B, Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. *Proc Natl Acad Sci USA* **85:** 8381–8385, 1988.
17. Wooster R, Sutherland L, Ebner T, Clarke D, Da Cruz e Silva O and Burchell B, Cloning and stable expression of a new member of the human liver phenol/bilirubin: UDP-glucuronosyl-transferase cDNA family. *Biochem J* **278:** 465–469, 1991.
18. Remmel R and Burchell B, Validation and use of cloned, expressed human drug-metabolizing enzymes in heterologous cells for analysis of drug metabolism and drug–drug interactions. *Biochem Pharmacol* **46:** 559–566, 1993.
19. Ebner T and Burchell B, Substrate specifities of two stably expressed human liver UDP-glucuronosyltransferases of the UGT1 gene family. *Drug Metab Dispos* **21:** 50–55, 1992.
20. Cho H and Harvey RG, Synthesis of hydroquinone diacetate from polycyclic aromatic quinones. *J Chem Soc Perkin Trans* **1:** 836–839, 1976.
21. Seidel A, Bochnitschek W, Glatt HR, Hodgson RM, Grover PL and Oesch F, Activated metabolites of chrysene: synthesis of 9-hydroxy-chrysene-1,2-diol and the corresponding bay-region syn- and anti-triol epoxides. In: *Polynuclear Aromatic Hydrocarbons: Measurement, Means, and Metabolism* (Eds. Cooke M, Loening K and Merrits J), pp. 801–817. Battelle Press, Columbus, OH, U.S.A., 1991.
22. Fournel-Gigleux S, Sutherland L, Sabolovic N, Burchell B and Siest G, Stable expression of two human UDP-glucuronosyl-transferase cDNAs in V79 cell cultures. *Mol Pharmacol* **39:** 177–183, 1991.
23. Bock KW, Forster A, Gschaidmeier H, Brück M, Münzel P, Schareck W, Fournel-Gigleux S and Burchell B, Paracetamol glucuronidation by recombinant rat and human phenol UDP-glucuronosyltransferases. *Biochem Pharmacol* **45:** 1809–1814, 1993.
24. Bock KW and White INH, UDP-glucuronyltransferase in perfused rat liver and in microsomes: Influence of phenobarbital and 3-methylcholanthrene. *Eur J Biochem* **46:** 451–459, 1974.
25. Otani G, Abou-El-Makarem MM and Bock KW, UDP-glucuronosyl-transferase in perfused rat liver and in microsomes. III. Effects of galactosamine and carbon tetrachloride on the glucuronidation of 1-naphthol and bilirubin. *Biochem Pharmacol* **25:** 1293–1297, 1976.
26. Lilienblum W, Walli AK and Bock KW, Differential induction of rat liver microsomal UDP-glucuronosyltransferase activities by various inducing agents. *Biochem Pharmacol* **31:** 907–913, 1982.
27. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
28. Sutherland L, Senafi S, Ebner T, Douglas JC and Burchell B, Characterisation of human bilirubin UDP-glucuronosyltransferase stably expressed in hamster lung fibroblast cell cultures. *FEBS Lett* **308:** 161–164, 1992.
29. Orzechowski A, Schrenk D, Bock-Hennig BS and Bock KW, Glucuronidation of carcinogenic arylamines and their *N*-hydroxy derivatives by rat and human phenol UDP-glucuronosyltransferases of the UGT1 gene complex. *Carcinogenesis* **15:** 1549–1553, 1994.
30. Gschaidmeier H and Bock KW, Radiation inactivation

analysis of microsomal UDP-glucuronosyltransferases catalysing mono- and diglucuronide formation of 3,6-dihydroxybenzo(*a*)pyrene and 3,6-dihydroxychrysene. *Biochem Pharmacol* **48:** 1545–1549, 1994.

31. Iyanagi T, Molecular basis of multiple UDP-glucuronosyltransferase isoenzyme deficiencies in the hyperbilirubinemic rat (Gunn rat). *J Biol Chem* **266:** 24048–24052, 1991.
32. Dutton GJ, *Glucuronidation of Drugs and Other Compounds*, pp. 23–28. CRC Press, Boca Raton, FL, U.S.A., 1980.
33. Senafi SB, Clarke DJ and Burchell B, Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation. *Biochem J* **303:** 233–240, 1994.
34. Radominska A, Little J, Pyrek JS, Drake RR, Igari Y, Fournel-Gigleux S, Magdalou J, Burchell B, Elbein AD, Siest G and Lester R, A novel UDP-Glc-specific glucosyltransferase catalyzing the biosynthesis of 6-*O*-glucosides of bile acids in human liver microsomes. *J Biol Chem* **268:** 15127–15135, 1993.
35. Jin CJ, Miners JO, Burchell B and Mackenzie PI, The glucuronidation of hydroxylated metabolites of benzo(*a*)pyrene and 2-acetylaminofluorene by cDNA-expressed human UDP-glucuronosyltransferases. *Carcinogenesis* **14:** 2637–2639, 1993.
36. Mackenzie PI, Rodbourn L and Iyanagi T, Glucuronidation of carcinogen metabolites by complementary DNA-expressed uridine 5′-diphosphate glucuronosyltransferases. *Cancer Res* **53:** 1229–1533, 1993.