Pergamon

Biochemical Pharmacology, Vol. 47, No. 8, pp. 1445–1448, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(94)E0006-7

SHORT COMMUNICATIONS

Tissue-specific constitutive and inducible expression of rat phenol UDP-glucuronosyltransferase

(*Received* 28 *September* 1993; *accepted* 24 *December* 1993)

Abstract—To investigate constitutive and inducible expression of rat phenol UDP-glucuronosyltransferase (UGT1A1) in liver and extrahepatic tissues, a selective cDNA probe for its unique exon 1 was utilized. 6-Hydroxychrysene was used as a functional probe of UGT1A1 activity. Constitutive expression of UGT1A1 was low in liver, but high in kidney, testis, epididymis and ovary. After treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 10 μg/kg for 7 days) the UGT1A1 mRNA level was markly increased in liver (*ca.* 10-fold), and only moderately enhanced (up to 2-fold) in extrahepatic tissues where constitutive enzyme expression was high. UGT activity toward 6-hydroxychrysene was strongly inducible in liver (*ca.* 9-fold) and only moderately inducible in extrahepatic tissues (up to 2-fold). The results suggest complex tissue-specific regulation of UGT1A1 including positive and negative transcriptional factors and marked inducibility by TCDD in liver.

Key words: phenol UDP-glucuronosyltransferase; rat tissues; TCDD induction; gene expression

UGT* isozymes represent members of a large supergene family present in vertebrates [1, 2]. Two families have been characterized. Family 1 consists of at least four isozymes of the UGT1A or phenol/bilirubin UGT gene complex. All members of the UGT1A complex share four common exons 2–5 at the 3'-end and differ in their unique exons 1 coding for the N-terminus of the protein, which determines substrate specificity. UGT isozymes are formed by differential splicing of this large gene complex [3, 4]. Family 2 consists of multiple steroid UGTs with broad substrate specificity. The UGT1A gene complex probably has been conserved during evolution since a similar organisation has been found in rats [4] and humans [3]. PAH-inducible rat phenol UGT has been shown to be encoded by the UGT1A gene complex [4]. Due to inconsistencies in the proposed nomenclature rat phenol UGT is operationally termed UGT1A1, in line with previous publications [5, 6].

Indirect evidence suggested that UGT1A1 may be widely distributed in various tissues. To substantiate these findings, constitutive and inducible expression was compared in rat liver and extrahepatic tissues. Since simple phenols such as 4-nitrophenol appear to be overlapping substrates of most UGTs, 6-hydroxychrysene was used as a more selective substrate of PAH-inducible UGT due to its high induction factor in rat liver, its chemical stability and relative safety compared with other PAH phenols [7, 8]. UGT activity toward 6-hydroxychrysene, previously shown to be a substrate of rat UGT1A1 [5, 6], has been compared with UGT1A1 mRNA levels in various tissues of adult male and female Wistar rats. TCDD was selected as the inducer because of its potency and wide tissue distribution.

Materials and Methods

Chemicals. Guanidine thiocyanate was purchased from Fluka (Buchs, Switzerland). The two primers were obtained from Appligene (Illkirch, France), 2'-deoxynucleotide 5'-triphosphates from Pharmacia (Uppsala, Sweden) and the AmpliTaq DNA Polymerase from Perkin Elmer Cetus (Norwalk, CT, U.S.A.). The random primed labeling DNA kit was purchased from Boehringer (Mannheim, F.R.G.). All other chemicals were provided either from Merck (Darmstadt, F.R.G.) or Serva (Heidelberg, F.R.G.). TCDD was a gift of Dr H. Hagenmaier (Institute of Organic Chemistry, University of Tübingen, F.R.G.). 6-Hydroxychrysene was obtained from Dr A. Seidel (Institute of Toxicology, University of Mainz, F.R.G.).

Animal tissues. Adult male and female Wistar rats (200 g) were treated with TCDD (10 μg/kg, s.c., dissolved in corn oil) and tissues were removed after 7 days. Excised organs were pooled from 10 treated and untreated animals and used for total RNA and poly(A)$^+$ mRNA preparation. Microsomes were prepared as described previously [9]. Protein was determined according to the method of Lowry *et al.* [10], using bovine serum albumin as protein standard.

Enzyme assays. The described method was used for the assay of UGT activity toward 6-hydroxychrysene [5]. In brief, 6-hydroxychrysene (0.2 mM) and microsomal protein (0.2 mg) were incubated in the presence of 0.2 M Tris–HCl, pH 7.4, 5 mM $MgCl_2$, and Brij 58 (0.5 mg/mg protein) in a total volume of 500 μL. The reaction was stopped by rapid mixing with chloroform (2 mL) and centrifugation. An aliquot of the aqueous supernatant (200 μL) was added to 1.6 M glycine–NaOH buffer, pH 10.3 (500 μL). Fluorescence was determined using a Perkin Elmer LS5B spectrophotometer using 330 and 360 nm as excitation and emission wavelengths, respectively. Fluorescence intensity was calibrated using glucuronides isolated by HPLC. Ethoxyresorufin *O*-deethylase was determined according to Burke and Mayer [11].

Preparation of the synthetic UGT1A1 DNA probe. UGT1A1 DNA probe was synthesized by polymerase chain reaction as described before [12]. Amplified cDNAs corresponded to the expected 280 bp fragment (nucleotide 71–350) of UGT1A1 exon 1.

P4501A1 cDNA and β-actin probes. The full length P4501A1 cDNA insert (2.2 kb) was kindly provided by Dr Thomas Friedberg (Institute of Toxicology, University of

* Abbreviations: UGT, UDP-glucuronosyltransferase (EC 2.4.1.17); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PAH, polycyclic aromatic hydrocarbons; EROD, 7-ethoxyresorufin *O*-deethylase.

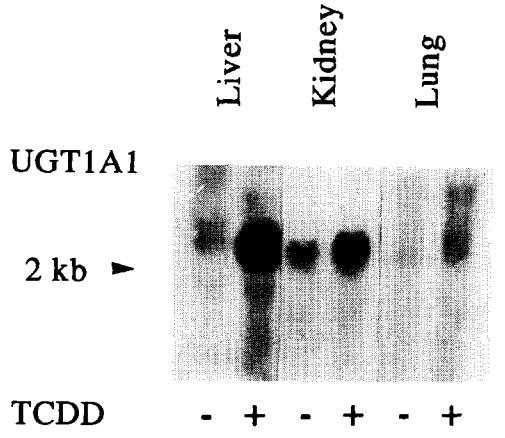

Fig. 1. Northern blot analysis of total RNA in different tissues of female rats. Each lane contained 20 μg of total RNA as judged from ethidium bromide staining and from rehybridization of the filter with a β-actin probe (not shown).

Mainz, F.R.G.). The cDNA encoding rat P4501A1 was obtained from a cDNA library prepared from rat liver DNA and inserted into the Bam HI site of pUC19 [13]. The β-actin probe (2 kb cDNA insert), derived from chicken, was a generous gift from Dr Martin Krönke (Clinical Research Group "BCWTI", Max Planck Society, Göttingen, F.R.G.).

Northern blot analysis. Total RNA was extracted from rat tissues by the guanidine thiocyanate method of Chirgwin *et al.* [14] and poly(A)$^+$ RNA was prepared as described elsewhere [15]. Total mRNA samples (20 μg) or poly(A)$^+$ RNA (5 μg) were denatured with 2.2 M formaldehyde, 50% formamide and 1× MOPS-buffer (20 mM 3-*N*-morpholinopropane sulfonic acid, 5 mM sodium acetate, 1 mM EDTA, pH 7.0) by heating for 3 min, electrophoresed on a 1% agarose gel containing 2.2 M formamide, transferred to a Nylon membrane (Hybond N, Amersham Buchler) in 20× SSC (3 M NaCl, 0.3 M sodium citrate buffer, pH 7.0) by capillary blotting and were covalently bound to the membrane by UV radiation. The membrane was then prehybridized in 6× SSC, 50% deionized formamide, 10× Denhardt's solution, 0.5% sodium dodecyl sulfate, 0.4 mg/mL denatured herring sperm DNA at 44° for 24 hr, and hybridization was performed at 44° for 48 hr using the selective cDNA probe for exon 1 of UGT1A1. The cDNA probes were labeled with [^{32}P]dCTP using the random primed labeling system. Washing of the membrane was carried out twice in 2× SSC, 0.1% SDS at 50° for 20 min. The membrane was exposed for 3–7 days at −70° to Kodak XAR-5 film with intensifying screens. Loading of equal amounts of total RNA and poly(A)$^+$ RNA and intactness of the mRNA were checked by ethidium bromide staining of the gels, from optical densities of 260 nm and from rehybridization with β-actin. The relative amounts of mRNA were estimated by densitometric scanning of autoradiogram of the northern blot.

Results

Using the selective cDNA probe and total RNA samples,

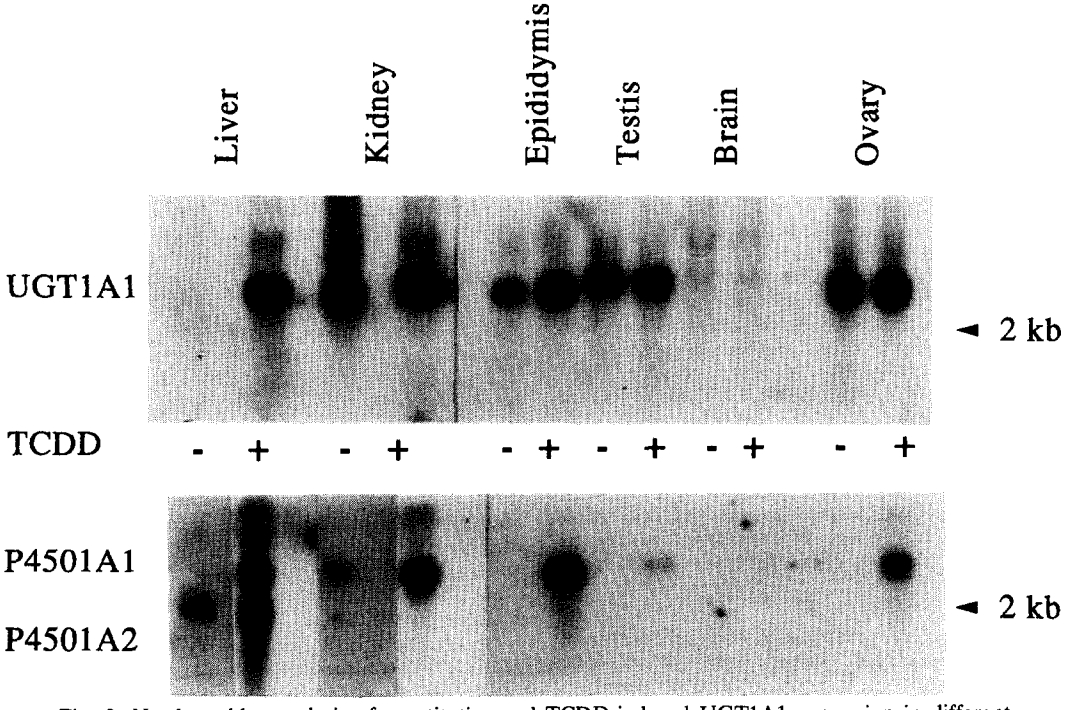

Fig. 2. Northern blot analysis of constitutive and TCDD-induced UGT1A1 expression in different tissues of male rats (except the ovary). Poly(A)$^+$ RNA samples (5 μg) were prepared for analysis as described in Materials and Methods and were hybridized with the UGT1A1 probe (upper panel) and with P4501A1 cDNA (lower panel). Equal loading of RNA samples was verified by rehybridizing of the northern blot with β-actin (not shown).

Table 1. UGT activity in microsomal fractions from various tissues of TCDD-treated rats and untreated controls toward 6-hydroxychrysene

Organ	UGT activity (nmol/min/mg protein)		
	Untreated controls	TCDD-treatment	
Males			
Liver	7.0 ± 1.0	50.7 ± 6.5	(7.2)
Kidney	6.4 ± 1.0	9.3 ± 0.5	(1.5)
Testis	1.9 ± 0.2	2.6 ± 0.5	(1.4)
Epididymis	1.1 ± 0.1	1.7 ± 0.2	(1.5)
Lung	0.9 ± 0.5	1.2 ± 0.2	(1.3)
Brain	0.4	0.3	
Females			
Liver	5.2 ± 0.6	53.8 ± 8.5	(10.3)
Kidney	6.0 ± 0.8	10.6 ± 2.1	(1.8)
Ovary	1.9 ± 0.6	3.2 ± 2.3	(1.7)

Data represent means ± SD (N = 3). The induction factor is given in parentheses. Data in brain represent the average of duplicate determinations.

UGT1A1 expression was found to be low in liver, but highly inducible by TCDD (*ca.* 10-fold; Fig. 1). The enzyme was constitutively expressed in kidney and only moderately induced by TCDD. No appreciable sex differences in UGT1A1 expression were found. UGT1A1 mRNA was also detectable in lung. Similar findings were observed when poly $(A)^+$ samples were analysed. With the exception of the kidney where constitutive expression was highly variable among individual rats, high constitutive UGT1A1 expression was observed in epididymis, testis and ovary and only moderate induction by TCDD (Fig. 2). A low level of expression was also found in brain after TCDD treatment.

Differences in the regulation of the P450 isozymes and UGT1A1 were also observed. UGT1A1 was co-induced with P4501A1/1A2 in liver, kidney, epididymis and ovary (Fig. 2). As expected P4501A2 was constitutively expressed in liver. Constitutive expression of P4501A1 was low in extrahepatic tissues where UGT1A1 showed high constitutive expression. EROD activity (largely reflecting P4501A1/1A2 activities) was also measured comparatively. EROD activity (pmol/min/mg protein) was similar in tissues of male and female rats; it was 30 in livers of untreated male rats and undetectable (<2) in extrahepatic tissues. TCDD treatment increased EROD activity to 2936, 1417, 110, 235 and 30 in liver, kidney, lung, testis and brain, respectively (not shown). UGT activity towards 6-hydroxychrysene was strongly inducible in liver (*ca.* 9-fold) and only moderately inducible in extrahepatic tissues (up to 2-fold; Table 1).

Discussion

Whereas constitutive expression of UGT1A1 was found to be low in liver and markedly inducible by TCDD treatment, constitutive expression was predominant in extrahepatic tissues in which UGT1A1 expression was only moderately inducible by TCDD. UGT1A1 appeared to be co-induced with cytochrome P4501A1/1A2 in liver, kidney, epididymis and ovary (Fig. 2). In contrast constitutive expression of cytochrome P4501A2 was high in liver, as shown previously [16]. Furthermore, UGT1A1 and cytochrome P4501A1 clearly differed in their constitutive expression in extrahepatic tissues which was low for cytochrome P4501A1. The low expression of UGT1A1 mRNA observed in brain tissue is probably restricted to microvessels where the isozyme seems to be involved in the enzymatic blood–brain barrier [17].

UGT activity toward 6-hydroxychrysene was also studied because 6-hydroxychrysene has been shown to be a substrate of V79 cell-expressed rat UGT1A1 [5, 6]. While the induction factors of UGT1A1 mRNA and UGT activity in liver appeared to be similar, discrepancies between mRNA levels and enzyme activity in different tissues were obvious. For example, comparable UGT1A1 mRNA levels were found in liver and kidney of TCDD-treated rats (based on both total RNA and poly$(A)^+$ RNA). However, UGT activity toward 6-hydroxychrysene was quite different. A variety of factors may be responsible for the discrepancies between UGT1A1 mRNA levels and enzyme activity in different tissues. In a previous study [6], UGT activity toward 6-hydroxychrysene was studied in the V79 cell-expressed human phenol UGT HlugP1 (considered to be the orthologous isozyme to rat UGT1A1) and HlugP4. It was found that 6-hydroxychrysene was conjugated by HlugP4 with higher affinity ($K_m = 0.02$ mM) than by HlugP1 ($K_m = 0.14$ mM). It is therefore conceivable that UGT isozymes other than UGT1A1 markedly contribute to glucuronidation of 6-hydroxychrysene in different tissues. Suggestive evidence for still undetected TCDD-inducible UGTs has been obtained previously [5].

The results suggest complex tissue-specific regulation of UGT1A1 with different levels of constitutive expression in various tissues. Furthermore the findings are in line with elements of co-regulation of UGT1A1 and cytochrome P4501A1/1A2 by the Ah receptor [18, 19]. However, more work is needed to elucidate the mechanisms responsible for tissue-specific regulation of UGT1A1 and its inducibility.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft. The expert technical assistance of Mrs G. Eisenhardt, Mrs B. Kaltschmitt and Ms S. Beck is gratefully acknowledged.

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