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Induction of UDP-Glycosyltransferase Family 1 Genes in Rat Liver: Different Patterns of mRNA Expression with Two Inducers, 3-Methylcholanthrene and β-Naphthoflavone

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ABSTRACT. Uridine diphosphate (UDP)-glucuronosyltransferases (UGTs), presently called UDP-glycosyltransferases, catalyse the detoxification of many toxic and carcinogenic compounds. Glucuronidation is also a major metabolic pathway for numerous drugs. The *UGT1A6* gene (formerly known as *UGT1*06* and *UGT1A1*) has been suggested to belong to the aryl hydrocarbon (Ah) gene battery, which consists of several genes encoding for drug-metabolising enzymes regulated by dioxin and other ligands of the Ah receptor. In this study, we analysed the localisation of *UGT1A6* expression in rat liver by *in situ* hybridisation to mRNA. Two different RNA probes were used, one which was specific to *UGT1A6* and the other against the C terminal sequence shared by all *UGT1* genes. In this study, no UGT1A6 mRNA was detected in the control animals. However, other gene(s) of the UGT1 family were expressed in the perivenous region surrounding the central veins as detected by hybridisation with the probe against the common region of the *UGT1* genes. Treatment with the lower dose (5 mg/kg) of 3-methylcholanthrene (3MC) induced expression of *UGT1A6* perivenously. Treatment with the higher dose (25 mg/kg) of 3-Methylcholanthrene resulted in a more panacinar expression pattern. In contrast to the perivenous induction observed with 3-methylcholanthrene, treatment with 15 mg/kg of β-naphthoflavone (BNF) resulted in strong induction in the periportal region. The results reveal an inducer-specific pattern of *UGT1A6* expression similar to that demonstrated earlier for other Ah battery genes, namely *CYP1A1*, *CYP1A2*, *GSTYα* and *ALDH3*. The finding further supports the notion that common factors regulate the regional hepatic expression of Ah battery genes. BIOCHEM PHARMACOL **56**;5:569–575, 1998.

KEY WORDS. UGT1A6; UGT1A1; UGT1*06; gene expression; *in situ* hybridization; 3-methylcholanthrene; β-naphthoflavone; periportal; perivenous hepatocytes

Glucuronidation is a major detoxification pathway in mammals. Glucuronide formation is catalysed by a family of UDP[‖]-glucuronosyltransferases, according to the new nomenclature UDP-glycosyltransferases [1], with a large number of endobiotic and xenobiotic compounds as substrates [2]. UGTs participate in the metabolism of many toxic and carcinogenic compounds. For instance, they play a major role in the elimination of nucleophilic metabolites of carcinogens, such as phenols and quinols of PAHs [3]. Glucuronidation is also a major metabolic pathway for numerous drugs [4].

In mammals, three UGT families (UGT1, UGT2 and UGT8) have been described [1]. UGT1 family enzymes are responsible for glucuronidation of bilirubin and phenols, whereas UGT2 family enzymes have steroids and bile acids as substrates. The gene locus encoding rat UGT family 1 enzymes has been characterised [5]. There are nine unique first exons which encode isoform-specific NH_2-terminal portions of each polypeptide. Each isoform of family 1 UGTs is formed by alternative utilisation of isoform-specific promoters, which initiate the synthesis of the unique first exons. These are spliced with the four commonly used exons (exons II–V), which encode the identical C terminal region present in all family 1 members [5].

One of the UGT1 family enzymes, UGT1A6 (formerly

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‖ *Abbreviations:* ALDH, aldehyde dehydrogenase; Ah receptor, aryl hydrocarbon receptor; BNF, β-naphthoflavone; CYP, cytochrome P450; GST, glutathione S-transferase; 3MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; RT-PCR, reverse transcriptase polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UDP, uridine diphosphate; UGT, UDP-glycosyltransferase; and XRE, xenobiotic responsive element.

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known as UGT1*06 and UGT1A1), catalyses glucuronidation of planar phenols. Phenolic compounds of many drugs are glucuronidated by UGT1A6 [1], and this isoform also participates in the detoxification of carcinogenic aromatic hydrocarbons (e.g. benzo(a)pyrene) and aromatic amines and their metabolites [2, 6]. The *UGT1A6* gene is expressed in rat and man mainly in the liver but also in other organs, such as kidney, lung and intestine [5, 7, 8]. The *UGT1* gene complex has been cloned and characterised only recently, and, because of the overlapping substrate specificities, it has been difficult to develop isoenzyme-specific assays or to raise specific antibodies.

UGT1A6 has been suggested to belong to the so-called Ah gene battery [9]. The Ah gene battery in mammals consists of several genes encoding for drug-metabolising enzymes. The Ah battery is best defined in mouse, in which it has been shown to contain at least six genes: two genes of oxidative metabolism, i.e. CYP genes *Cyp1a1* and *Cyp1a2*, as well as four genes participating in detoxification, i.e. NAD(P)H:menadione oxidoreductase (*Nmo1*), glutathione *S*-transferase Ya (*GSTYa*), "class 3" aldehyde dehydrogenase (*Ahd4*), and UDP-glucuronosyltransferase 1a6 (*Ugt1a6*; corresponding to rat *UGT1A6*) [9]. All these genes appear to be regulated positively by inducers such as TCDD and other ligands of the Ah receptor. Other components such as a 90-kDa heat shock protein (hsp90) and Ah receptor nuclear translocator (Arnt) are also involved in the induction process. The Ah receptor-Arnt heterodimer complex initiates transcriptional activation by binding to the XREs located in the promoter regions of the Ah-responsive genes [9, 10, 11].

It has been demonstrated that, in the rat liver, many of the xenobiotic-metabolising genes are not expressed uniformly, but exhibit characteristic zonated expression patterns [12, 13]. Previously, it has been shown that after induction by TCDD, 3MC and other common inducers, the expression of two members of the Ah battery, *CYP1A1* and *CYP1A2*, predominantly occurs in the centrilobular (perivenous) region [14, 15]. In contrast to this, we and others have previously observed that one of the inducers, BNF, induced *CYP1A1* expression at hepatocytes mainly in the region surrounding the portal veins (periportal area) [15–17]. Our studies on the expression of the *CYP1A1* gene by mRNA *in situ* hybridisation and amplification of both *CYP1A1* and *CYP1A2* mRNA by RT-PCR from perivenous and periportal cell lysates showed that the differential region-specific induction occurs at the pretranslational level in the rat liver [17]. Recently, similar regionally different distribution patterns were seen for expression of other Ah battery genes, *GSTYa* and *ALDH3* [18].

In the present study, we used *in situ* hybridisation to investigate the expression of the *UGT* family 1 genes in rat liver. We used two different UGT1 probes, one which was specific to *UGT1A6* and the other complementary to a fragment of the commonly used exons of the *UGT1* genes, to localise mRNA expression in untreated animals and in animals treated with 3MC and BNF. Our aim was to study hepatic induction of *UGT1* genes, especially *UGT1A6*. Moreover, we wanted to examine whether *UGT1A6* would show a similar region-specific expression pattern as observed previously for *CYP1A1*, *CYP1A2*, *GSTYa* and *ALDH3*.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 190 to 250 g and fed with standard R3 diet (Ewos AB) were treated with 5 or 25 mg/kg of 3MC (Sigma Chemical Co.), 15 mg/kg of BNF (Aldrich Chemical Co. Inc.) or vehicle only (corn oil, 5 mL/kg), by i.p. injection once a day for 3 consecutive days. The experiments were approved by the local committee for animal experiments.

cDNA CLONING

In order to generate the probes for *in situ* hybridisation, rat UGT1A6 cDNA was cloned by the standard RT-PCR method [19]. Total RNA was isolated from Wistar rat liver as described earlier [20]. Primer for the RT reaction, based on the published rat UGT1A6 cDNA sequence [21], was 5′-TCAGTGGGTCTTGGATTTGTGTGATTTC-3′and the 5′-end primer for the PCR reaction was 5′-GAAAG GATGGCTTGCCTTCTTCCTGCT-3′. The PCR product was cloned into pGEM3 vector (Promega) to yield plasmid pOGL 911, and the DNA sequence of the insert was determined with the Sequenase 2.0 sequencing kit (United States Biochemical).

In Situ *Hybridisation*

The probe for the *UGT1A6*-specific sequences was generated from the pOGL 911 plasmid by *Hin*dIII restriction enzyme digestion. The larger of the two DNA fragments obtained was isolated from the agarose gel and religated. This plasmid, pOGL 910, contained the vector and, in addition, a 225-bp DNA fragment from the 5′-end of the rat UGT1A6 cDNA coding sequence. The probe common for all rat UGT1 family genes was prepared by digestion of pOGL 911 plasmid with *Eco*RI restriction enzyme, which yielded two DNA fragments. The larger fragment containing the vector and a 619-bp sequence from the 3′ end of the UGT1A6 cDNA coding sequence was isolated from agarose gel and religated to give plasmid pOGL 909. Figure 1 shows the location of the specific and common region probes.

Single-stranded ^{35}S-labeled antisense (complementary to mRNA) RNA probes were synthetised by using the Riboprobe Gemini system II kit (Promega) as recommended by the supplier and described in detail elsewhere [17]. Human CYP1A1 sense probe was used as a negative, nonrelevant control [22]. *In situ* hybridisation was carried out as described earlier [17]. Briefly, 6-μm thick cryosections were fixed with 4% paraformaldehyde and ice-cold ethanol,

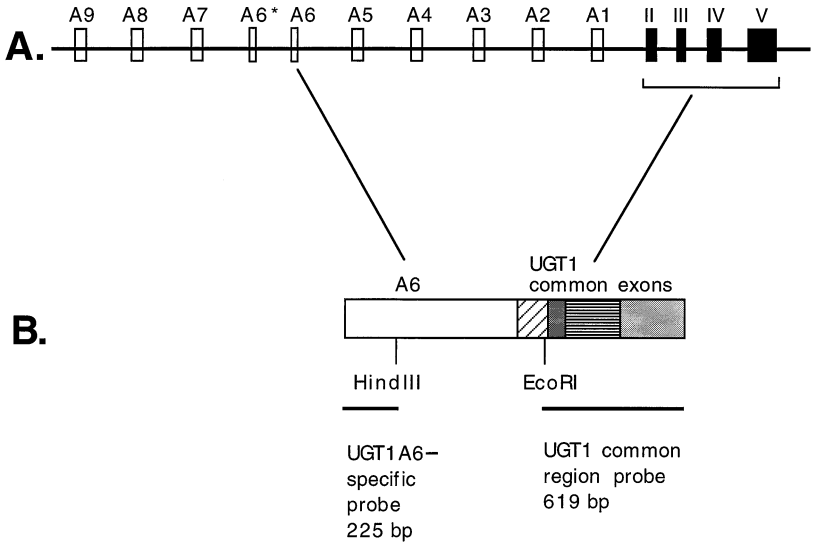

FIG. 1. The schematic structure of the rat *UGT1* gene locus (according to Emi *et al.* [23]) is illustrated in panel A. The boxes indicate exons. The structure of the rat *UGT1A6* (*UGT1*06/UGT1A1*) cDNA is depicted in panel B. The blank box indicates the *UGT1A6*-specific first exon, the hatched and stippled boxes indicate the common exons (II-V) for UGT1 family isoenzymes. *Hin*dIII and *Eco*RI restriction sites used to generate the *in situ* probes are shown in the middle. The location of UGT1A6-specific probe as well as UGT1 common region probe are shown at the bottom.

acetylated with 0.2% acetic anhydride in 0.1 M triethanolamine, pH 8.0, and hybridized for 16–18 hr at 63°. After posthybridisation washes, the slides were dipped in Kodak NTB2 emulsion (Eastman Kodak Co.), exposed for 14 days at +4°, and developed as described [17].

RESULTS

The *in situ* hybridisation experiments were performed with two RNA probes complementary to the rat *UGT1* gene mRNAs. First, we used a 650-bp fragment complementary to the mRNA of the common C-terminal part, thus able to recognise all the family 1 mRNAs. The second probe was specific for *UGT1A6*, consisting of a 225-bp fragment complementary to the mRNA of the specific NH2-terminal region of the UGT1A6 isoform (Fig. 1). In the experiments, uninduced rats and animals treated with 5 mg/kg and 25 mg/kg of 3MC and 15 mg/kg of BNF were used.

In untreated control animals, the probe for the UGT1 common exons showed hybridisation, which was restricted to the perivenous region. The intensity of the signal ranged from a faint signal observed in two rats to a more intense hybridisation observed in one animal (Fig. 2A). In contrast, no hybridisation was observed with the probe specific for the UGT1A6 mRNA in any of the uninduced animals (data not shown).

In the animals treated with 3MC, the lower dose (5 mg/kg) induced mRNA expression detectable with the probe specific for *UGT1A6* in areas surrounding the central veins (Fig. 2B). The treatment also appeared to have induced other forms of the UGT1 mRNAs, as the hybridisation was more intense with the probe common for the UGT1 family (Fig. 2C). After treatment with the higher dose (25 mg/kg) of 3MC, hybridisation spread toward the panacinar pattern, being less intense only in the periportal area (Fig. 2D and 2E).

In contrast to the predominantly perivenous induction observed after treatment with 3MC, 15 mg/kg of BNF resulted in induction of UGT1 mRNA expression in the periportal areas (Fig. 3A and 3B). A similar, but slightly less intense, periportal induction was observed with the *UGT1A6*-specific probe (Fig. 3C). In one animal, faint, ambiguous hybridisation was also observed in the perivenous area (data not shown). Sections hybridised with the control probe (human sense *CYP1A1*) were negative, showing only a faint uniform background (data not shown).

DISCUSSION

In the present study, we investigated expression of *UGT* family 1 genes, in particular *UGT1A6*, in rat liver with *in situ* hybridisation to mRNA. In the three uninduced animals, UGT/A6 expression was undetectable. However, when serial sections were hybridised with the probe against the commonly used exons of the *UGT1* genes, hybridisation ranging from faint to relatively intense was detected in the perivenous region of the liver, indicating that other gene(s) of the *UGT1* family were expressed perivenously in the untreated animals. The presence of UGT1A1 (formerly UGT1B1) mRNA is likely to have contributed to the hybridisation signal intensities observed with the probe complementary to the common exons, because UGT1A1 is known to be constitutively expressed and abundant in rat liver [5].

Earlier results on the expression of the UGT1A6 isoform in untreated rats are conflicting. Emi and coworkers [5] reported previously that expression of the *UGT1A6* (earlier UGT1A1), but not *UGT1A7* (earlier *UGT1A2*), was detected by RT-PCR in the livers of untreated rats. From the various UGT1 forms, UGT1A1 (earlier UGT1B1) was found, however, to be the major form expressed. Interestingly, in a more recent study they found that the levels of UGT1A6 mRNA were almost undetectable in untreated rat hepatocytes in culture [23]. Another study demonstrated that the hepatic expression of *UGT1A6* was low in un-

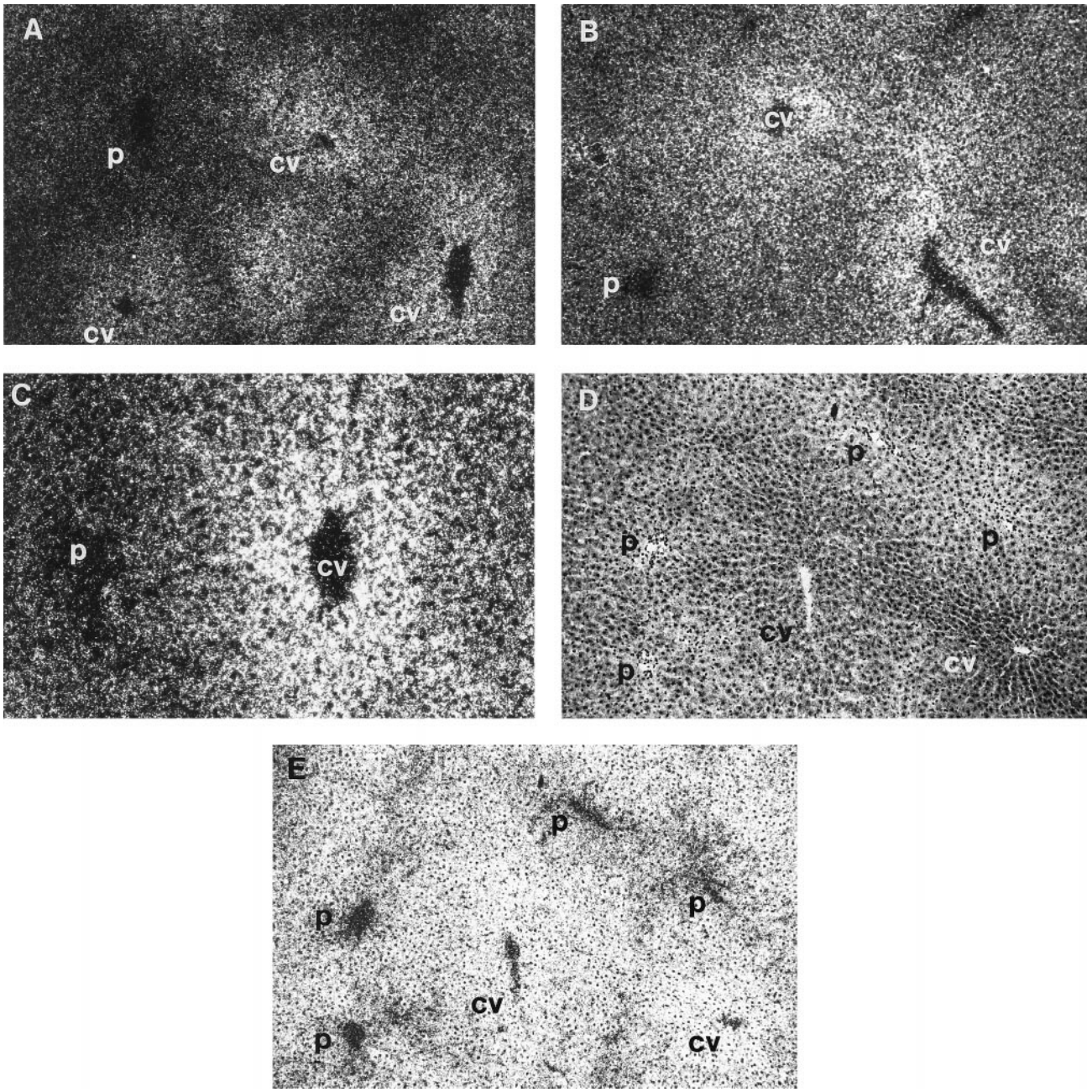

FIG. 2. Localisation of UGT1A6 and other UGT1 family mRNAs by *in situ* hybridisation in liver sections from untreated rats and rats treated with 5 mg/kg or 25 mg/kg of 3MC. Terminal central venules (cv) and portal venules (p) are indicated in each photomicrograph. Dark-field illumination was used except for (D) (bright-field). Original magnifications were 100× (A, B, D and E) and 200× (C). The probe complementary to the common exons of the UGT1 family showed clear hybridisation in the perivenous region in an untreated animal (A). After treatment with 5 mg/kg of 3MC, perivenous induction was observed with the probe specific for UGT1A6 (B) and with the probe for the common exons of the UGT1 family (C). The treatment with a higher dose (25 mg/kg) of 3MC resulted in mRNA expression which was more panacinar, as shown with the probe against the common exons of the UGT1 family (D, bright-field; E, dark-field).

treated animals and markedly inducible by TCDD treatment, whereas in the extrahepatic tissues the expression was predominantly constitutive and only moderately inducible by TCDD [7]. Northern blotting studies indicated no UGT1A6 mRNA in the livers of untreated rats [24]. In mice, Ugt1a6 (corresponding to rat *UGT1A6*) was reported to be constitutively expressed in the livers of uninduced animals, and a 2- to 3-fold increase was observed after TCDD treatment [25]. According to our results, *UGT1A6* expression was undetectable in rat liver by *in situ* hybridisation.

In the present study, the hybridisation with both *UGT1A6*-specific probe and the probe for the commonly used exons of UGT1 showed a perivenous expression pattern in the livers of 3MC-treated rats. A higher dose of 3MC resulted in a more homogeneous expression pattern.

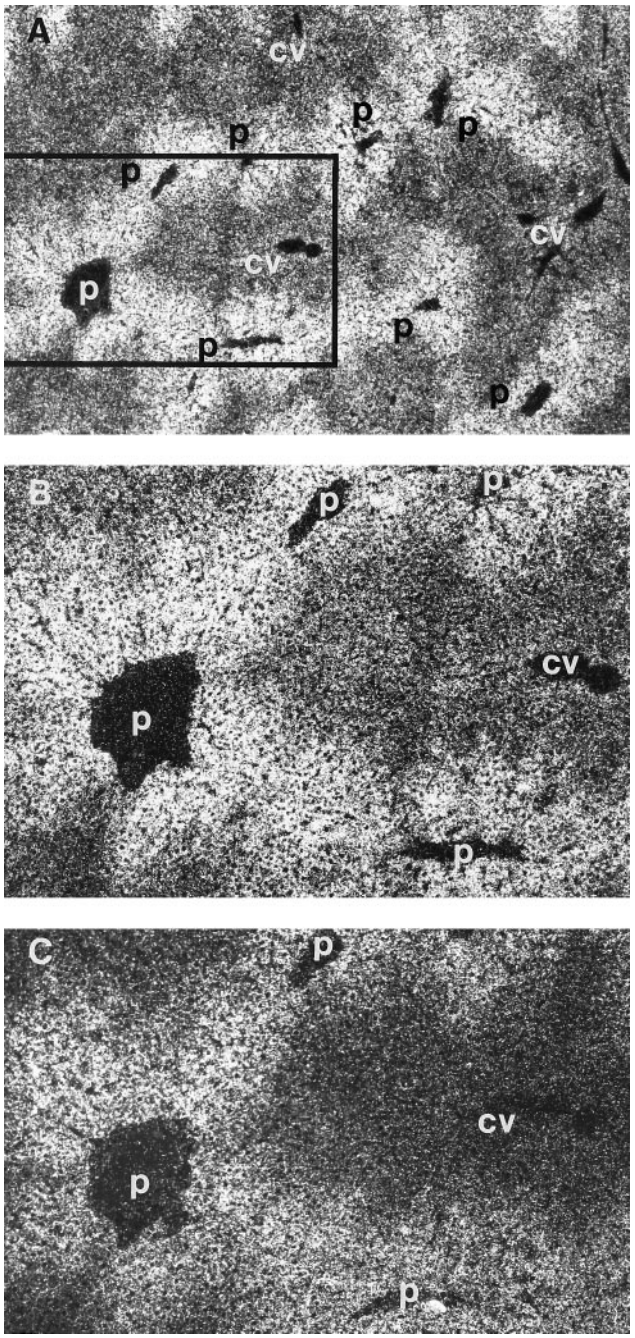

FIG. 3. Treatment with 15 mg/kg of BNF resulted in mRNA induction in the periportal region of the liver as detected by the probe against the common region of the UGT1 family (dark-field). A lower magnification (50×) showing several periportal areas (A). A higher magnification (100×) of the framed area shown in 3A (B). Hybridisation with the probe specific for UGT1A6 showed a similar, but less intense, expression pattern in a serial section (C).

In contrast, in BNF-treated animals, an intense induction in the opposite periportal region was observed. Similar to the control animals, the animals treated with either 3MC or BNF showed hybridisation that was more intense with the probe against the commonly used exons. Both rat UGT1A6 and UGT1A7 isoforms have been reported to be induced by 3MC in the liver [5, 23, 26]. Thus, the more intense hybridisation observed with the probe for the common region of the *UGT1* gene complex, as compared to the *UGT1A6*-specific probe, may partly reflect the expression of *UGT1A7*. Furthermore, as UGT1 forms other than UGT1A6 seemed to be expressed at variable intensities in the uninduced control animals, this "background" expression may also contribute to the hybridisation intensity observed in the induced animals. This probably explains the faint binding observed in the perivenous region in one BNF-treated animal when hybridised with the probe common for the UGT1 family. The probe against the common region was longer (619 bp) than the *UGT1A6*-specific probe (225 bp), which may contribute to the differences observed in the intensities of the hybridisation signal.

We have previously observed inducer-specific regional expression of cytochrome P450 genes *CYP1A1* and *CYP1A2*, glutathione S-transferase Ya (*GSTYa*), and the tumor-associated aldehyde dehydrogenase 3 (*ALDH3*) in the rat liver [17, 18]. Our present data demonstrate that *UGT1A6* shares this inducer-specific difference in regional induction. These results support the notion that within the liver, common factors regulate the regional expression and induction of Ah battery genes. The perivenous induction after the low dose of 3MC is similar to the response observed for other common inducers [15, 16]. The periportal induction observed after treatment with BNF seems to be a unique exception to this pattern. The regulatory factors which could explain the opposite expression patterns after pretreatments by 3MC and BNF are yet to be examined. It has been shown that many of the genes induced by planar aromatic compounds such as 3MC and BNF possess one or several xenobiotic responsive element core consensus sequence(s) in their promoters. The XRE core consensus sequence (GCGTG) has been found in rat *CYP1A1* [27], *UGT1A6* [23], *GSTYa* [28] and NQO_1 [29]. The induction of the rat *UGT1A6* gene by 3-methylcholanthrene has been shown to be dependent on the XRE located between nucleotides -134 and -129 [23]. In addition to XRE, other response elements, such as antioxidant response element (ARE), have been described in some of the Ah battery genes [30].

The spreading of the expression in the liver by the higher doses, as observed here and in our previous studies, indicates that these genes show opposite sinusoidal gradients in sensitivity to the studied inducers. The acinar distribution of the expression of the Ah receptor does not seem to explain the deviant induction pattern observed after BNF treatment, since a consistent perivenous expression pattern of the Ah receptor was observed both in the control animals and after pretreatment with BNF or 3MC [31]. Arnt mRNA was expressed in all liver cells, i.e. no zonation was found [31]. The deviant regional induction pattern by BNF suggests that this inducer may regulate the expression of *UGT1A6* and other Ah battery genes by a mechanism different from the normal Ah receptor-dependent regulatory pathway.

To our knowledge, this is the first study describing the localisation of UGT1A6 mRNA expression in rat liver. Previously, *p*-nitrophenol (4-nitrophenol) and 1-naphthol were used to measure UGT activities assumed to represent the UGT1A6 isoform. According to an earlier report, both enzyme activity (*1*-naphthol) and immunohistochemistry indicated that 3MC-inducible forms of UDP-glucuronosultransferases were preferentially located in centrilobular hepatocytes [32]. Furthermore, in uninduced animals, the *p*-nitrophenol UGT form was found to be expressed predominantly in the perivenous hepatocytes [33, 34]. More recently, a perivenous dominance was reported for UGT activities toward 1-naphthol and bilirubin, suggested to represent UGT1A6 and UGT1A1 isoforms, respectively [35]. However, in addition to UGT1A6, other rat UGT isoenzymes are known to metabolise *p*-nitrophenol and *1*-naphthol [1, 36, 37] and may have contributed to the glucuronidation activities observed in these studies. Finally, in human liver samples, *in situ* hybridisation was recently used to assess the distribution of phenol and bilirubin UGT mRNAs. No significant zonation in the pattern of expression of either of the mRNAs was observed, but both were found to be evenly distributed [38].

At present, little is known about the tissue- and drug-specific induction of UGT1 family enzyme isoforms. As the *UGT1* gene cluster is regulated by several structurally different isoform-specific promoters, more studies are needed to reveal the molecular mechanisms behind the observed induction and regional expression patterns of the genes.

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