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Proximal HNF1 element is essential for the induction of human UDP-glucuronosyltransferase 1A1 by glucocorticoid receptor

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Abbreviations:
UGT, UDP-glucuronosyltransferase
PB, phenobarbital
PBREM, PB response enhancer module
DE, distal element
DEX, dexamethasone
GR, human glucocorticoid receptor
GRE, glucocorticoid responsive element
CAR, constitutive androstane receptor
PXR, human pregnenolone xenobiotic receptor

ABSTRACT

Previous study showed noinduction of the reporter gene (−3174/+14) of UGT1A1 in HepG2 by bilirubin, but induction by dexamethasone (DEX). This induction was enhanced seven-fold by the co-expression of human glucocorticoid receptor (GR) and was inhibited by a GR antagonist, RU486, indicating stimulation by DEX-GR. Meanwhile, we could not detect stimulation by β-estradiol, phenobarbital or rifampicin (RIF) in the presence of GR. We investigated the position playing a role in this induction by GR in the promoter region of UGT1A1 using deletion mutants, and clarified the essential sequence (−75/−63) for the binding site of hepatocyte nuclear factor 1 (HNF1). However, GR did not bind directly to this sequence, because UGT-PE2 did not compete for binding to a glucocorticoid responsive element (GRE) probe in an electrophoretic mobility shift assay (EMSA) method. Labeled [^{32}P]DNA probe of HNF1 binds with nuclear extracts as shown by the EMSA. This shift of the complex of probe–protein was not inhibited by unlabeled GRE but was inhibited by unlabeled HNF1 element. This shift was not influenced by the addition of anti-GR, but was supershifted by the addition of anti-HNF1. GR did not stimulate the induction of HNF1, because we detected no-elevation of the mRNA level of HNF1 by reverse transcription-polymerase chain reaction (RT-PCR). Therefore, the induction of UGT1A1 by DEX-GR did not depend on the elevation of HNF1 but on the interaction of GR with HNF1 or the activation of HNF1 through the transcription of other proteins. Also given the lack of evidence of binding of DEX-GR to HNF1 in the EMSA, the data suggest that the mechanism of DEX-GRE effect on HNF1 is indirect by whatever mechanisms.

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HNF, hepatocyte nuclear factor
BIL, bilirubin
RIF, rifampicin
NE, nuclear extracts
PE, proximal element
PCN, pregnenolone 16α-carbonitrile
RT-PCR, reverse transcription-polymerase chain reaction

1. Introduction

During the metabolism of most xenobiotics, such as drugs, non-nutrient chemicals in foods, and pollutants, phase I metabolizing enzymes functionalize lipophilic drugs by adding active residues such as OH to the drugs, and then phase II metabolizing enzymes conjugate with water-soluble substances, such as UDP-glucuronic acid (UDPGA) for UDP-glucuronosyltransferase (UGT), sulfuric acid for sulfotransferase, and glutathione for glutathione S-transferase, to further functionalize the drugs [1]. These soluble conjugates are excreted in bile and urine by transporters. UGT is the most functional of the phase II enzymes. UGT transfers the glucuronic acid in UDPGA to ligands, such as steroid hormones, catecholamines, and bilirubin (BIL), to make water-soluble glucuronides [2]. The C-terminal portion of UGT also plays a role, such as binding with UDPGA and with membrane. Meanwhile, the N-terminal half of UGT plays roles in ligand recognition, providing substrate specificity and oligomerization [3]. UGT is a membrane-bound enzyme; UGT in microsomal preparations is activated by the addition of certain detergents, such as Triton X-100, suggesting that it internalized to inner microsomal vessels [4–6]. UGT has also species specificity and organ specificity. UGT is mostly found in the liver [7], but is also found in the kidney, gastric duct, lung, and brain [8].

There are many UGT species and they are classified into UGT1 and UGT2 families, with a molecular mass of 50–57 kDa [9]. The human UGT1A subfamily gene is found on chromosome 2, and nine kinds of UGT1 can be made by alternative splicing between several exon 1 and a constant exon 2–5 [10]. Also, the UGT2 family gene is found on chromosome 4 [11]. From a genetic map of the UGT1A subfamily, four pseudo genes of exon 1 have been declared [12]. UGT1A1 is a main enzyme in the conjugation of BIL and a defect in UGT1A1 causes hyperbilirubinemia and jaundice, such as Crigler–Najjar syndrome type II and Gilbert's syndrome. We previously analyzed the promoter region (−3174/+14) of the human UGT1A1 gene in relation to Gilbert's syndrome [13].

Regarding the enhancer region of UGT1A1, which is activated by phenobarbital (PB), a drug for the treatment of jaundice, PB response enhancer element (PBREM) was found at position −3483/−3194 as a functional site [14,15]. This transcriptional stimulation by PB was involved a mechanism like that found in CYP2B [16,17]. In this region of UGT1A1, three elements for binding nuclear transcriptional factors, NR1 (−3472/−3457), gtNR1 (−3366/−3351) and NR3 (−3272/−3257), have been reported and gtNR1 showed strong binding in a gel-shift mobility assay. This PBREM region also contains the xenobiotic response element (XRE) (−3319/−3300) [18], the element for binding pregnenolone xenobiotic receptor (PXR) (−3430/−3285) [19], which is equivalent to SXR (−3424/−3410) [18]. PBREM is activated by nuclear orphan receptor human constitutive androstane receptor (CAR) [20]. The XRE in PBREM reflects the stimulation of the expression of UGT1A1 by 2,3,7,8-tetrachlordibenzo-*p*-dioxin and β-naphthoflavone binding with arylhydrocarbon receptor [18]. Such stimulation of the expression of UGT1A6 by polycyclic aromatic hydrocarbon, 2,3,7,8-tetrachlordibenzo-*p*-dioxin and oltpraz has also been reported [21,22]. It has been suggested that the nuclear receptor CAR/RXR expresses the hepatic effects of PB and is a regulator of BIL clearance [23]. Meanwhile, Sugatani et al. [24] reported that double defects among the three mutations of T-3263G in PBREM $(TA)_7TAA$, and G71R in the exon are associated with mild hyperbilirubinemia (Gilbert's syndrome).

We found two transcriptional regulatory elements in the promoter region (up to −3174) by conducting transient transfection assays; one was the distal element (DE, −1346/−1204) and the other was the proximal element (PE, −97/−54). PE consisted of two regions, an E-box (−88/−79) and an HNF-1 site (−75/−63) [13]. Bosma et al. [25] and Monaghan et al. [26] reported that one-third of patients with Gilbert's syndrome had a TATA box mutation, i.e. $(TA)_7TAA$ instead of $(TA)_6TAA$. Sato et al. [27] compared the promoter activity of the two TATA boxes, did but not find a substantial difference. These results suggest that the TATA box mutation itself is not the major cause of the syndrome Some patients with Gilbert's syndrome had simultaneous mutations in the TATA box and in the coding region (G71R) [27]. Koiwai et al. [28] considered that the high frequency of the syndrome, compared to the frequency of the gene, might depend on the dominant negative phenomenon.

In this report, we show the induction of UGT1A1 expression by GR with dexamethasone.

2. Materials and methods

2.1. Materials

The drugs used were dexamethasone (DEX) and phenobarbital (PB) from Wako Chemicals, rifampisin (RIF) and β-estradiol (β-Est) from Sigma Chemicals, and bilirubin (BIL), pregnenolone 16α-carbonitrile (PCN) and RU486 [29] (mifeprostone) from