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## **Regular Article**

# Effect of Retinoids on UDP-Glucuronosyltransferase 2B7 mRNA Expression in Caco-2 Cells

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**Summary:** Human UDP-glucuronosyltransferase 2B7 (UGT2B7) is one of the major isoforms involved in the glucuronidation of endogenous compounds and xenobiotics. This isoform is the only human UGT shown to glucuronidate retinoids and their oxidized derivatives. In this study, the effects of *all-trans* retinoic acid (*at*RA), 9-*cis* RA, and the RAR agonist TTNPB, on UGT2B7 and UGT2B15 mRNA expression in Caco-2 cells have been examined. Each of these retinoids significantly suppressed UGT2B7 mRNA expression in a concentration-dependent manner with IC<sub>50</sub> values of 3.5, 0.3, and 0.2  $\mu$ M, respectively. However, no inhibition was observed when two other UGTs, UGT2B15 or -1A6, were exposed to *at*RA, 9-*cis* RA, or TTNPB, demonstrating that the inhibitory effect of retinoids might be specific for the UGT2B7 isoform. Further, experiments with oxidized *at*RA derivatives, 4-OH-*at*RA, 4-oxo-*at*RA, and 5,6-epoxy-*at*RA showed that these RA degradation products have no inhibitory effect on UGT2B7 mRNA expression. These data lead us to hypothesize that biologically active forms of RA suppress the expression of UGT2B7 in intestinal cells. This information provides a new pathway by which retinoids may enhance their own toxicity when accumulated in the body at pharmacological concentrations by down-regulating the enzymes involved in their biotransformation into soluble derivatives.

## Keywords: UDP-glucuronosyltransferase (UGT); retinoid; suppression; Caco-2 cells

### Introduction

Retinoids are potent regulators of a variety of physiological processes including development, cell differentiation and proliferation, apoptosis, homeostasis, and reproduction.<sup>1)</sup> all-trans retinoic acid (*a*tRA) and 9-*cis* retinoic acid (9-*cis* RA) have been recognized as important signaling molecules and physiological ligands for the several nuclear receptors.<sup>2)</sup> Retinoids exert most of their function via interaction with two types of nuclear retinoid receptors, the retinoic acid receptors (RAR) and the retinoid X receptors (RXR), which act as ligand-activated transcription factors controlling the expression of a number of target genes.<sup>3,4)</sup> RARs can bind both *at*RA and 9-*cis* RA, but RXRs can only bind 9-*cis* RA.<sup>5)</sup> RXRs are also known to be a heterodimer partners for a number of nuclear receptors.

Although RARs and RXRs are the major regulators of retinoid-mediated pathways, other pathways have also been found to be activated by retinoids. *at*RA has been found to down-regulate the transcriptional activities of AP-1 transcription complex.<sup>6</sup> Furthermore, retinoids have been shown to alter the activity of MAP kinase pathways.<sup>7</sup> However, attention needs to be paid to the cells and genes involved, since all these signaling pathway have been shown to be cell-type dependent and target gene selective.

Interactions between retinoids and drug metabolizing enzymes have been demonstrated previously.<sup>8)</sup> This is especially true in relation to cytochrome P450s (CYPs),

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This work was supported by Tobacco Settlement funds from UAMS and a grant from the National Institutes of Health (DK60109) to A.R-P. Abbreviations: 4-OH RA, 4-hydroxy; AP-1, activator protein-1; *at*RA, *all trans* retinoic acid; CYP, cytochrome P450; FXR, farnesoid X receptor; GR, glucocorticoid receptor; LCA, lithocholic acid; Oct-1, octamer binding protein-1; RAR, retinoic acid receptor; RXR, retinoid X receptor; TR, thyroid receptor; TTNPB, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetra-methyl-2-naphthylenyl)-1-propenyl]benzoic acid; UGT, UDP-glucuronosyltransferase.

which are regulated by retinoids and also actively metabolize them into their oxidized derivatives. The total number of CYPs that are regulated *in vivo* by retinoids is not known; however, it has been suggested that CYP1A1, CYP3A and, most importantly, CYP26 and its variants are induced by and in turn metabolize these compounds.<sup>9–11)</sup> These enzymes are differentially expressed in various tissues, with the liver, intestine, and brain being the sites of highest expression.<sup>12)</sup> The role of the intestine in the metabolism of retinoids is clearly recognized.<sup>13)</sup> Dietary retinol can be oxidized in enterocytes to form *at*RA, which can be further metabolized by CYP26 to form various oxidized derivatives.<sup>14)</sup> It has also been shown that intestinal cells are the site of both CYP gene regulation and the metabolism of *at*RA.<sup>10,11,14)</sup>

UDP-Glucuronosyltransferases (UGTs) are quantitatively the most abundant Phase II drug metabolizing enzymes and are involved in the biotransformation of the greatest number of endogenous and exogenous compounds of any drug metabolizing enzyme. However, studies of UGTs as enzymes responsible for the biotransformation of retinoids are very limited. Our laboratory has carried out studies with recombinant human human UGTs that demonstrated that UGT2B7 is the only isoform able to glucuronidate atRA, 4-hydroxy (OH) atRA, 4-oxo atRA, and 5,6-epoxy atRA with activities similar to those found in human liver microsomes.<sup>15)</sup> We proposed that UGT2B7 might be involved in controlling intracellular levels of retinoids and their biological fate. Our later publication confirmed the role of UGT2B7 in retinoid metabolism and further showed that both liver and all segments of intestine actively glucuronidate atRA, although the rate of atRA glucuronidation in liver was comparatively higher than that of intestine.<sup>16)</sup> It was calculated that, under normal physiological conditions, glucuronidation of retinoids in humans occurs at a concentration of 5–17 nM.<sup>17)</sup>

In this report, a continuation of our previous retinoidrelated metabolic studies,<sup>15,16)</sup> we have begun investigating of the role of retinoids in the regulation of UGT2B7 expression. In order to examine the effect of retinoids on this isoform, Caco-2 and HepG2 cells were exposed to increasing concentrations of biologically active retinoids, including atRA, 9-cis RA, and 13-cis RA. These experiments showed dramatic suppression of UGT2B7 mRNA expression in the presence of these retinoids in Caco-2 but not HepG2 cells. However, similar experiments using the oxidized derivatives of atRA showed no suppressive effects of these catabolic products. To clarify whether this down-regulation is isoform specific, we carried out similar experiments testing the effects of the active retinoids on the expression of two other human UGT isoforms, UGT1A6 and -2B15. These compounds had no suppressive effect on the expression of either isoform; however, the levels of UGT2B15 were slightly

up-regulated.

Based on these data, we hypothesize that exposure to pharmacological concentrations of biologically active retinoids induces a rapid down-regulation of UGT2B7 expression in intestinal but not hepatic cells. The different pattern of regulation between these two tissues may be an indication of tissue-specific mechanisms of regulation of UGT2B7 expression and is shown to be specific to this retinoid glucuronidating enzyme. The possible mechanism for this suppression is discussed here.

#### Methods

**Materials:** *all-trans* retinoic acid, *9-cis* RA, 4-OH *at*RA, 4-oxo *at*RA, 5,6-epoxy *at*RA, and (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetra-methyl-2-naphthylenyl)-1-propenyl] benzoic acid (TTNPB) (chemical structures are shown in **Fig. 1**) were all purchased from Sigma-Aldrich (St. Louis, Mo, USA). All procedures using retinoids were performed under yellow light to minimize photoisomerization.

**Cell culture:** Caco-2 (ATCC HTB-37) and HepG2 (ATCC HB-8065) cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and kept in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>-air. Chemicals were dissolved in DMSO (Sigma-Aldrich) and cells we treat at indicated concentrations.

**Cell viability assay:** Cell viability was determined by Trypan Blue exclusion. Caco-2 cells were plated in 6well plates at a density of  $5 \times 10^5$  cells per well and cultured for 24 hr. *at*RA, 9-*cis* RA and TTNPB at concentrations corresponding to those used in UGT mRNA expression level studies were then added. After an additional 48 hr incubation, the cells were released from the plates with trypsin/EDTA (Invitrogen), incubated with 0.4% Trypan Blue (Mediatech, Herndon, VA, USA) for 5 min, and counted using a hemocytometer. Since the monolayers were not washed prior to trypsin-EDTA treatment or scraping of adherent cells, cells that detached during incubation with treated chemicals are detected.

**RNA preparation:** Total RNA was isolated from cell cultures using a phenol and guanidine isothiocyanate RNA extraction method (Trizol; Invitrogen), following the instructions of the supplier. To avoid any contamination of the RNA by genomic DNA, DNase treatment was performed using RQ1 RNase-Free DNase (Promega, Madison, WI, USA). cDNA was synthesized by mixing 1  $\mu$ g of total RNA from each sample with 100 pmol random hexamer primers in 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, containing 100 U M-MLV reverse transcriptase, 20 U RNase inhibitor, and 1 mM each dNTP (Promega) in a total volume of 20  $\mu$ l. The samples were incubated at 37 °C for 60 min



Fig. 1. Chemical structures of retinoids used.

 Table 1. PCR primers and conditions for semi-quantitative analysis of UGT isoforms

Target Transcript	F: Forward primer 5'-3' R: Reverse primer 5'-3'	Annealing Temp (°C)	Amplicon Size (bp)	No. of cycles on Caco-2 cDNA
UGT2B7	F: ttgcaccaggatgtctgtgaa R: taccctctccatgaaagtcatttga	56	643	32
UGT2B15	F: gactgtgttgacatcttcggcttct R: ccagtagctcaccacagggattaag	54.7	326	32
UGT1A6	F: gaagaaccgttaccaatcat R: gaccattgatcccaaaga	55.4	670	35
GAPDH	F: acccactcctccacctttg R: ctcttgtgctcttgctggg	64	178	25

Nucleotide sequences are reported for primer pairs used in RT-PCR analysis of UGTs expressions in Caco-2 cells.

and then heated at 95 °C for 5 min to inactivate the reverse transcriptase. The reaction mixture was diluted to 100  $\mu$ l with sterile diethylpyrocarbonate-treated H<sub>2</sub>O.

**RT-PCR analysis:** Primers for UGT2B7, UGT2B15, UGT1A6 and GAPDH are described in **Table 1**. PCR reactions were performed as follows: a 10  $\mu$ l aliquot of cDNA was added to a reaction mixture containing 10 mM Tris-HCl buffer (pH 8), 20 mM KCl, 0.1% Triton X-100; 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 50 pmol of each primer and 2 units of Taq DNA polymerase (Promega), in a total volume of 50  $\mu$ l. Amplification of the ubiquitously expressed GAPDH cDNA was performed under the same conditions in separate experiments. The specificity of all primer pairs was confirmed through sequencing of the PCR products. For each primer pair, we performed PCR with different cycle numbers and plotted these data to form a standard curve. We chose the cycle that was found to be within the nonsaturable range of amplification for use in further experiments. All other conditions were kept consistent unless significant changes in mRNA level were observed. The PCR products were resolved by 2% agarose gel electrophoresis and detected with ethidium bromide. The bands were visualized under UV light and photographed by a computed-assisted camera. Quantification of each band was performed by densitometric analysis by using NIH Image software (NIH, Bethesda, MD).

**Statistical methods:** GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA, USA) was used for data analysis. The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Measurements that vary significantly from the control are indicated \*, p < 0.05; \*\*, p < 0.01).

#### Results

**Effect of** *at***RA**, *9-cis* **RA**, and **TTNPB on UGT2B7 mRNA expression:** To determine whether *at*RA, a substrate for UGT2B7, can affect UGT2B7 gene expression in cultured cell systems, Caco-2 and HepG2 cells



**Fig. 2.** Effect of retinoids on UGT2B7 mRNA expression in Caco-2 cells Caco-2 cells were treated with *at*RA, *9-cis* RA and TTNPB at the indicated concentrations for 48 hours. Total RNA was isolated and RT-PCR analysis was performed to evaluate the expression level of UGT2B7 mRNA. The bar graphs show the relative expression of UGT2B7 mRNA (normalized to GAPDH expression). The IC<sub>50</sub> values for retinoid inhibition of UGT2B7 mRNA expression were calculated. The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Measurements that vary significantly from the control are indicated (\*, p < 0.05; \*\*, p < 0.01).

were treated for 48 hr with various concentrations of atRA, and 9-cis RA, as indicated in Figure 2. Caco-2 cells were also treated with low concentrations of the RAR agonist, TTNPB, under the same conditions. After treatment, total RNA was isolated from the cells and UGT2B7 mRNA levels were analyzed by semi-quantitative RT-PCR. atRA treatment of Caco-2 cells resulted in a significant dose-dependent decrease in UGT2B7 mRNA levels as compared with vehicle-treated (0.1% DMSO) cells (Fig. 2), with an IC<sub>50</sub> about 3.5  $\mu$ M. Incubation of Caco-2 cells with 9-cis RA had a similar effect on UGT2B7 mRNA levels but the decrease began at a much lower concentration, resulting in an IC<sub>50</sub> of is 0.2  $\mu$ M, significantly lower than that of *at*RA. The synthetic *at*RA analog, TTNPB, had a similar effect on UGT2B7 mRNA levels, with a low IC<sub>50</sub> of 0.3  $\mu$ M. These data showed that the RAR agonist TTNPB is the most potent suppressor of UGT2B7 mRNA expression in Caco-2 cells. On the other hand, in parallel experiments carried out in HepG2 cells under identical conditions, UGT2B7 mRNA expression was not affected by either retinoid or TTNPB (data not shown).

Effect of oxidized derivatives of retinoic acid on UGT2B7 mRNA expression: *at*RA is metabolized *in vivo* by CYP26 and others to form 4-OH *at*RA, 4-oxo *at*RA, 5,6-epoxy *at*RA, all of which are generally accepted as inactive metabolites. *14,18*) To test the response of

UGT2B7 gene expression to these oxidized retinoids, Caco-2 cells were treated with 4-OH *at*RA, 4-oxo *at*RA, and 5,6-epoxy *at*RA at the same concentrations used for *at*RA. UGT2B7 mRNA levels were evaluated using semiquantitative RT-PCR and normalized to GAPDH mRNA expression. As the data in **Figure 3** show, neither 4-OH *at*RA, 4-oxo *at*RA, nor 5,6-epoxy *at*RA induced or suppressed UGT2B7 mRNA levels, whereas *at*RA consistently suppressed UGT2B7 mRNA expression. These data further demonstrate that UGT2B7 mRNA suppression is specific for *at*RA and *9-cis* RA, which are biologically active, while the three oxidized metabolites, the inactive intermediates of the retinoid detoxification pathway, have no visible effects.

Effect of retinoids on UGT1A6 and UGT2B15 mRNA expression: To answer the question of whether retinoid mediated suppression of UGT2B7 is an observation related specifically to this isoform, the mRNA expression of UGT2B15 and UGT1A6 mRNA was examined under conditions identical to those used for UGT2B7 (Fig. 4). Treatment with *at*RA, 9-*cis* RA, and TTNPB had no effect on UGT1A6 mRNA expression except at the highest concentrations used, which corresponds with the decreases in cell viability seen in Figure 5. On the other hand, treatment with *at*RA and TTNPB significantly increased the mRNA expression of UGT2B15 up to concentrations of 0.1  $\mu$ M and 10  $\mu$ M,



**Fig. 3.** Effect of *at*RA and its oxidized derivatives on UGT2B7 mRNA expression in Caco-2 cells Caco-2 cells were treated with each retinoid at the concentrations shown in the figure  $(0.1-50 \mu M)$  for 48 hours. Total RNA was isolated and RT-PCR analysis was performed to evaluate mRNA expression level of UGT2B7. The results are shown as the relative mRNA expression (normalized to GAPDH expression). The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Measurements that vary significantly from the control are indicated (\*, p < 0.05; \*\*, p < 0.01).



Caco-2 cells were treated with *at*RA at the indicated concentration for 48 hours. Total RNA was isolated and RT-PCR analysis was performed to evaluate UGT2B15 and UGT1A6 mRNA expression levels. The results are shown as relative mRNA expression (normalized to GAPDH expression). The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Measurements that vary significantly from the control are indicated (\*, p < 0.05; \*\*, p < 0.01).

respectively. No effect of *9-cis* RA on UGT2B15 mRNA expression was observed under the current experimental conditions.

**Effect of retinoids on Caco-2 cell viability:** Retinoids are also known to be toxic to cells at pharmacological concentrations. To exclude the possibility that these retinoids might disrupt the Caco-2 cell membrane, cell viability was assayed using a Trypan Blue exclusion assay. As shown in **Figure 5**, only the highest concentrations

of retinoids, which were far above pharmacological conditions, decreased Caco-2 cell viability. All other concentrations of retinoids, including those that significantly suppressed UGT2B7 mRNA expression or induced UGT2B15 mRNA expression, were well-tolerated by the cell.

### Discussion

In these studies, we report that human UGT2B7



Fig. 5. Effect of retinoids on Caco-2 cell viability

Caco-2 cells were plated in 6-well plates at a density of  $5 \times 10^5$  cells per well and cultured for 24 hr. *at*RA, *9-cis* RA, and TTNPB at were then added the indicated concentrations and cells were incubated for an additional 48 hr. The percentage viability was determined by Trypan Blue exclusion. The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Measurements that vary significantly from the control are indicated (\*, p < 0.05; \*\*, p < 0.01).

mRNA expression is negatively regulated by the biologically active retinoids, *at*RA and *9-cis* RA, and their synthetic derivative, TTNPB, in Caco-2 cells. This dosedependant decrease in UGT2B7 mRNA levels seems to be specific to this UGT isoform, since it is not observed with the two other UGT isoforms tested, UGT1A6 and -2B15.

The different effects of *at*RA, *9-cis* RA and TTNPB on UGT2B7 mRNA expression could be due to their different functions in the cell. *9-cis* RA is a unique ligand for the retinoid X receptor (RXR) and *9-cis* RA and *at*RA are both ligands for RAR. As for TTNPB, it is a strong agonist for RAR. These biologically actively compounds are involved in different signaling pathways. The receptors activated by these ligands could effect the regulation of totally different sets of genes, and therefore, the net effect of this pathway on UGT2B7 expression could be different. Although similar, it is unlikely that their effects on 2B7 gene regulation would be identical.

Three P450-oxidized catabolic products of atRA, 4-OH-, 4-oxo-, and 5,6-epoxy-RA, showed no effect on the mRNA expression of this isoform, One concern that needs to be taken into consideration is whether the oxidized compounds can cross the cell membranes in Caco-2 cells. However, there is evidence that these oxidized RAs can cross the membranes of Cos-7 cells<sup>19</sup> and that oxidized metabolites of linoleic acids can cross in both colon cell lines, Caco-2 and HCT-116.<sup>20)</sup> Therefore, it is reasonable to make the assumption that oxidized retinoids can also cross in Caco-2 cells, but are not able to cause the suppressive effects, which are more likely specific to the biologically active retinoids. The lack of suppression seen with these compounds provides important proof that the suppressive effects are specific to biologically active retinoids and are not simply artifacts of the system.

Additionally, no suppressive response to retinoids was observed in HepG2 cells, indicating that the regulation of this isoform is tissue-specific with the major site of this action being the intestine: the first organ exposed to dietary retinoids and orally administered retinoid-based drugs. It is recognized that UGTs are expressed in a tissue-specific manner. Therefore, we can speculate that there are additional factors involved, such as the existence and/or regulation of different nuclear receptors or signaling pathways. These could vary in the liver and colon and differentially mediate the UGT regulation, including the observed suppression of UGT2B7. It is also possible that other factors that we have not recognized, can be involved (i.e., the presence of the retinoid acid binding protein (RABP), etc).

A majority of atRA enters the body in the form of vitamin A, or other dietary products<sup>13)</sup> and enters the organism via the intestinal tract. Our previous studies have provided information that retinoids are actively metabolized by UGT2B7 in the human intestinal tract.<sup>16)</sup> The product of this biotransformation, retinoyl- $\beta$ -glucuronide, is found in the liver, kidney, intestinal mucosa, and several other tissues. However, of all these tissues, the intestinal mucosa seems to be the most active in synthesizing and retaining retinoyl- $\beta$ -glucuronide *in vivo*<sup>21)</sup>.

It is recognized that, in addition to a large number of beneficial processes initiated by retinoids, this class of compounds can also be responsible for many deleterious effects on the body, when they either exceed physiological levels or are present in inadequate amounts.<sup>22)</sup> Because of this, one can easily speculate on the consequences of the dramatic decrease of UGT2B7 expression seen in Caco-2 cells in response to retinoids. Most obviously, since UGT2B7 is involved in the elimination of excess retinoids and/or the termination of their biologicall activity, the suppression of this enzyme could result in the accumulation of excess retinoids, leading to toxicity in the cell and organism and an increase in teratogenesis and hypervitaminosis A syndrome.<sup>23,24</sup> UGT2B7 is also an indispensable component of the overall detoxification network. In addition to its function in modulating the activity of biologically active compounds, it also plays an essential role in controlling steady-state concentrations of compounds such as fatty acids, bile acids, prostaglandins, steroid hormones, and retinoids,<sup>25)</sup> which are ligands for nuclear receptors, such as RXR and RAR, and other signaling pathways. A lack of UGT2B7 would allow for prolonged interactions of active retinoids with nuclear receptors and signaling pathways, potentially resulting in non-specific activation of a variety of genes. Therefore, impairment of this isoform's activity could have far reaching effects on the homeostasis of many nuclear receptor-dependant systems throughout the organism.

In addition, a decrease in UGT2B7 could result in insufficient biosynthesis of retinoyl- $\beta$ -glucuronide, which may itself possess biologically activity. In fact, it has been shown that retinoyl- $\beta$ -glucuronide is more active than atRA or retinol in modulating differentiation in certain cell types and inducing differentiation of human promyelocytitic leukemia cells (HL-60) without being hydrolyzed to retinoic acid.<sup>26)</sup> In cell differentiation, retinoyl- $\beta$ -glucuronide might act as a carrier to transfer the retinoyl moiety to appropriate protein, as a transport agent from the cytosol to the nucleus, or as a slow-release precursor of retinoic acid.<sup>21)</sup> On the other hand, retinoyl- $\beta$ -glucuronide might serve a protective role against the teratogenic events induced by retinoic acid. Retinoyl- $\beta$ -glucuronide is much less teratogenic than atRA and crosses the placenta to the fetus at a much slower rate.<sup>27)</sup> Therefore, suppression of the formation of this compound could result in a decrease in the bioavailability of this important regulator.

From these results, we can only speculate on the putative mechanism of the observed UBT2B7 mRNA suppression in response to biologically active retinoids. Since all the major physiological effects of retinoids are mediated by nuclear receptors, one has to acknowledge the possible role of the nuclear receptors in this process. Several recent studies have demonstrated that in some cases ligand-activated hormone and orphan receptors, such as the glucocorticoid receptor (GR), thyroid receptor (TR), and farnesoid X receptor (FXR), can suppress gene transcription, producing deleterious effects,<sup>28-31)</sup> and mechanisms accounting for this negative regulation have been proposed. A picture emerges of negative transcriptional regulation by nuclear receptors involving two basic mechanisms. One major form of this regulation occurs as a result of interaction between nuclear receptors and other transcriptional factors, including AP-1 and Oct-1.<sup>32)</sup> Ligand-activated GR and retinoic acid receptor were shown to interact with AP-1 in a DNA-independent manner and repress the induction of gene expression by AP-1.<sup>33,34)</sup> Another form based on the binding of ligand activated nuclear receptors to specialized negative response elements has been observed for GR,<sup>31</sup>) TR,<sup>28</sup>) the Vitamin D receptor,<sup>35)</sup> and most recently FXR.<sup>29,30)</sup> Growing evidence has been collected that ligand-activated FXR is involved in the negative regulation of a number of target genes encoding drug transporters, and drug

metabolizing enzymes.<sup>29,30)</sup> The most significant examples are P450 7A1<sup>36)</sup> and cholesterol 12 $\alpha$ -hydroxylase.<sup>37)</sup> Evidence has also been presented showing suppression of apolipoprotein A-I by activated FXR binding to negative FXR response elements.<sup>29)</sup>

The phenomenon of nuclear receptor mediated negative transcriptional regulation has also been observed for UGTs.<sup>30</sup> These studies from our laboratory revealed that lithocholic acid (LCA) dramatically decreased UGT2B7 mRNA expression in Caco-2 cells.<sup>30)</sup> We demonstrated that the mechanism of this negative regulation is mediated by the interaction of LCA-activated FXR with a specific negative FXR response element in the UGT2B7 gene promoter. We cannot currently conclude that UGT2B7 gene suppression by retinoids and, consequently, the metabolism of these compounds in intestinal cells, is regulated through a retinoid-specific receptor, such as RAR or RXR, but the fact that the RAR agonist, TTNPB, shows the highest potency of UGT2B7 gene suppression does suggest that this regulation might be at the transcriptional level. Experiments are in progress to identify the exact mechanism involved.

In summary, the present studies provide another novel example of the suppression of UGT2B7 in response to pharmacological concentrations of its substrate, in this case biologically active retinoids. It is important to emphasize that the inhibition of the constitutively expressed human UGT2B7, an isoform that is recognized as one of the most effective detoxification enzyme widely expressed in the body, may significantly contribute to the frequently observed toxicity of retinoids.

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