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IN2001 Regulates *CYP3A4* Gene Expression in Hep G2 Cells

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ABSTRACT **: Cytochrome P450 3A4 (CYP3A4) is the most abundant CYPs in human liver, comprising approximately 30% of the total liver CYPs contents and is involved in the metabolism of more than 60% of currently used therapeutic drugs. The expression of *CYP3A4* is induced by a variety of structurally unrelated xenobiotics including the antibiotic rifampicin and endogenous hormones, and might be mediated through steroid and xenobiotic receptor (SXR) system. The molecular mechanisms underlying regulation of *CYP3A*4 gene expression have not been understood. In order to gain the insight of the molecular mechanism of *CYP3A4* gene expression, study has been undertaken to investigate if the histone deacetylation is involved in the regulation of *CYP3A4* gene expression by proximal promoter or not. Also SXR was investigated to see if they were involved in the regulation of *CYP3A4* proximal promoter activity. HepG2 or Hepa-I cells were transfected with a plasmid containing~1kb of the *CYP3A4* proximal promoter region (–863 to +64 bp) cloned in front of a reporter gene, luciferase, in the presence or absence of SXR or hER. Transfected cells were treated with CYP3A4 inducers such as rifampicin, PCN and RU 486, or with estradiol, in order to examine the regulation of *CYP3A4* gene expression in the presence or absence of trichostatin A (TSA). In HepG2 cells, CYP3A4 inducers and estradiol increased significantly the luciferase activity by *CYP3A4* proximal promoter, only when TSA was co-treated after SXR cotransfection. In the case of Hepa-I cells, CYP3A4 inducers and estradiol increased modestly the luciferase activity when TSA was co-treated, but this increment was not enhanced by SXR cotransfection in contrast to HepG2 cells. Taken together, these results indicated that the inhibition of histone deacetylation was required to SXR-mediated increase in *CYP3A4* proximal promoter region when rifampicin, or PCN was treated. Further a trans-activation by SXR may demand other species-specific transcription factors.**

Key words : ***CYP3A4*, PCN, Rifampicin, RU486, SXR, HDAC, IN2001, Hepa I**

Introduction

The CYP3A4 is involved in the metabolism of an extensive range of xenobiotics and endogenous substrates. The expression of *CYP3A4* is also induced by a variety of structurally unrelated xenobiotics including the antibiotic rifampicin and endogenous hormones and this induction is mediated by SXR. The molecular mechanism underlying regulation of *CYP3A4* gene expression has been studied by several groups. According to the literatures, the minimal DNA element for SXR interaction, ER-6 or DR-3, induced the SXR-mediated response by CYP3A4 inducers and *CYP3A4* proximal promoter associated with an enhancer element, *XREM*, also induced that. Goodwin *et al.* reported that $p(ER6)_3$*-tk-Luc* exhibited a 2- to 4-fold activation by rifampicin in the presence of cotransfected *SXR* and 5'-flanking regions of *CYP3A4*, such as –1252 to +53 bp and –362 to +53 bp, were not responded to rifampicin in HepG2 cells (Goodwin *et al.*, 1999). When 5'-flanking region of *CYP3A4* (–362 to +53 bp) was fused with *XREM* region (–7836 to –7607 bp), however, rifampicin exhibited a 40- to 50-fold induction with *SXR* cotransfection (Goodwin *et al.*, 1999; Luo *et al.*, 2002). Hashimoto *et al.* demonstrated that CAT activities were scarcely detected, when HepG2 cells were transfected with a few CAT-gene-containing plasmids such as *p–1105 CAT* (–1105 to +71 bp) and *p–362 CAT* (–362 to +71 bp), and thus they converted the promoter region of *CYP3A4* gene into the SV40 promoter and then detected activities of CAT. The activity, however, was higher in *p–362 CAT* (–362 to –94 bp) and lower in *p–1105 CAT* (–1105 to –94 bp) than control vector. (Hashimoto *et al.*, 1993).

Post-translational modification of the histones of chromatin has a fundamental role in regulating gene expression. The amino-terminal tails of the histones are subject to post-translational modification by acetylation of lysine, methylation of lysine and arginine, phospho-

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rylation of serine and ubiquitination of lysine (Jenuwein *et al.*, 2001; Zhang *et al.*, 2001; Agolioti *et al.*, 2002; Richards *et al.*, 2002). Enzymes involved in theses epigenetic events include histone deacetylases (HDACs) and histone acetyl transferases (HATs). These enzymes determine the pattern of histone acetylation, which together with other dynamic sequential post-translational modifications might represent a code that can be recognized by non-histone proteins forming complexes involved in the regulation of gene expression. Hyperacetylation of histones is associated with transcriptional activity or the potential for activity, whereas histone hypoacetylation is correlated with transcriptionally silent chromatin and heterochromatin (Wu *et al.*, 1997). HDACs are also involved in the reversible acetylation of non-histone proteins (e.g. p53, tubulin and various transcription factors) and this acetylation regulates the functional activity of these proteins.

The mechanism of gene repression or activation is not well understood and might result from either direct or indirect effects of histone acetylation or from the increase in acetylation of proteins other than histones (e.g. transcription factors). A few hypothetical models for the regulation of transcriptional activity by protein acetylation were suggested by other report (Pazin *et al.*, 1997). In the first model, modification of charged lysine residues in the histone tails by acetylation increases the access of transcription factors to the DNA template. While there is not a perfect correlation between acetylation and transcriptional activity, it is possible that the changes in core histone structure that occur upon acetylation may facilitate transcription but not be sufficient to induce transcription of an inactive gene. In fact, core histone acetylation could be involved at the level of transcriptional competence (i.e., the facility by which a gene can be transcribed) rather than transcriptional induction. In such instances, core histone acetylation may contribute to the establishment and/or maintenance of a transcriptionally competent state in which the chromain is in an altered ("open") conformation. In the second model, the specific acetylation of a lysine residue (of a histone or non-histone protein) might create a recognition site for the binding of another factor and then transduce a signal. The third model postulates that acetylation of non-histone proteins, such as a transcription factors of the basal transcription machinery, might affect the activity of the protein. These models are not mutually exclusive and may partially overlap.

Transcriptional repression by a sequence-specific DNA-binding factor can be mediated by the recruitment of a deacetylase to the promoter region (Fig. 5). HDACs do not bind directly to DNA but are recruited to DNA by protein complexes that differ in their subunit composition (Khochbin *et al.*, 2001). For example, HDACs 1 and 2 have been found in complexes with Sir 3, NuRD (Nucleosome remodeling and deacetylation) and N-coR (nuclear receptor co-repressor). The N-terminal tails of HDACs 4, 5 and 7 interact with myogenic transcription factor-2, which is involved in muscle differentiation (McKinsey *et al.*, 2001). HDACs can be inhibited by a structurally diverse group of small molecules. HDAC inhibitors can be divided into several structural classes, including hydroxamates, cyclic peptides, aliphatic acids, benzamides and electrophilic ketones (Marks *et al.*, 2003). Trichostatin A (TSA) was the first natural product hydroxamate to be discovered to inhibit HDACs directly (Yoshida, *et al.*, 1990). HDAC inhibitors activated or repressed the expression of a small number of genes, that is 2-5% of the expressed genes (Butler *et al.*, 2002; Suzuki *et al.*, 2002; Rogione *et al.*, 2001; Van *et al.*,

The regulation of native *CYP3A4* proximal promoter activity was not understood. Recently it has been reported that histone deacetylation is related with the expression of *CYP1A* and *CYP1B* (Nakajima *et al.*, 2003). Therefore the present study was aimed at investigating the role of the histone deacetylation on the regulation of *CYP3A4* gene expression by proximal promoter in the presence or absence of SXR. In addition, whether the native *CYP3A4* proximal promoter was regulated by endogenous hormone specific receptor, hER, was investigated. For this study, hepatic cells were chosen because CYP3A4 was mostly expressed in the liver and tissue-specific transcription factors might be related with *CYP3A4* regulation. Moreover, two kinds of cells were used to compare the response in different species. HepG2 cells or Hepa-I cells were transfected with a plasmid containing~1kb of the *CYP3A4* proximal promoter region (–863 to +64bp) which was cloned in front of luciferase gene as a reporter, in the presence or absence of SXR. Transfected cells were treated with various chemicals such as rifampicin, PCN and RU 486, or with estradiol, in the presence or absence of TSA and then cells were lysed and assayed for the luciferase activity using luciferin.

Materials and Methods

Materials

Rifampicin (Rif), RU486 (RU), pregnenolone 16α-carbonitrile (PCN), HC-toxin was purchased from Sigma-Aldrich (St. Louis, MO). Agarose was purchased from FMC and LipofectAMINE from Gibco-BRL and Hind III, *Sma I* from BMS, while pGL3 basic vector and luciferase assay system were ordered from Promega. Trichostatin A was provided from Dr. Dae-kee Kim, Ewha Womans University, Korea. 4-Dimethylamino-*N*-[4-(2-hydroxycar-bamoyl-vinyl) benzyl]benzamide (IN2001) and IN2002 were synthesized.

Plasmids

ph*CYP3A4*-Luc

The chimeric *CYP3A4* luciferase reporter plasmid, *phCYP3A4-Luc*, was constructed in our laboratory from *phCYP3A4-lacZ* . The *phCYP3A4-lacZ* was a gift from Dr. Yongkyu Kim of the National Institute of Toxicological Research (NITR), Korea. The *hCYP3A4* promoter, from base pairs –863 to +64, was generated by polymerase chain reaction (PCR) using the DNA template isolated from the human lymphocyte. The PCR sense and antisense primers used were 5'-CATGC CCTGTC TCTCCTTTAGC-3' (corresponding to nucleotides –863 to –843) and 5'-CCTT TCAGCTCTGTGTTGCTC-3' (corresponding to nucleotides +64 to +44), respectively. The amplification cycle profile included denaturation at 93°C for 1 minute, annealing at 54°C for 2 minutes, and extension at 72°C for 1 minute. The product (928-bp) was then subcloned into the *pGEM-T* vector, and the resulting construct digested with *EcoR I* to obtain a 943-bp, with additional 13-bp, cloning restriction eyzyme site sequence. The 943-bp fragment was then cloned into the *EcoR I Sal I* site in the 5' to 3' orientation with respect to the *lacZ* transcription unit in the *pCMV* plasmid (*phCYP3A4-lacZ*). For *phCYP3A4-Luc*, *phCYP 3A4-lacZ* was digested with *EcoR I* and was prepared *CYP3A4* insert DNA containing~1kb of *CYP3A4* proximal promoter. *pGL3-basic* vector, a promoter-less luciferase reporter vector, was linearized with *Sma I*. *CYP3A4* insert DNA was cloned into a linearized *pGL3-basic* vector at *Sma I* site. Finally, *phCYP3A4-Luc*, *CYP3A4* luciferase reporter plasmid, containing ~1kb (–863 to +64 bp) of the *CYP3A4* proximal promoter was prepared.

pSAP-SXR

The pSAP-SXR expression plasmid was a gift from Dr. Yongkyu Kim of NITR, Korea. The pSAP-SXR contained SXR cDNA, under the control of the liver-specific human SAP promoter. The SXR gene sequence was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) with a full-length of mRNA isolated form HepG2 cells. The primers used for the amplification were the SXR sense and antisense primers, 5'-CAAGG ACAGCAGCATGACAGTC-3' (corresponding to nucleotides +36 to +57 bp) and 5'-GCTCATCTACCTGTG ATGCCGAA-3' (corresponding to nucleotides +1472 to +1450 bp), respectively. The amplified SXR was 1437 bp in length, which was then cloned into the pGEM-T vector. The pGEM/SXR construct was digested with *EcoR I* to clone it into the *EcoR I* site of *pBluscript SK+* (pBS-SXR). Separately, the liver-specific human serum amyloid P component (SAP) gene promoter was purified from *pLG I*-SAP by digestion with *Hind III/BamH I*, and the SXR fragment then inserted into the site *Hind III/BamH I* of pBluscript SK+ (pBR-SAP*)*. The *pLG I-SAP* plasmid was a gift Dr. Kenichi Yamamura of the Kumamoto University, Japan. Following digestion of *pLG I*-SAP with *Xho I/Spe I*, this fragment was inserted into the *Xho I/Spe I* cut pTet-Splice that had the tetracycline operator sequence eliminated by digestion with *Xho I/Spe I* enzymes (pSAP-Splice). Finally, the SXR fragment isolated from pBS-SXR with *Spe I* was cloned into the *Spe I* site of pSAP-Splice downstream of the *SAP* promoter. The SXR sequence was linked to the SV40 polyadenylation signal of *pTet-Splice*.

Cell culture and transfection

Hepa I mouse liver cell lines were grown in Minimum Essential Media (DMEM) supplemented with 10% (v/v) fetal bovine serum and penicillin-streptomycin (100 U/mL). For the transfection of *phCYP3A4-Luc*, HepG2 cells were seeded in multi-well plates. ph*CYP3A4* –Luc and/or pSAP-SXR and LipofecTAMINE (Invitrogen) were mixed in serum-free medium and incubated at room temperature for 45 min before the addition to each well. Cells were incubated for at least 5 hr before adding normal DMEM containing 20% fetal bovine serum. The details were followed as supplier's manual.

Chemical treatment

Hepa I cells were rinsed with PBS three-times before the administration of various chemicals. Stock solutions of chemicals were made in dimethylsulfoxide (DMSO)

as a vehicle and control cells were treated with 0.1% DMSO or chemicals for 24 hr. Final concentration of DMSO did not exceed 0.2%.

Luciferase reporter assay

Luciferase assays were performed using the Luciferase Assay System (Promega). Briefly, the transfected cells were lysed with reporter lysis buffer. The lysates was incubated with luciferase substrate, luciferin and luciferase activity was determined by the luminometer. Protein assay of cell extracts was carried out using the Micro BCA protein assay reagent kit (Pierce) and an ELISA Reader (Bio-rad). Luciferase activity was normalized to protein content. The data are presented as the fold induction of control cells that were treated with 0.1% DMSO.

Results

CYP3A4 proximal promoter response to known CYP3A4 inducers in HepG2 cells

To investigate whether *CYP3A4* proximal promoter was regulated by CYP3A4 inducers such as rifampicin, PCN and RU-486, transient transfection experiments were performed in HepG2 cells. Reporter gene, *phCYP 3A4-Luc,* containing 928 bp of the *CYP3A4* proximal promoter (–863 to +64 bp) was transfected. Then CYP3A4 inducers (0.25 to 25 μM) were treated for 24 hr and the activity of luciferase was measured. Rifampicin didn't increase the activity. When PCN was treated with 0.25, 2.5 and 25 μM, the activity appeared to be slightly increased to 1.2-, 1.2- and 1.3-fold respectively compared to control (Fig. 1). In RU-486, the activity appeared to be increased slightly to 1.1-, 1.3- and 1.5-fold at the same concentration with PCN. But both of them didn't show statistically significant changes. These results indicated that CYP3A4 inducers didn't increase gene expression by *CYP3A4* proximal promoter.

SXR effect on the *CYP3A4* proximal promoter activity in response to the inducers.

CYP3A4 expression by proximal promoter was progressively increased, when the increasing amount of expression plasmid for SXR was cotransfected with *phCYP3A4-Luc* (data not shown). Therefore, to examine whether SXR enhanced the *CYP3A4* stimulation by CYP3A4 inducers at proximal promoter region, *phCYP 3A4-Luc* was cotransfected with SXR expression plasmid. Transfected cells were treated with CYP3A4

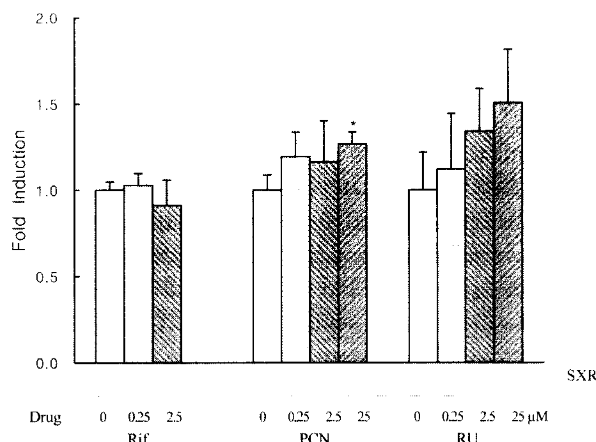

Fig. 1. Effects of CYP3A4 inducers on *CYP3A4* proximal promoter in *phCYP3A4-Luc* transfected HepG2 cells Reporter genes containing 928 bp of the *CYP3A4* promoter (–863 to +64 bp) were constructed as described in *materials and methods.* HepG2 cells were transiently transfected with *phCYP3A4-Luc* and exposed to various concentrations of CYP3A4 inducers such as rifampicin (Rif), pregnenolone 16-carbonitrile (PCN) and RU486 (RU) for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment * : Significantly different from control at $p < 0.05$.

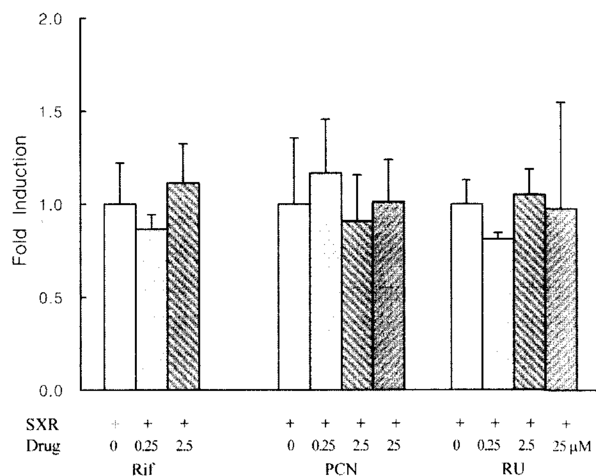

Fig. 2. Effects of CYP3A4 inducers on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected HepG2 cells HepG2 cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR.* Cells were exposed to various concentrations of CYP3A4 inducers such as rifampicin (Rif), pregnenolone 16-carbonitrile (PCN) and RU486 (RU) for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment.

inducers (0.25 to 25 μM) for 24 hr and the activity of luciferase was measured. Rifampicin, PCN and RU-486 didn't increase the activity at all treated concentrations compared to control (Fig. 2). These results showed that CYP3A4 inducers also didn't increase gene expression by *CYP3A4* proximal promoter under SXR cotransfection.

HDAC inhibitors increase *CYP3A4* proximal promoter activity in HepG2 cells

It is known that the degree of histone acetylation in the promoter region of genes might control the transcriptional activity of these promoters and has been reported that histone acetylation affected to the induction of some CYPs. To investigate whether a weak response to CYP3A4 inducers in proximal promoter region was related with a chromatin structure, *phCYP3A4-Luc* was cotransfected with SXR expression plasmid into HepG2 cells and then treated with TSA, HC-toxin, IN2001 or IN2002, as known histone deacetylase inhibitors for 24 hr. Treatment concentrations were 0.05, 0.5 and 5 μM. All of them increased significantly the luciferase activity by *CYP3A4* proximal promoter ($p < 0.05$, Fig. 3). TSA increased the activity to 4.9-, 2.3- and 1.7-fold at respective concentration and it showed a maximum response at 0.05 M. HC-toxin increased significantly the activity to 3.5-, 8- and 3.9-fold at each concentration. Also IN2001 increased to 2.5-, 5-, 2.7-fold and IN2002 to 2.8-, 7-, 3.4-fold. These results indicated that the inhibition of histone deacetylation increased markedly *CYP3A4* expression by proximal promoter and thus a remodeling of chromatin structure might be related with *CYP3A4* stimulation in proximal promoter region.

CYP3A4 proximal promoter response to known CYP3A4 inducers in Hepa I cells

To investigate whether *CYP3A4* proximal promoter was regulated by CYP3A4 inducers such as rifampicin and PCN, *phCYP3A4-Luc* was transiently transfected into Hepa I cells. Cells were treated with rifampicin (0.1 to 10 μM) or PCN (0.25 to 25 μM) for 24 hr and the activity of luciferase was measured. Neither rifampicin nor PCN increased proximal promoter activity at the range of treated concentrations (Fig. 4). These results indicated that *CYP3A4* proximal promoter does not respond to CYP3A4 inducers, such as rifampicin and PCN in Hepa I cells and the lack of responsiveness to rifampicin and PCN were observed in HepG2 cells (Kim J.Y. *et al.* 2004).

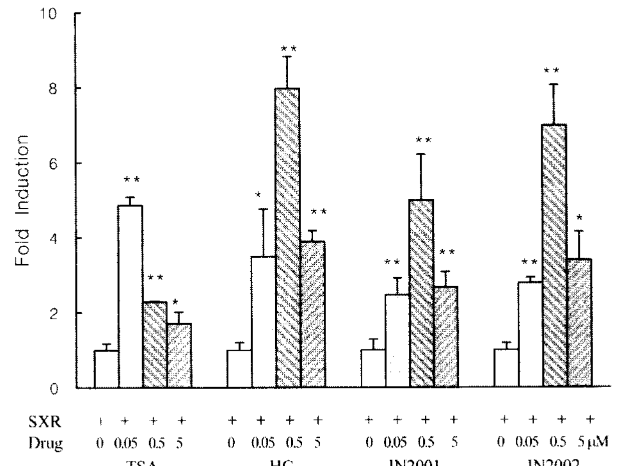

Fig. 3. Effects of HDAC inhibitors on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected HepG2 cells HepG2 cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR*. Cells were exposed to various concentrations of HDAC inhibitors such as TSA, HC-toxin (HC), IN2001 and IN2002 for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment * : Significantly different from control at $p < 0.05$, ** : Significantly different from control at $p < 0.01$.

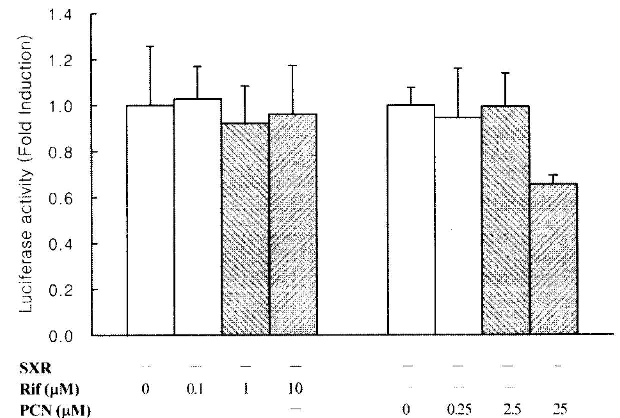

Fig. 4. Effects of CYP3A4 inducers *on CYP3A4* proximal promoter in *phCYP3A4-Luc* transfected Hepa I cells Hepa-I cells were transfected with *phCYP3A4-Luc*. Cells were exposed to various concentrations of CYP3A4 inducers such as rifampicin (Rif), and pregnenolone 16-carbonitrile (PCN) for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment.

SXR effect on the CYP3A4 proximal promoter activity in response to the inducers

To examine whether SXR enhanced *CYP3A4* proximal promoter activity that was stimulated by CYP3A4

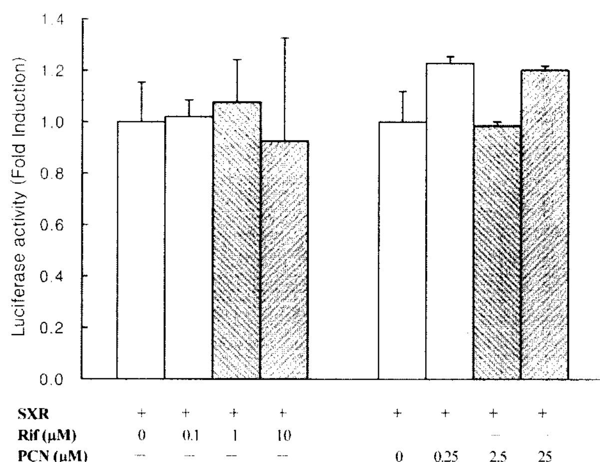

Fig. 5. Effects of CYP3A4 inducers on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected Hepa I cells Hepa-I cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR*. Cells were exposed to various concentrations of CYP3A4 inducers such as rifampicin (Rif) and pregnenolone 16-carbonitrile (PCN) for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment.

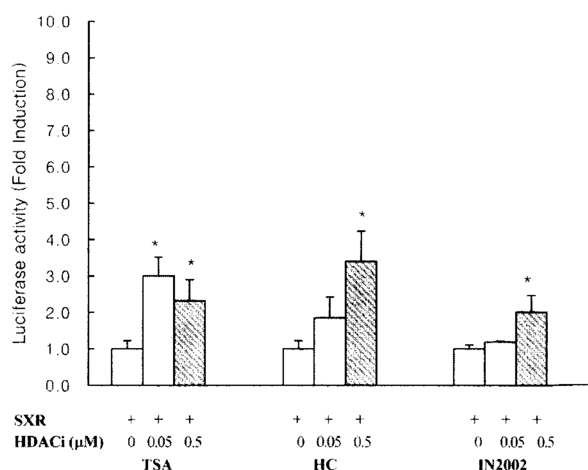

Fig. 6. Effects of HDACs inhibitors on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected Hepa I cells Hepa-I cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR*. Cells were exposed to various concentrations of HDAC inhibitors such as TSA, HC-toxin (HC) and IN2002 for 24 hr. Control cells were treated with 0.1% DMSO. The luciferase activity was measured using luciferin. Data present mean ± S.D. of triplicate transfections from a single representative experiment * : Significantly different from control at $p < 0.05$.

inducers such as rifampicin and PCN, *phCYP3A4-Luc* was cotransfected with pSAP-SXR expression plasmid. These transfected Hepa I cells were treated with CYP3A4 inducers such as, 0.1, 1 and 10 μM rifampicin and 0.25, 2.5 and 25 μM for PCN for 24 hr and the activity of luciferase was measured. As shown in Fig. 5, SXR did not affect the responsiveness of *CYP3A4* proximal promoter to rifampicin (Fig. 4, 5). These results showed that the activation of *CYP3A4* proximal promoter with rifampicin and PCN might require other transcription factors besides SXR in Hepa I cells and this phenomenon was similar to those in HepG2 cells.

HDAC inhibitors increase *CYP3A4* proximal promoter activity in Hepa I cells

To investigate whether no response of *CYP3A4 proximal promoter* to CYP3A4 inducers in proximal promoter region was related to a chromatin structure, *phCYP3A4-Luc* was cotransfected with pSAP-SXR expression plasmid into Hepa-I cells and then were treated with TSA, HC-toxin or IN2002, as known histone deacetylase inhibitors for 24 hr. 0.05, 0.5 and 5 M TSA increased the activity significantly to 3-, 2.3- and 1.9-fold, respectively ($p < 0.05$, Fig. 6). 0.05, 0.5 and 5 μM HC-toxin increased the proximal promoter activity to 1.9-, 3.4- and 2.4-fold respectively and the maximum response was shown at 0.05 μM HC-toxin. 0.05, 0.5 and 5 μM IN2002 increased to 1.2-, 2- and 1.1-fold, respectively and the maximum response was shown at 0.05 μ IN2002. These results indicated that the inhibition of histone deacetylation increased markedly *CYP3A4* gene proximal promoter activity. Therefore a remodeling of chromatin structure might be related with *CYP3A4* stimulation in proximal promoter region same as HepG2 cells.

Discussion

The ability of xenobiotics to regulate *CYP3A4* gene expression has been studied using *in vitro* reporter gene expression system, Ogg *et al.* reported that rifampicin, PCN, and dexamethasone increased the reporter gene activity by *CYP3A4* proximal promoter in HepG2 cells and these increments were enhanced significantly with hGR cotransfection (Ogg *et al.*, 1999). They used a reporter gene in which –1087 to –57 bp of the *CYP3A4* regulatory region was inserted into the pCMV-cSPAP plasmid. From their results, 50 μM of rifampicin increased the activity to 2.4-fold and 100 μM of PCN increased to 1.45-fold over untreated control cells. Other study demonstrated using the same plasmid as Dr. Ogg had used that 50 μM of rifampicin increased

the promoter activity to 13-fold and this stimulation was enhanced to 26.7-fold with pSAP-SXR cotransfection (Gibson *et al.*, 2000). In contrast, Goodwin *et al.* reported that rifampicin did not stimulate the promoter activity of 5'-flanking regions of *CYP3A4*, such as –1252 to +53 bp and –362 to +53 bp, in HepG2 cells and neither pSAP-SXR cotransfection stimulate the promoter activity of 5'-flanking regions of *CYP3A4*, such as –1252 to +53 bp and –362 to +53 bp, in HepG2 cells (Goodwin *et al.*, 1999). When 5'-flanking region of *CYP3A4* (–362 to +53 bp) was fused with XREM region (–7836 to –7607 bp), however, 5 μM of rifampicin exhibited 2- to 4-fold increment and 40- to 50-fold increment with SXR cotransfection (Goodwin *et al.*, 1999; Luo *et al.*, 2002). Similar studies reported that rifampicin treatment did not increase the 5'-flanking fragments of *CYP3A4* (–263 to +11 bp) with a cotransfection of SXR, and rifampicin stimulated the promoter activity of XREM (–7836 to –7208 bp) markedly to 6-fold in the presence of SXR (Drocourt *et al.*, 2001; Pascussi *et al.*, 2001). In the present study, any stimulation of CYP3A4 inducers was unable to be elicited from *phCYP3A4-Luc* containing –863 to +64 bp of the *CYP3A4* proximal promoter without TSA. This contradicts some earlier reports by Ogg *et al.* that a plasmid containing~1kb (–1087 to –57 bp) of the 5'-flanking region of *CYP3A4* conferred xenobiotic responsiveness on a reporter gene. At this moment, it is not clear whether this nucleotide sequence difference counts for the discrepancy between two studies. Importantly, these studies used the potent cytomegalovirus promoter as a minimal promoter, whereas the present study used the native *CYP3A4* proximal promoter. Some similar results with this study were reported using a plasmid containing the native *CYP3A4* proximal promoter. Hashimoto *et al.* and others suggested that silencer elements might be existed in this proximal region of the *CYP3A4* promoter, possibly –1105 to –362 bp or –57 to +64 bp, and repress a basal transcriptional activity ((Hashimoto *et al.*, 1993; Goodwin *et al.*, 1999). Moreover, they suggested that other transcription factors were required for maximal responsiveness. SXR was known to regulate the expressions of various CYP enzymes including CYP3A4, CYP3A11, and CYP2C8 (Staudinger *et al.*, 2001; Xie *et al.*, 2001). SXR belongs to the subfamily of the nuclear hormone receptors which repress gene transcription in the absence of ligands via the interaction with nuclear corepressors such as N-coR and SMRT (silencing mediator for retinoid and thyroid receptors) and heterodimerize with RXRs and mediate ligand-dependent transcription (Horlein *et al.*, 1995; Chen *et al.*, 1995). Recent studies revealed that the various inducers of the CYP3A4 recruit the nuclear receptor coactivators such as steroid receptor coactivator-1 (SRC-1) to LBD of SXR and this interaction of SXR with SRC-1 was also confirmed in studies from Takeshita *et al* (Kliewer *et al.*, 1998; Lehmann *et al.*, 1998; Jones *et al.*, 2000; Takeshita *et al.*, 2002). Therefore the relative balance of SXR interaction with coactivators and corepressors may determine the specific promoter activity. It was reported that interaction of SXR with SMRT in HepG2 cells may require other cell-specific factor or post-transcriptional modification (Takesihita *et al.*, 2002; Synold *et al.*, 2001). Other study reported that a corepressor protein interacted with Sin3A and mediated silencing through the recruitment of histone deacetylases (Mathur *et al.*, 2001). Thus the effect of histone deacetylation was examined on transcriptional regulation of the *CYP3A4* gene. The present study indicated that the inhibition of histone deacetylation enhanced the *CYP3A4* induction by proximal promoter and affected to a trans-activation of SXR specifically in Hepa I cells. Taking these data into consideration, one could hypothesized that CYP3A4 promoter is repressed by silencer elements through an interaction between corepressors and histone deacetylase and TSA inhibits this repression by mediating changes in a chromatin structure and then SXR can interact with *CYP3A4* promoter and eventually mediate stimulations by CYP3A4 inducers. In conclusion, the inhibition of histone deacetylation was required to SXR-mediated stimulation in *CYP3A4* proximal promoter region when rifampicin or PCN were treated Hepa I cells.

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