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Trichostatin A, a Histone Deacetylase Inhibitor Stimulate *CYP3A4* Proximal Promoter Activity in Hepa-I Cells

Mee Ryung Ahn, Dae-Kee Kim, and Yhun Yhong Sheen

College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

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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. However, the molecular mechanisms underlying regulation of *CYP3A4* gene expression have not been understood. Thus, this study has been carried out to gain the insight of the molecular mechanism of *CYP3A4* gene expression, investigating if the histone deacetylation is involved in the regulation of *CYP3A4* gene expression by proximal promoter. Also SXR was investigated to see if they were involved in the regulation of *CYP3A4* proximal promoter activity. Hepa-I cells were transfected with a plasmid containing ~1 kb of the human *CYP3A4* proximal promoter region (863 to +64 bp) cloned in front of a reporter gene, luciferase, in the presence or absence of SXR. Transfected cells were treated with CYP3A4 inducers such as rifampicin, PCN and RU 486, in order to examine the regulation of *CYP3A4* gene expression in the presence or absence of trichostatin A (TSA). In Hepa-I cells, CYP3A4 inducers increased modestly the luciferase activity when TSA was co-treated, but this increment was not enhanced by SXR cotransfection. 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, IN2002, Hepa I

INTRODUCTION

A small subset of constitutively expressed human CYPs is of particular interest because they are quantitatively the most important forms and catalyze a range of biologically important reactions. These include CYP3A, which is the predominant CYP expressed in human liver, comprising approximately 30% of the total CYPs content (Watkins, 1994). The *CYP3A* subfamily contains four members, *CYP3A4, CYP3A43, CYP3A5* and *CYP3A7* (Pascussi *et al.*, 2003). CYP3A7 was isolated from fetal liver tissue and it appeared to be expressed mainly in fetal liver and in adult endometrium and placenta. During the first week after birth, a major developmental change occurs with *CYP3A7* virtually disappearing, at the same time with transcriptional activation of the *CYP3A4* gene. *CYP3A5* is expressed polymorphically, with 20% of individual adults showing appreciable hepatic expression (Guengerich *et al.*, 1999). The CYP3A4 isoform is the most prevalent in adults and 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 steroid and xenobiotic receptor (SXR). The molecular mechanism underlying regulation of *CYP3A4* gene expression has been studied by several groups using cotransfection strategies (Goodwin *et al.*, 1999; Luo *et al.*, 2002). 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 the SXR-mediated response by CYP3A4 inducers. Goodwin *et al.* reported that *p(ER6)$_3$-tk-Luc* exhibited a 2- to 4-fold activation by rifampicin in the presence of SXR and 5'-flanking regions of *CYP3A4*, such as −1252 to +53 bp and −362 to +53 bp,

Correspondence to: Yhun Yhong Sheen and Dae-Kee Kim, College of Pharmacy, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-ku, Seoul 120-750, Korea
Tel: 82-2-3277-3028/3025, Fax: 82-2-3277-2851
E-mail : yysheen@mm.ewha.ac.kr/dkkim@ewha.ac.kr

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 in the presence of SXR (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). This suggested the possible repressive regulation of *CYP3A4* gene expression.

Transcriptional repression by a sequence-specific DNA-binding factor can be mediated by the recruitment of a deacetylase to the promoter region. Histone deacetylase (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 (Butler *et al.*, 2002; Suzuki *et al.*, 2002; Van *et al.*, 1996). The acetylation state of several of non-histone proteins is increased following exposure to inhibitors of HDACs, including p-53, α-tubulin, Hsp90, MyoD and GATA-1 (Polevoda and Sherman, 2002; Kouzarides *et al.*, 2000; Freiman *et al.*, 2003; Yu *et al.*, 2002). In addition to effects on transcription, HDAC inhibitors can cause changes in protein expression that might be the result of acetylation. For example, depsipeptide, HDAC inhibitor, causes an increase in acetylation of the chaperone protein Hsp90, with a consequent decrease in binding of client proteins and also, therefore, results in degradation of these proteins (Yu *et al.*, 2002). It is possible that a combination of transcriptional and post-translational effects leads to alter protein expression. Taken together, HDAC inhibition leads to changes in transcriptional activity and protein stability through increased acetylation of proteins (Marks *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. Hepa I cells were transfected with a plasmid containing ~1 kb 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, 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 16á-carbonitrile (PCN) and HC-toxin was purchased from Sigma-Aldrich (St. Louis, MO). Agarose was purchased from FMC and LipofectAMINE from Gibco-BRL and Hind III and *Sma I* from BMS, while pGL3 basic vector and luciferase assay system were ordered from Promega. Trichostatin A, 4-dimethylamino-*N*-[4-(2-hydroxycarbamoyl-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 *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'-CATGCCCTGTCTCT CCTTTAG-3' (corresponding to nucleotides -863 to -843) and 5'-CCTT TCAGCTCTGTGTTGCTC-3' (corresponding to nucleotides +44 to +64), 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*, *phCYP3A4-lacZ* was digested with *EcoRI* and was prepared *CYP3A4* insert DNA containing ~1 kb 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 ~1 kb (863 to +64 bp) of the *CYP3A4* proximal promoter was prepared.

pSAP-SXR

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 ACAGCAGCA TGACAGTC-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.

Statistical analysis

Statistical analysis of data was carried out by student t-test.

RESULTS

***CYP3A4* proximal promoter response to known CYP3A4 inducing chemicals**

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 h and the activity of luciferase was measured. Neither rifampicin nor PCN increased proximal promoter activity at the range of treated concentrations (Fig. 1). 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).

Influence of SXR on the *CYP3A4* proximal promoter activity in response to the inducing chemicals

To examine whether SXR enhanced *CYP3A4* proximal promoter activity that was stimulated by CYP3A4 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. 2, SXR did not affect the responsiveness of *CYP3A4* proximal promoter to rifampicin (Fig. 1, 2). These results showed that the activation of *CYP3A4* proximal promoter with rifampicin

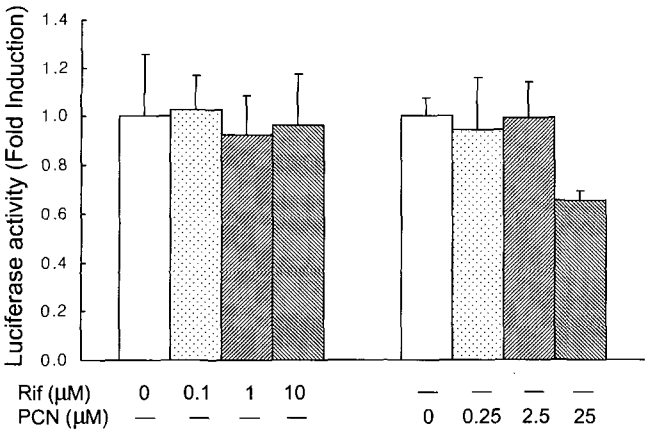

Fig. 1. Effects of CYP3A4 inducers *on CYP3A4* proximal promoter in *phCYP3A4-Luc* transfected Hepa-I cells. Hepa-I cells were transfected with *phCYP3A4-Luc*. Cells were exposed to various concentrations of CYP3A4 inducers such as rifampicin (Rif), and pregnenolone 16α-carbonitrile (PCN) for 24 hr. 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.

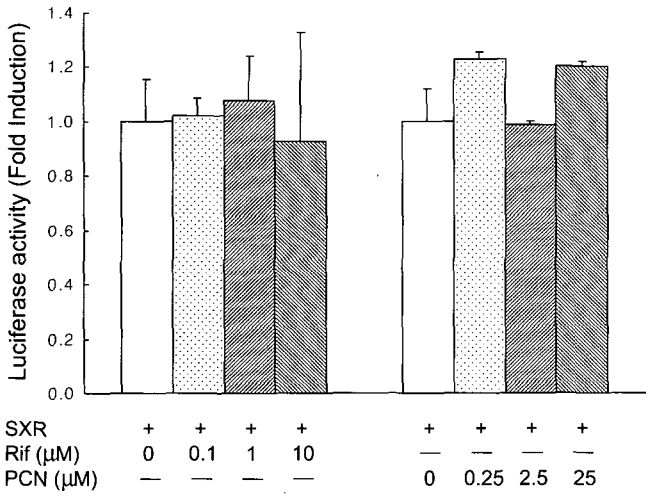

Fig. 2. Effects of CYP3A4 inducers on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected Hepa-I cells. Hepa-I cells were transfected by *phCYP3A4-Luc* along with pSAP-SXR. Cells were exposed to various concentrations of *CYP3A4* inducers such as rifampicin (Rif) and pregnenolone 16α-carbonitrile (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.

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

To investigate whether no response of *CYP3A4*

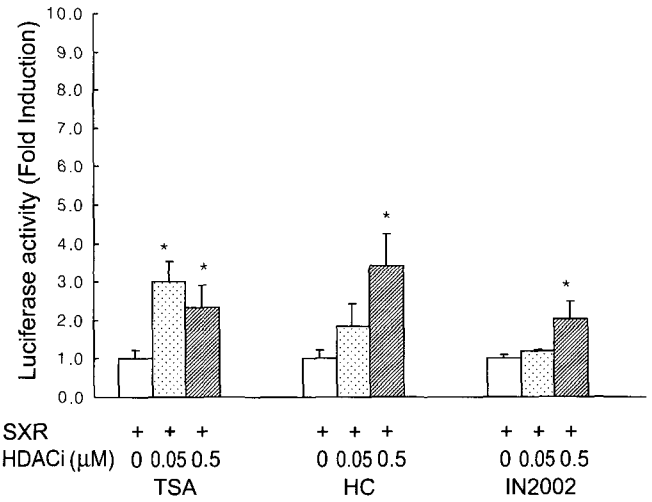

Fig. 3. Effects of HDACs inhibitors on *CYP3A4* proximal promoter in *phCYP3A4-Luc/SXR* cotransfected Hepa-I cells. Hepa-I cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR*. Cells were exposed to various concentrations of HDAC inhibitors such as TSA, HC-toxin (HC) and IN2002 for 24 hr. Control 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 $p < 0.05$

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 h. 0.05 and 0.5 μM TSA increased the activity significantly to 3-, 2.3-, and 1.9-fold, respectively ($p<0.05$, Fig. 3). 0.05 and 0.5 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 and 0.5 μM IN2002 increased to 1.2-, 2-, and 1.1-fold, respectively and the maximum response was shown at 0.05 μM 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.

TSA effect on *CYP3A4* proximal promoter activity in the absence of SXR transfection

To investigate whether TSA affects *CYP3A4* regulation by CYP3A4 inducers in proximal promoter region, an experiment was performed under the same condition except TSA co-treatment. *phCYP3A4-Luc* was transfected into Hepa-I cells, followed by treatment with rifampicin or PCN in the presence of TSA for 24 h. TSA was treated with the concentration of 0.05 μM and rifampicin was treated with 0.1, 1, and 10 μM and PCN was treated with

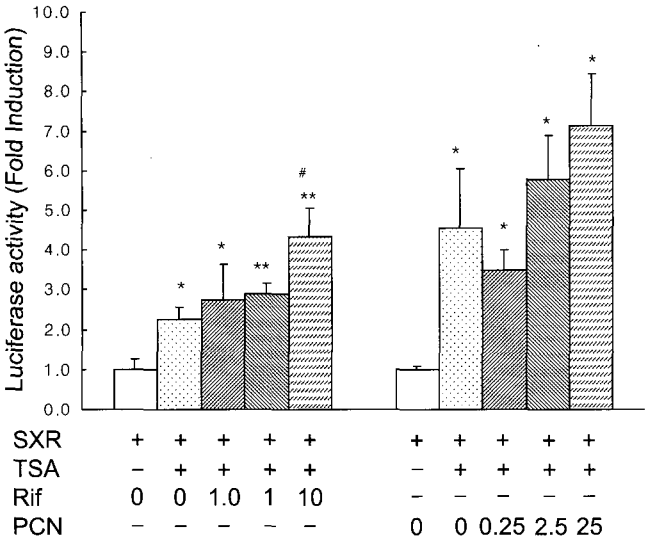

Fig. 4. Effects of TSA on *CYP3A4* regulation by *CYP3A4* inducers in *phCYP3A4-Luc* transfected Hepa-I cells. Hepa-I cells were transfected with *phCYP3A4-Luc*. Cells were exposed to various concentrations of *CYP3A4* inducers such as rifampicin (Rif) and pregnenolone 16α-carbonitrile (PCN) in the presence of 0.05 mM TSA 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 $p < 0.01$, #: Significantly different from TSA at $p < 0.05$.

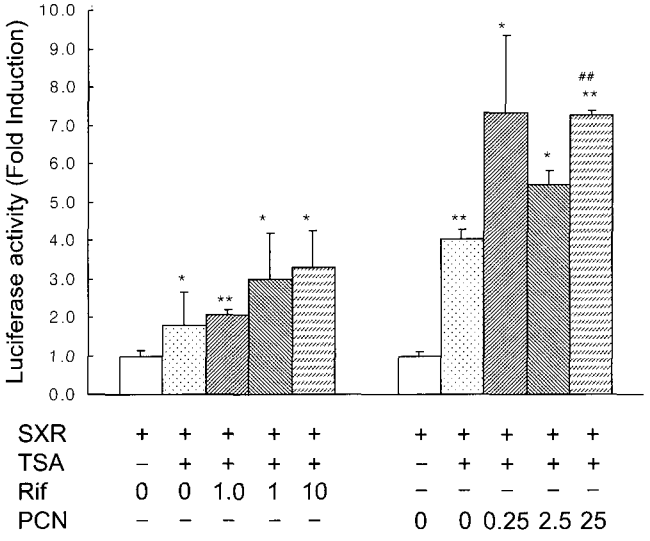

Fig. 5. Effects of TSA on *CYP3A4* regulation by *CYP3A4* inducers in *phCYP3A4-Luc/SXR* cotransfected Hepa-I cells. Hepa-I cells were transfected by *phCYP3A4-Luc* along with *pSAP-SXR*. Cells were exposed to various concentrations of *CYP3A4* inducers such as rifampicin (Rif) and pregnenolone 16α-carbonitrile (PCN) in the presence of 0.05 mM TSA 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 $p<0.01$, ##: Significantly different from TSA at $p < 0.01$.

0.25, 2.5, and 25 μM. TSA alone increased the activity about 2- to 5-fold compared to control (Fig. 4). When rifampicin was combined with TSA, the activity was increased to 2.7-, 2.9-, and 4.3-fold respectively while TSA increased 2.3-fold. The response of rifampicin was statistically significant at 10 μM ($p<0.05$). In the case of PCN, when it was co-treated with TSA, the activity was increased to 3.5-, 5.8-, and 7.1-fold during TSA increased to 4.5-fold. These results showed that the response of CYP3A4 inducers was increased by TSA in proximal promoter region.

TSA effect on *CYP3A4* proximal promoter activity in the presence of SXR transfection

To identify whether TSA affected the SXR-mediated *CYP3A4* regulation by CYP3A4 inducers in proximal promoter region, *phCYP3A4-Luc* was cotransfected with pSAP-SXR expression plasmid and transfected cells were treated with both CYP3A4 inducers and TSA for 24 hr. TSA alone increased the luciferase activity about 2- to 4-fold compared to control. When 0.1, 1, and 10 μM rifampicin were treated with TSA concomitantly, the luciferase activity appeared to be increased modestly in a dose-dependent manner to 2.1-, 3-, and 3.3-fold, respectively, while TSA resulted in 1.8-fold increase. In case of PCN, 0.25, 2.5, and 25 μM PCN, increased luciferase activity to 7.3-, 5.5-, and 7.3-fold over control while TSA alone increased 4-fold ($p<0.05$, Fig. 5). These results demonstrated that the response of CYP3A4 inducers was increased by TSA in proximal promoter region, however, SXR didnt mediate the stimulation of CYP3A4 inducers in Hepa-I cells differently from 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., 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 ~1 kb (–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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