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Pregnane X receptor is required for IFN-α-mediated CYP3A29 expression in pigs

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ABSTRACT

Pregnane X receptor (PXR) has been identified as a central mediator for coordinate responses to xenobiotic and drug metabolism, and is the major transcriptional regulator of cytochrome P-450 (CYP). Interferon (IFN)-α is known to induce antiviral mechanisms and exert immune regulatory capacity in various cell types. Here, we used primary porcine hepatocytes and a cultured hepatocyte cell line to identify the metabolic role of PXR in IFN-α-mediated CYP3A29 expression. We found that IFN-α could activate PXR in both time- and dose-dependent manners in pigs. Activation of PXR significantly increased CYP3A29 mRNA and protein expression. Meanwhile, the expression of CYP3A29 induced by IFN-α occurred after the increase of PXR expression in porcine hepatocytes. In addition, the IFN-α-induced CYP3A29 expression was blocked by PXR knockdown. The PXR-overexpressed cells (transfected with porcine PXR) increased CYP3A29 mRNA and protein expression. Furthermore, in animal experiments, we found that IFN-α increased both CYP3A29 mRNA and protein levels. Collectively, our results suggest that PXR plays an important role in IFN-α-mediated CYP3A29 expression in porcine hepatocytes.

1. Introduction

The cytochrome P450 (CYP) enzymes are encoded by a large family of genes and can metabolize a wide range of exogenous and endogenous compounds. CYP3A4 is an important CYP isoform that is responsible for metabolizing more than 50% of clinical medicines in humans [1–3]. Its activity seriously affects drug interactions [4]. Studies on CYP3A4 are limited to *in vitro* experiments due to the lack of an experimental animal model. The pig is similar to humans in terms of the cardiovascular, gastrointestinal, urogenital, and renal systems, and the skin as well [5]. Recent pharmacodynamic studies demonstrated that the mouse is not an ideal model for human drug studies, and thus the pig has been considered as an appropriate animal model for drug metabolic studies [6]. It is well known that pigs have the main enzymes of drug metabolism [7]. It is believed that the properties of porcine CYP3A29 are similar to human CYP3A4 [8–11]. In addition, pigs can also be exposed to drugs or pollutants, so studies on porcine CYP3A29 are important for a better understanding of drug interactions *in vivo*.

Interferon (IFN)-α belongs to the type I IFN family of cytokines, and the type I IFNs are potent antiviral agents that inhibit viral replication and confer cellular resistance to viral infection. In addition to their antiviral effects, IFNs are pluripotent and possess multiple functions, including the modulation of cellular metabolism, control of growth and differentiation, regulation of immune function, and inhibition of tumor development [12–14].

Recent works have demonstrated that Pregnane X receptor (PXR) belongs to a superfamily of nuclear receptors that regulates target gene transcription in a ligand-dependent manner [15]. Some chemicals modulate the expression of CYPs through PXR-dependent mechanisms [16]. In addition, recent studies have revealed that some biological drugs can activate PXR, which is involved in CYP3A transcription in the liver [17]. Although the regulatory roles of PXR in CYP expression have been elucidated, the issue whether PXR is involved in IFN-α-mediated CYP3A29 expression has not been addressed.

The present study aimed to elucidate the role of IFN-α in the regulation of CYP3A29 expression. Using quantitative reverse transcription-polymerase chain reaction (qRT-PCR), Western blot analysis, transient luciferase reporter assays, and *in vivo* animal experiments, here we demonstrated that the nuclear receptor PXR is the response element for IFN-α-mediated CYP3A29 expression.

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2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from GIBCO (Life Technologies, Grand Island, NY). 5,6-dichlororibosidyl-benzimidazole (DRB) was from Sigma–Aldrich (St. Louis, MO). Recombinant swine IFN-α was purchased from PeproTech (Rehovot, Israel). Antibodies against CYP3A and β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). PXR antibody was purchased from GeneTex, Inc. (Irvine, CA). All other chemicals were of analytical grade.

2.2. Plasmid construction

PXR was amplified from pig liver cDNA and was cloned into the pMD 18-T vector (TAKARA, Otsu, Shiga, Japan). The insert in pMD 18-T-pPXR was cut out by double digestion with *BamHI* and *XhoI*, and recloned into pcDNA3.1 (Invitrogen, Life Technologies, Grand Island, NY) to generate pcDNA3.1-pPXR. The CYP3A29 promoter (−1473/+80) was amplified from the pig liver genome and cloned into the pRL-Basic vector (Promega, Madison, WI) to generate pCYP-Luc. The PXR shRNA construct was purchased from Genepharma (Shanghai, China).

2.3. Isolation and culture of pig hepatocytes and Hep-Li cells

Our experimental protocols were approved by the Institutional Animal Care and Use Committee of Center for Animal Experiment Huazhong Agriculture University and followed the guidelines for humane care of laboratory animals. Hepatocytes were isolated from Chinese experimental miniature pigs using a modified four-step collagenase perfusion method as previously described [18,19]. Briefly, the pigs were fasted for 12 h before surgery. After anesthetization with 1.5 ml/kg of 3% pelltobarbitalum natricum, the liver was removed from the abdominal cavity. The portal cannula was placed, and the liver was perfused for 15 min with perfusion buffer (9 g/L NaCl, 0.42 g/L KCl, 2.1 g/L $NaHCO_3$, 0.9 g/L glucose, and 4.78 g/L Hepes) at 37 °C at a flow rate of 20 ml/min. The liver was further perfused for 35 min with the perfusion buffer containing 0.5% EDTA at 37 °C, and then perfused with D-Hank's buffer containing 0.5% collagenase at 37 °C. After digestion for 10 min, the liver capsule was disrupted and its parenchyma was suspended in Hank's buffer. The cell suspension was filtered and washed twice by centrifugation at 500 rpm for 3 min. The viability of isolated hepatocytes was 91.4 ± 1.2% (trypan blue dye exclusion, $n = 4$). Approximately 5×10^6 cells were plated into a 6-well plate in 3 ml of DMEM containing 20% FBS, 10 mg/L insulin, 100 U/ml penicillin, and 100 μg/ml streptomycin. The hepatocytes were incubated overnight in a humidified chamber maintained at 37 °C with 5% CO_2.

A recent study demonstrated that the immortalized porcine hepatocyte cell line (Hep-Li cell line) showed similar characteristics to primary porcine hepatocytes [20]. The Hep-Li cell line was obtained from Zhejiang University (Hangzhou, China) and cultured in high-glucose DMEM containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin.

2.4. Animal treatment and microsome preparation

This study was performed using six large white Landrace hybrid pigs (13–15 kg body weight, aged 5 weeks). The animals were housed for 14 days with free access to food and water. Three pigs were treated with 100,000 IU/kg IFN-α in phosphate-buffered saline (PBS) solution and sacrificed after 24 h, while three control pigs were treated with PBS only. Microsome preparation was performed as previously described [21].

2.5. Total RNA isolation and reverse transcription

Total RNA was isolated from primary pig hepatocytes, Hep-Li cells, and pig liver by using the HP Total RNA Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's instructions. The integrity of the total RNA was examined on a NanoDrop spectrophotometer (ND-2000, Thermo Scientific, Wilmington, DE). Reverse transcription was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Beijing, China) with random hexamers as recommended by the manufacturer.

2.6. Real-time quantitative RT-PCR analysis

Real-time quantitative RT-PCR was carried out using a Bio-Rad IQ5 and Bio-Rad IQ5 Optical System software version 1.0CR (Bio-Rad, Hercules, CA). The β-actin used as a reference gene was amplified in parallel with the target gene. Detection of real-time RT-PCR products was done by using the IQ™SYBR® Green Supermix kit (Bio-Rad) following the manufacturer's instructions. Five microliters of cDNA (the equivalent of 25 ng of total RNA) was used as a template for the PCR, which was carried out at 94 °C for 1 min, followed by 45 cycles of 94 °C for 5 s, 58 °C for 35 s, and 72 °C for 35 s. The sequences for the primers were as follows: CYP3A29 sense, 5′-CCTGAAATTAACCACGCAAGGGCT-3′; CYP3A29 antisense, 5′-TCTGGGATGCAGCTTTCTTGACCA-3′; PXR sense, 5′-ATTGATTTGCGTGGATGCTGAACTG-3′; PXR antisense, 5′-TGTAGTCCCAGTATTCCAGCCTCGT-3′; β-actin sense, 5′-TCTGGCACCACACCTTCT-3′; β-actin antisense, 5′-TGATCTGGGTCATCTTCTCAC-3′.

2.7. Western blot analysis

The protein concentration was determined by the BCA assay (Pierce, Thermo Scientific). Western blot analysis was performed as previously described [17]. Briefly, the protein was separated by 10% SDS polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene fluoride membrane. Immunoblotting was performed with anti-pig CYP3A29 (1:500 dilution) and anti-pig PXR (1:500 dilution) polyclonal antibodies. Immunoreactive bands were visualized with the Clarity™ Western ECL Substrate (Bio-Rad) and quantified by a DNR Bio Imaging System (DNR Industries, Jerusalem, Israel).

2.8. PXR knockdown and overexpression

In the RNAi experiment, Hep-Li cells were plated in a 12-well plate at a density of 1×10^6 cells/ml and transfected with the PXR shRNA plasmid (500 ng/well) or the same amount of control vector for 24 h. The same procedure was used for the PXR overexpression experiment by replacing the shRNA plasmid with the pig PXR or control plasmid. The medium was changed at 24 h. The transfected cells were treated with IFN-α (100 ng/ml) or the same volume of PBS for 24 h, and the expression of CYP3A29 and PXR was examined by real-time PCR.

2.9. Transfection and reporter assay

Transient transfection was performed by using Lipofectamine 2000 (Invitrogen). Cells were transfected with 100 ng/well of the CYP3A29 promoter luciferase reporter plasmid (pCYP-Luc) and 50 ng/well of the Renilla luciferase plasmid (pRL-TK) (Promega). After 24 h, the cells were treated with IFN-α and harvested at different time points. The luciferase activity was measured using the

Dual-luciferase Assay System (Promega) according to the manufacturer's instructions. The relative firefly luciferase activity was normalized to the Renilla luciferase activity.

2.10. Statistical analysis

Data are presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC). The statistical analysis was carried out using one-way analysis of variance followed by Duncan's multiple comparison tests. $p < 0.05$ was considered statistically significant.

3. Results

3.1. IFN-α induces PXR and CYP3A29 expression in primary pig hepatocytes and Hep-Li cells

Here, we examined whether IFN-α alters the expression of PXR and CYP3A29. Primary porcine hepatocytes and Hep-Li cells were isolated and treated for 24 h with IFN-α at various concentrations. The mRNA expression of CYP3A29 and PXR was upregulated in a dose-dependent manner in primary pig hepatocytes and Hep-Li cells after IFN-α treatment (Fig. 1A and B). Moreover, similar to the mRNA expression trend, the expression of CYP3A29 and PXR was also upregulated by IFN-α treatment in a concentration-dependent manner (Fig. 1C).

3.2. Transcriptional expression of CYP3A29 is regulated by IFN-α

To further investigate the mechanism of IFN-α-induced CYP3A29 mRNA expression, the RNA synthesis inhibitor 5, 6-dichlororibosidyl-benzimidazole (DRB) was used for the transcriptional inhibition assay [16]. Hep-Li cells were treated with IFN-α and DRB for 9 h separately and together. The qRT-PCR results showed that DRB abolished the IFN-α-induced transcriptional CYP3A29 upregulation in Hep-Li cells (Fig. 2A), indicating that IFN-α upregulated CYP3A29 expression through its promoter. To further verify whether PXR is involved in this regulation, co-transfection was performed in Hep-Li cells using a CYP3A29 luciferase

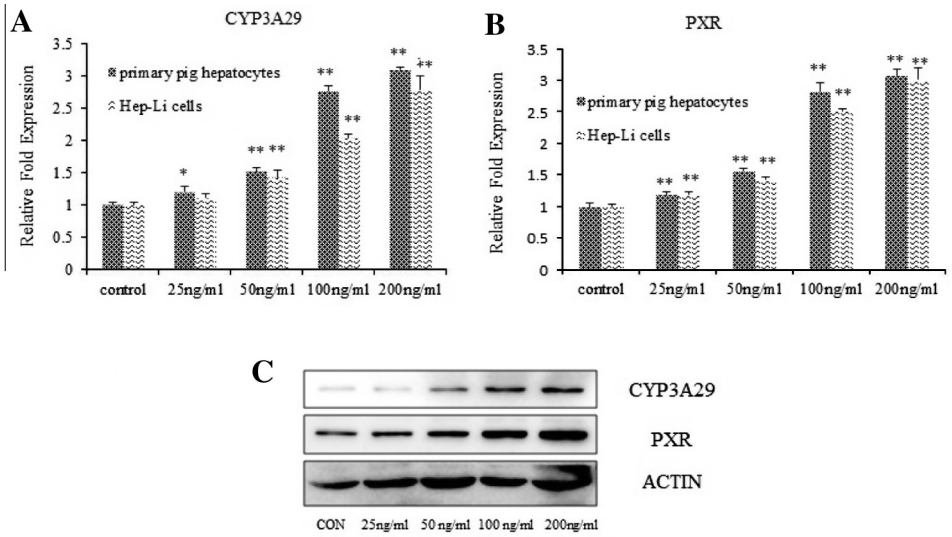

Fig. 1. Effects of IFN-α on the expression of PXR and CYP3A29 in primary porcine hepatocytes and Hep-Li cells. (A and B) Primary hepatocytes and Hep-Li cells were treated with IFN-α (25 ng/ml, 50 ng/ml, 100 ng/ml, and 200 ng/ml) or the same volume of PBS for 24 h. Total RNA was prepared and analyzed for the expression of CYP3A29 (A) and PXR (B) by RT-qPCR. The statistical analysis was made compared to corresponding controls. (C) Effects of IFN-α (25 ng/ml, 50 ng/ml, 100 ng/ml, and 200 ng/ml) on the protein expression of CYP3A29 and PXR by Western blot analysis. Values are mean ± SD of three independent tests. ($^{*}p < 0.05$, $^{**}p < 0.01$).

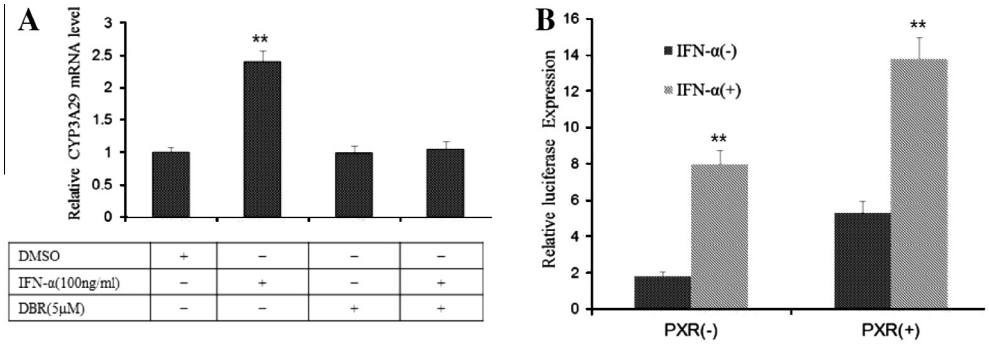

Fig. 2. Effect of IFN-α on CYP3A29 expression. (A) The promoting effect of IFN-α on the expression of CYP3A29 mRNA is blocked by DRB in primary porcine hepatocytes. Porcine hepatocytes were treated with 100 ng/ml IFN-α for 9 h in the absence or presence of 5 μM DRB. Total RNA was prepared and analyzed for the expression level of CYP3A29 by qRT-PCR. The statistical analysis was determined by the comparison to the control. (B) Expression of the CYP3A29-Luc promoter reporter. Hep-Li cells were transiently transfected with 50 ng of CYP3A29-Luc and 5 ng of Null-*Renilla reniformis* luciferase plasmid in the presence and absence of PXR using Lipofectamine 2000. The transfected cells were treated with 100 ng/ml IFN-α or the same volume of PBS for 24 h. Luciferase activity was examined by a Dual-Luciferase reporter assay system, and the reporter activity was normalized based on the Null-*Renilla reniformis* luminescence signal. Values are mean ± SD of four independent tests ($^{*}p < 0.05$, $^{**}p < 0.01$).

reporter and a PXR expression vector. Hep-Li cells were transfected with CYP3A29-luc and the Renilla plasmid with or without the PXR plasmid, and then treated with 100 ng/ml IFN-α or PBS. After incubation for 24 h, the cells were lysed to determine the luciferase activity. IFN-α significantly activated the activity of the CYP3A29 luciferase promoter reporter (Fig. 2B).

3.3. PXR is involved in IFN-α-induced CYP3A29 upregulation in hepatocytes

The activity of the CYP3A29 luciferase promoter by IFN-α in the cells transfected with the PXR expression vector was greater (13.79-fold) than that without the PXR expression vector (7.95-fold), and the basal activity of CYP3A29-luciferase after transfection with the PXR vector was greater (2.97-fold) than that without the PXR vector (Fig. 2B).

To further verify the role of PXR in the upregulation of CYP3A29 in response to IFN-α, we mediated the expression of PXR by siRNA knockdown and overexpression experiments. In the knockdown experiment, Hep-Li cells were transfected with PXR siRNA for 24 h. After treatment with IFN-α (100 ng/ml) or PBS for 24 h, the expression of CYP3A29 and PXR in cells was analyzed using qRT-PCR. The results indicated that the mRNA level of PXR in the cells transfected with PXR siRNA was significantly reduced, which was approximately 70% less than that in the control cells (Fig. 3A, left). Moreover, the upregulation of CYP3A29 mRNA by IFN-α was blocked after PXP knockdown (Fig. 3A, right). In the overexpression experiment, the Hep-Li cells were transfected the PXR construct or empty vector for 24 h. After treatment with IFN-α (100 ng/ml) or PBS for 24 h, the PXR overexpression significantly increased CPY3A29 mRNA expression, and the upregulation of CPY3A29 mRNA expression by PXR overexpression was further enlarged by IFN-α treatment (Fig. 3B). These results suggest that PXR is required for IFN-α-mediated expression of CYP3A29 in hepatocytes.

3.4. Apparent role of PXR in vivo

Since the activation of PXR could result in the expression of CYP3A29 *in vitro*, animal experiments were performed to investigate whether PXR could regulate CYP3A29 *in vivo*. As shown in Fig. 4A, IFN-α could upregulate mRNA expression of PXR and CYP3A29. The level of CYP3A29 expression in pigs injected with IFN-α was significantly greater than those injected with PBS (2.15-fold), and the expression of PXR mRNA had the same pattern as that of CYP3A29 mRNA. The protein expression of PXR and CYP3A29 was also elevated after IFN-α treatment (Fig. 4B).

4. Discussion

IFN-α and antiviral drugs are commonly used to treat chronic hepatitis B viral infection [22]. Zhang et al. suggested that the Janus tyrosine kinase-STAT (JAK-STAT) pathway may play a major role in mediating the effects of IFN-alpha against HBV [23]. Studies have focused on the classical pathway of interferon, but the effects of interferon and drug metabolizing enzymes have been rarely studied. Currently, the study on CYP450 enzyme mostly focuses on the impact of chemical substances or herbal products on its expression and its molecular mechanism [24–26], studies regarding biological macromolecular drugs are quite a few. The pig has been considered as an appropriate animal model for the investigation of drug absorption and disposition because its physiology shows high similarities to humans; they have both quantitative and qualitative similarities and dissimilarities regarding pharmacokinetics [6]. Our study showed that IFN-α significantly enhanced CYP3A29 mRNA and protein expression via PXR in both primary pig hepatocytes and cultured pig hepatocytes. Moreover, the increase in CYP3A29 mRNA after IFN-α treatment was blocked by adding an RNA synthesis inhibitor, suggesting that the expression of CYP3A29 by IFN-α occurs at the transcriptional level, which was

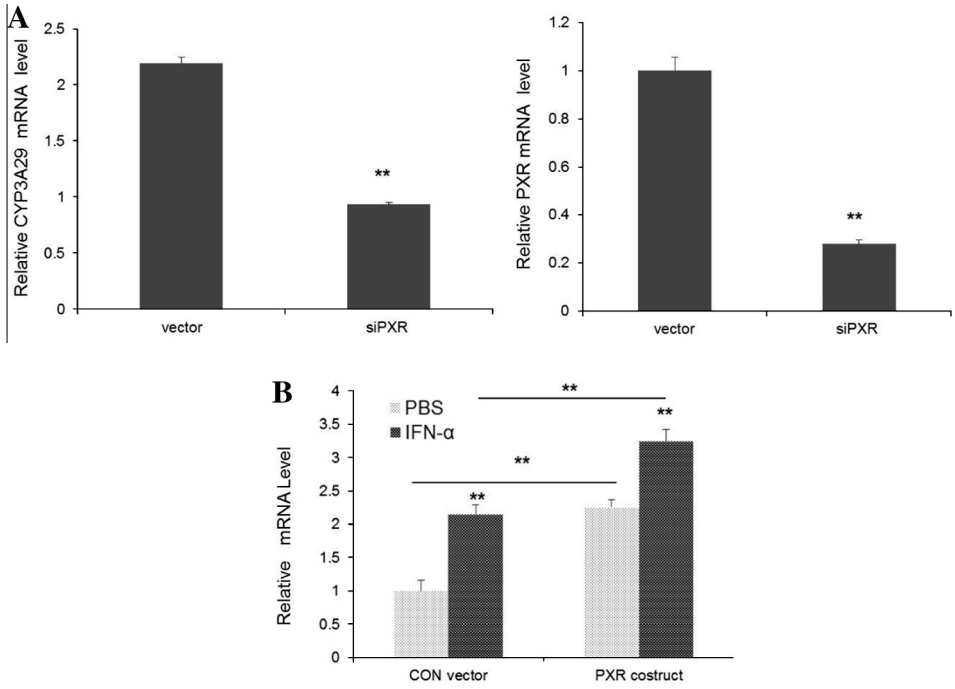

Fig. 3. Effect of PXR on CYP3A29 expression. (A) Effect of PXR knockdown on the expression of CYP3A29. Hep-Li cells were transfected with the PXR shRNA construct or the corresponding vector for 24 h, and the transfected cells were treated with IFN-α (100 ng/ml) or the same volume of PBS for 24 h. Total RNA was prepared and analyzed for the expression levels of CYP3A29 (left) and PXR (right) by qRT-PCR. (B) Effect of PXR overexpression on CYP3A29 expression. Hep-Li cells were transfected with the PXR construct or control vector for 24 h, and then treated with IFN-α (100 ng/ml) or the same volume of PBS for 24 h. Total RNA was prepared and analyzed for CYP3A29 mRNA expression by qRT-PCR. All experiments were repeated three times, and the data were expressed as mean ± SD (**$p < 0.01$).

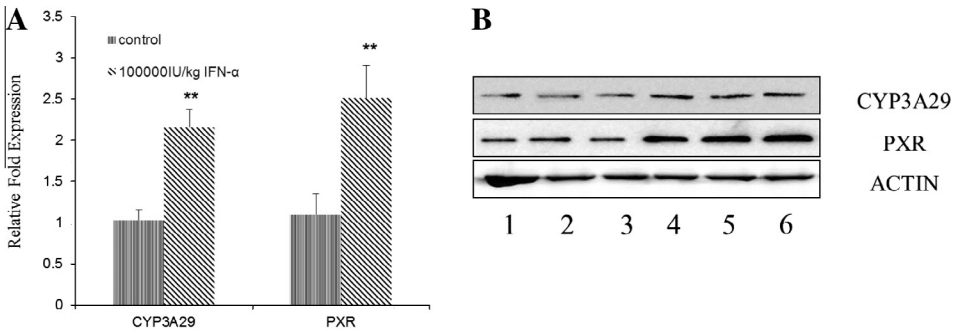

Fig. 4. Effect of IFN-α on the expression of PXR and CYP3A29 in the pig liver. Three pigs were treated with 100,000 IU/kg IFN-α in PBS or the same volume of PBS for 24 h. (A) Total RNA was prepared and analyzed for the expression of CYP3A29 and PXR by qRT-PCR. (B) Microsome preparation was performed and analyzed for the expression of CYP3A29 and PXR by Western blot analysis. Lanes 1–3: three control pigs were treated with PBS; Lanes 4–6: three pigs were treated with 100,000 IU/kg IFN-α.

further confirmed by a luciferase reporter assay. It is well known that PXR plays a key role in regulating the expression of CYP3A [27,28], and the role of PXR in drug–drug interactions (DDIs) has been studied extensively over the last decades [29,30,25]. However, how PXR mediates the expression of the target genes remains unknown. The PXR response elements, such as PBREM and XREM, are conserved in many human genes, such as CYP3A4 [31]. Some studies have suggested that downregulation of CYPs (e.g., CYP2B, CYP3A) is due to the downregulation of PXR and constitutive androstane receptor (CAR) [32]; therefore, PXR expression may upregulate CYP3A29 expression. In our study, we demonstrated that PXR knockdown decreased CYP3A29 expression and that IFN-α significantly increased CYP3A29 in normal cells but not in the PXR knockdown cells, while PXR overexpression increased CYP3A29 expression. Furthermore, PXR is essential for the IFN-α-mediated upregulation of CYP3A29 expression in pig hepatocytes.

CYP3A29 plays an important role in drug metabolism in pig liver, and is a very useful model for the study of human CYP3A4. In the present study, we used the pig to demonstrate that IFN-α upregulated CYP3A29 expression via PXR and could increase the metabolic activity of the enzyme.

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References

[1] T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, F.P. Guengerich, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians, J. Pharmacol. Exp. Ther. 270 (1994) 414–423.
[2] C. Rodriguez-Antona, A. Gomez, M. Karlgren, S.C. Sim, M. Ingelman-Sundberg, Molecular genetics and epigenetics of the cytochrome P450 gene family and its relevance for cancer risk and treatment, Hum. Genet. 127 (2010) 1–17.
[3] S. Rendic, F.J. Di Carlo, Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors, Drug Metab. Rev. 29 (1997) 413–580.
[4] M. Shou, M. Hayashi, Y. Pan, Y. Xu, K. Morrissey, L. Xu, G.L. Skiles, Modeling, prediction, and in vitro in vivo correlation of CYP3A4 induction, Drug Metab. Dispos. 36 (2008) 2355–2370.
[5] M.W. Balk, Animal models: assessing the scope of their use in biomedical research, in: Proceedings of The Sixth Charles River International Symposium on Laboratory Animal, October 1985, Kyoto, Japan, Emerging models in the USA: swine, woodchucks, and the hairless guinea pig, Alan R. Liss Inc., New York, NY, 1987, pp 311–326.
[6] H.A. Thorn, A. Lundahl, J.A. Schrickx, P.A. Dickinson, H. Lennernas, Drug metabolism of CYP3A4, CYP2C9 and CYP2D6 substrates in pigs and humans, Eur. J. Pharm. Sci. 43 (2011) 89–98.
[7] G. Zamaratskaia, V. Zlabek, E. Ropstad, O. Andresen, In vitro and in vivo association of porcine hepatic cytochrome P450 3A and 2C activities with testicular steroids, Reprod. Domest. Anim. 47 (2012) 891–898.
[8] E. Anzenbacherová, J. Baranová, R. Zuber, A. Pechová, P. Anzenbacher, P. Soucek, J. Martínková, Model systems based on experimental animals for studies on drug metabolism in man: (mini)pig cytochromes P450 3A29 and 2E1, Basic Clin. Pharmacol. Toxicol. 96 (2005) 244–245.
[9] M. Yao, M. Dai, Z. Liu, L. Huang, D. Chen, Y. Wang, D. Peng, X. Wang, Z. Liu, Z. Yuan, Comparison of the substrate kinetics of pig CYP3A29 with pig liver microsomes and human CYP3A4, Biosci. Rep. 31 (2011) 211–220.
[10] P. Soucek, R. Zuber, E. Anzenbacherová, P. Anzenbacher, F.P. Guengerich, Minipig cytochrome P450 3A, 2A and 2C enzymes have similar properties to human analogs, BMC Pharmacol. 1 (2001) 11.
[11] P.H. Nissen, A.K. Winterø, M. Fredholm, Mapping of porcine genes belonging to two different cytochrome P450 subfamilies, Anim. Genet. 29 (1998) 7–11.
[12] I.L. Campbell, T. Krucker, S. Steffensen, Y. Akwa, H.C. Powell, T. Lane, D.J. Carr, L.H. Gold, S.J. Henriksen, G.R. Siggins, Structural and functional neuropathology in transgenic mice with CNS expression of IFN-alpha, Brain Res. 835 (1999) 46–61.
[13] H. Yoshiji, R. Noguchi, S. Kuriyama, J. Yoshii, Y. Ikenaka, Ombination of interferon and angiotensin-converting enzyme inhibitor, perindopril, suppresses liver carcinogenesis and angiogenesis in mice, Oncol. Rep. 13 (2005) 491–495.
[14] S. Radaeva, B. Jaruga, F. Hong, W.H. Kim, S. Fan, H. Cai, S. Strom, Y. Liu, O. El-Assal, B. Gao, Interferon-alpha activates multiple STAT signals and down-regulates c-Met in primary human hepatocytes, Gastroenterology 122 (2002) 1020–1034.
[15] X. Gu, S. Ke, D. Liu, T. Sheng, P.E. Thomas, A.B. Rabson, M.A. Gallo, W. Xie, Y. Tian, Role of NF-kappaB in regulation of PXR-mediated gene expression: a mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents, J. Biol. Chem. 281 (2006) 17882–17889.
[16] D. Shi, D. Yang, B. Yan, Dexamethasone transcriptionally increases the expression of the pregnane X receptor and synergistically enhances pyrethroid esfenvalerate in the induction of cytochrome P450 3A23, Biochem. Pharmacol. 80 (2010) 1274–1283.
[17] J. Yang, C. Hao, D. Yang, D. Shi, X. Song, X. Luan, G. Hu, B. Yan, Pregnane X receptor is required for interleukin-6-mediated down-regulation of cytochrome P450 3A4 in human hepatocytes, Toxicol. Lett. 197 (2010) 219–226.
[18] L.J. Li, W.B. Du, Y.M. Zhang, J. Li, X.P. Pan, J.J. Chen, H.C. Cao, Y. Chen, Y.M. Chen, Evaluation of a bioartificial liver based on a nonwoven fabric bioreactor with porcine hepatocytes in pigs, J. Hepatol. 44 (2006) 317–324.
[19] M. Maruyama, T. Totsugawa, T. Kunieda, T. Okitsu, N. Shibata, M. Takesue, Y. Kurabayashi, M. Oshita, S. Nakaji, M. Kodama, N. Tanaka, N. Kobayashi, Hepatocyte isolation and transplantation in the pig, Cell Transplant. 12 (2003) 593–598.
[20] X. Pan, W. Du, X. Yu, G. Sheng, H. Cao, C. Yu, G. Lv, H. Huang, Y. Chen, J. Li, L.J. Li, Establishment and characterization of immortalized porcine hepatocytes for the study of hepatocyte xenotransplantation, Transplant. Proc. 42 (2010) 1899–1906.
[21] J.Q. Clement, M.F. Wilkinson, Rapid induction of nuclear transcripts and inhibition of intron decay in response to the polymerase II inhibitor DRB, J. Mol. Biol. 299 (2000) 1179–1191.
[22] D. Rudin, S.M. Shah, A. Kiss, R.V. Wetz, V.M. Sottile, Interferon and lamivudine vs. interferon for hepatitis B e antigen-positive hepatitis B treatment: meta-analysis of randomized controlled trials, Liver Int. 27 (2007) 1185–1193.
[23] Q. Zhang, Y. Wang, L. Wei, D. Jiang, J.H. Wang, H.Y. Rao, L. Zhu, H. Chen, R. Fei, X. Cong, Role of ISGF3 in modulating the anti-hepatitis B virus activity of interferon-alpha in vitro, J. Gastroenterol. Hepatol. 23 (2008) 1747–1761.
[24] J.H. Lin, CYP induction-mediated drug interactions: in vitro assessment and clinical implications, Pharm. Res. 23 (2006) 1089–1116.
[25] I. Meijerman, J.H. Beijnen, J.H. Schellens, Herb-drug interactions in oncology: focus on mechanisms of induction, Oncologist 11 (2006) 742–752.

[26] A.A. Izzo, E. Ernst, Interactions between herbal medicines and prescribed drugs: an updated systematic review, Drugs 69 (2009) 1777–1798.
[27] B. Goodwin, E. Hodgson, C. Liddle, The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module, Mol. Pharmacol. 56 (1999) 1329–1339.
[28] J.M. Lehmann, D.D. McKee, M.A. Watson, T.M. Willson, J.T. Moore, S.A. Kliewer, The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions, J. Clin. Invest. 102 (1998) 1016–1023.
[29] G. Bertilsson, J. Heidrich, K. Svensson, M. Asman, L. Jendeberg, M. Sydow-Bäckman, R. Ohlsson, H. Postlind, P. Blomquist, A. Berkenstam, Proc. Natl. Acad. Sci. USA 95 (1998) 12208–12213.
[30] S.A. Kliewer, B. Goodwin, T.M. Willson, The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism, Endocr. Rev. 23 (2002) 687–702.
[31] B. Goodwin, M.R. Redinbo, S.A. Kliewer, Regulation of cyp3a gene transcription by the pregnane x receptor, Annu. Rev. Pharmacol. Toxicol. 42 (2002) 1–23.
[32] A.P. Beigneux, A.H. Moser, J.K. Shigenaga, C. Grunfeld, K.R. Feingold, Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response, Biochem. Biophys. Res. Commun. 293 (2002) 145–149.