Regular Article

Selective Suppressions of Human CYP3A Forms, CYP3A5 and CYP3A7, by Troglitazone in HepG2 Cells

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Summary: Troglitazone is an insulin sensitizer and also known as an agonist of peroxisome proliferator-activated receptor-γ (PPARγ). In the present study, we have studied the influence of troglitazone on CYP3A form expressions in HepG2 cells for a model of human tissue. Interestingly, constitutively expressed forms of cytochrome P450, CYP3A5 and CYP3A7, were suppressed by the pretreatment of troglitazone but not of the related thiazolidinediones, pioglitazone and rosiglitazone in this cell line. A major liver CYP3A form, CYP3A4, was not detected in this cell line with and without troglitazone treatment. The troglitazone-mediated suppressions of CYP3A5 and CYP3A7 were found to be independent of expression levels of nuclear transcriptional factors, PXR, RXRα and PPARγ. These results suggest that the selectively suppressive effects of troglitazone on CYP3A5 and CYP3A7 expressions may be caused by a novel pathway.

Key words: CYP3A5; CYP3A7; HepG2; troglitazone; suppression; transcriptional regulation

Introduction

The human cytochrome P450 3A (CYP3A) subfamily includes mainly three forms, CYP3A4, CYP3A5 and CYP3A7. Notably, CYP3A4 is a major P450 form in human livers and intestines. CYP3A4 is involved in metabolism of endogenous and exogenous compounds, and its expression is enhanced by treatment with chemicals such as rifampicin, clotrimazole or phenobarbital. Troglitazone has recently been shown to increase human urinary excretion of 6β-hydroxycortisol and also the expression level of CYP3A protein in primary cultures of human hepatocytes. Troglitazone has been used as an oral antidiabetic drug for treatment of type II diabetes and is also known as an agonist of the peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear transcriptional factor. However, the mechanism enhancing the human CYP3A form remains obscure.

The induction of CYP3A form in human-derived cell lines has been studied for models of human tissues. HepG2 cells are one of most useful cell lines as a human liver model because of the well-differentiated function of this human hepatoblastoma. Thus, we have examined the influence of troglitazone on microsomal CYP3A expression in HepG2 cells as the tool.

Materials and Methods

Chemicals

Troglitazone, pioglitazone and rosiglitazone were kindly donated by Sankyo Co. Ltd. (Tokyo, Japan). Rifampicin was purchased from Sigma (St. Louis, MO).

Cell Culture

Human hepatoma, HepG2 cells, were maintained in Dulbecco’s modified Eagle’s high-glucose medium supplemented with antibiotic-antimycotic solution (GIBCO BRL, Rockville, MD), MEM nonessential amino acid solution (GIBCO BRL) and 10% fetal bovine serum (FBS, Boehringer Mannheim, Germany) at 37°C under an atmosphere of 5% CO2. HepG2 cells were seeded at 1.0 × 10⁶ per well in 6-well plates, and cultured for 24 h. The medium was changed into FBS-free, but contained chemicals (10 μM in Fig. 1 and 1 μM and/or 10 μM in Fig. 2) or dimethylsulfoxide (DMSO, final 0.1%) as vehicle for a successive 48 h.

RT-PCR

Total RNAs were isolated by the AGPC method. To confirm the quality of RNA, the total RNAs were separated by electrophoresis in a denaturing 1% agarose gel. Reverse transcriptase reactions were carried out us-
Western blot analysis of microsomal protein isolated from HepG2 cells. Electrophoresis was performed in an 8% SDS-polyacrylamide gel and the blotted membrane was probed with anti-human CYP3A4 polyclonal antibody.\(^{12}\) (A) Western blot analysis of microsomal proteins from chemicals-treated (10 \(\mu\)M) HepG2 cells and intensities of detected bands. (B) Western blot analysis of microsomal proteins from rifampicin (10 \(\mu\)M) and/or troglitazone-treated (10 \(\mu\)M) HepG2 cells and intensities of detected bands. Band intensities were quantified by NIH Image software (version 1.59). CT; control (vehicle alone), RIF; rifampicin, TRO; troglitazone, PIO; pioglitazone, BRL; rosiglitazone.

**Western Blotting**

Western blotting was carried out according to the method as reported previously.\(^{11}\) Microsomal proteins (50 \(\mu\)g from HepG2 cells) were separated by SDS-PAGE using 8% gel. The blotted membrane was probed with a polyclonal anti-human CYP3A antibody\(^{12}\) and developed by an alkaline phosphatase method.\(^{13}\) The visualized bands were scanned and the intensities were calculated by use of the NIH Image software (version 1.59).

**Results**

To examine whether CYP3A protein expressions are altered by the treatment of HepG2 cells with troglitazone, microsomal CYP3A protein levels were analyzed by Western blotting (Fig. 1(A)). Human CYP3A protein content was decreased in the microsomal fraction from troglitazone-treated HepG2 cells (about 35% of control), although it was increased by the pretreatment with rifampicin, a typical CYP3A4 inducer. The CYP3A content was, however, unaltered by the treatments with other thiazolidinedione compounds, pioglitazone or rosiglitazone. Moreover, troglitazone-induced decrease of the CYP3A protein expression was not reversed fully by simultaneous treatment with rifampicin (Fig. 1(B)). These results suggest the selective suppression of the CYP3A expression by troglitazone, but not by pioglitazone and rosiglitazone in HepG2 cells.

Identification of CYP3A forms in HepG2 cells was
Fig. 2. RT-PCR analysis of mRNA expressions in troglitazone- and/or rifampicin-treated HepG2 cells. PCR products were separated by an agarose gel electrophoresis (1% gel/1 x TBE). PCR conditions and primer sequences used in this study were shown in Table 1. (A) Confirmation of the PCR primer specificity using subcloned human CYP3A cDNAs (line 1: pCMV3A4, line 2: pCMV3A5 and line 3: pCMV3A7) as templates (B) RT-PCR analysis of mRNA expressions. M; molecular weight maker

As shown in Fig. 2(A), these primers detected only a designated CYP3A form, without a cross-amplification under this PCR condition. A major adult human CYP3A form, CYP3A4 was not detected in HepG2 cells with or without pretreatment of rifampicin in the PCR experiment, which was in contrast to a previous study.6) Similar results were also obtained with troglitazone (Fig. 2(B)). These results may indicate a functional defect of the CYP3A4 expression and/or induction by troglitazone and rifampicin in HepG2 cells used in this experiment. On the other hand, CYP3A7, a fetal CYP3A form, was constitutively expressed in HepG2 cells. The CYP3A7 expression was decreased by the pretreatment with 10 μM of troglitazone, but was retained by simultaneous treatment with rifampicin and troglitazone (10 μM) (Fig. 2(B)). Another form, CYP3A5, was weakly expressed in HepG2 cells. The CYP3A5 expression is decreased by treatment of 10 μM of troglitazone similar to that observed with CYP3A7. Although the CYP3A5 expression was enhanced by the treatment with rifampicin in HepG2, the rifampicin induction was not reversed in the presence of 10 μM of troglitazone (data not shown). These results confirm that the CYP3A forms suppressed in troglitazone-treated HepG2 cells are CYP3A7 and CYP3A5, but not CYP3A4.

A steroid receptor, PXR (pregnane X receptor), has been reported to be a key regulator of CYP3A4 and CYP3A7 genes transactivations triggered by chemicals such as rifampicin.7,14–16) Thus, we examined whether or not troglitazone decreases PXR expression. The PXR mRNA was constitutively detected and the levels of that were not influenced by the pretreatment with troglitazone and/or rifampicin in HepG2 cells (Fig. 2(B)). In addition, specific mRNA levels of PPARγ were also not influenced by the treatment of troglitazone in HepG2 cells. Furthermore, specific mRNA levels of retinoid X receptorα (RXRα), which is a heterodimer partner of PXR and PPARγ, were not changed by this treatment (Fig. 2(B)).

Discussion

Hepatic dysfunction by troglitazone is a more serious problem than by other thiazolidinediones (TZDs).4) It is also reported that troglitazone, but not rosiglitazone, induces human liver CYP3A4.4) Camp et al. showed the distinct regulation of several genes by treatment with troglitazone and rosiglitazone in HepG2 cells using two-dimensional protein gel electrophoresis.17) They demonstrated the decreased expression of at least three proteins by troglitazone but not by rosiglitazone. These data suggest the distinct influences of these two TZDs. However, the reason that causes the difference among TZDs is not yet well understood.

In the present study, we have examined the influence...
of TZDs on expression of the human CYP3A forms in HepG2 cells. Interestingly, expression of CYP3A7 was suppressed in troglitazone-treated HepG2 cells but not in cells treated with other TZDs, pioglitazone or rosiglitazone. The suppression was observed at both protein and mRNA levels, and thus troglitazone possibly affects the transcriptional regulation of the CYP3A7 gene. PXR, which is a transactivator of the CYP3A7 gene, exists in HepG2 cells (Fig. 2B) and troglitazone is one of the activators of PXR. Nevertheless, CYP3A7 was suppressed by treatment of troglitazone in HepG2 cells. This suppression was not caused by down-regulation of PXR, PPARγ and RXRα as shown in Fig. 2B). Therefore, the suppression of CYP3A7 is likely to be caused by a novel pathway.

CYP3A5 expression was also suppressed in troglitazone-treated HepG2 cells. The suppression was not restored by simultaneous treatment with rifampicin, although the CYP3A7 suppression by troglitazone was restored by the same treatment. Thus, the reason for the incomplete recovery of CYP3A content may be the lack of CYP3A5 induction capability in troglitazone- and rifampicin-treated HepG2 cells.

In conclusion, we have shown the selective suppressive effect of troglitazone on CYP3A5 and CYP3A7 expressions in HepG2 cells without the down regulation of transcription factors such as PXR, PPARγ and RXRα. This is likely to be caused by a novel pathway.

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