Differential Effects among Thiazolidinediones on the Transcription of Thromboxane Receptor and Angiotensin II Type 1 Receptor Genes

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Peroxisome proliferator-activated receptor (PPAR)-γ ligands thiazolidinediones (TZDs) have recently been reported to be anti-hypertensive and anti-atherosclerotic. We have previously shown that one of the TZDs troglitazone significantly suppressed the transcription of both thromboxane receptor (TXR) and angiotensin II type 1 receptor (AT1R) genes in vascular smooth muscle cells (VSMCs) by activating PPAR-γ. In the present study, we compared the effects of troglitazone and other TZDs on the transcription of these genes. TXR and AT1R mRNAs in rat VSMCs were determined by semi-quantitative RT-PCR. Luciferase chimeric constructs containing either the 989-bp rat TXR gene promoter or the 1,969-bp rat AT1R gene promoter were transiently transfected into VSMCs. The cells were incubated with troglitazone, RS-1455 (a derivative of troglitazone which does not contain the hindered phenol resembling α-tocopherol), pioglitazone, or rosiglitazone for 12 h before harvesting. mRNA expression levels of TXR and AT1R were significantly decreased by troglitazone in contrast to rosiglitazone. TXR gene and AT1R gene transcription was significantly suppressed by troglitazone in a dose-dependent manner, while RS-1455 was less potent. Pioglitazone and rosiglitazone weakly suppressed the transcription of both genes in a manner almost similar to RS-1455. We have shown that troglitazone suppresses transcription of both the TXR and AT1R genes more potently than other TZDs. The structure of troglitazone and RS-1455 is identical except the hindered phenol, which is recently recognized to function as an antioxidant. Moreover, we have shown that the potency for activating PPAR-γ is almost identical between troglitazone and RS-1455. We therefore speculate that the strong transcriptional suppression of the TXR and AT1R genes by troglitazone may be mediated in part by its antioxidant effect. (Hypertens Res 2001; 24: 229-233)

Key Words: troglitazone, pioglitazone, rosiglitazone, PPAR-γ, α-tocopherol, antioxidant

Introduction

Thromboxane (TX) A2 and angiotensin (A) II both exert contraction and growth of vascular smooth muscle cells (VSMCs) via TX receptor (TXR) and AII type 1 receptor (AT1R), respectively (1, 2), both of which result in the progression of hypertension and atherosclerosis. Insulin sensitizing thiazolidinediones (TZDs) have been shown to be ligands for the nuclear receptor peroxisome proliferator
ator-activated receptor (PPAR-γ) (3). TZDs, especially troglitazone, have recently been shown to be anti-hypertensive and anti-atherosclerotic (4-6). We have also shown that troglitazone significantly suppressed the transcriptional activity of both TXR and AT1R genes in rat VSMCs (7, 8). The transcriptional suppression of these genes was most likely mediated by activating PPAR-γ, since the suppression was augmented by the overexpression of PPAR-γ (7, 8). We have also shown that activation of PPAR-γ suppresses the gene transcription of rat thromboxane synthase in macrophages via an interaction with NF-E2 related factor 2 (9). Troglitazone, but not other TZDs, has recently been characterized to function as an antioxidant by scavenging reactive oxygen species (10-14). Since troglitazone, but not other TZDs, contains a hindered phenol structurally related to α-tocopherol, it is speculated that the antioxidant effect of troglitazone may be mediated by the hindered phenol (14). In order to study the role of the hindered phenol in the transcriptional suppression of TXR and AT1R genes, we here compared the effects of several TZDs, troglitazone, pioglitazone (15), rosiglitazone (16), and RS-1455 (17), a derivative of troglitazone which lacks the hindered phenol (Fig. 1), on the transcription of TXR and AT1R genes.

Methods

Plasmids

Previously subcloned chimeric constructs containing either the 989-bp 5′-flanking region (FL) of the rat TXR gene promoter (TXR-luc) (18) or the 1,969-bp 5′-FL of the rat AT1R gene promoter (AT1R-luc) (19) fused upstream of luciferase cDNA was used for the transient transfection studies. The β-galactosidase control plasmid in pCMV was purchased from Clontech (Palo Alto, USA).

Semi-Quantitative RT-PCR

When cultured rat VSMCs reached 70% confluence, the media were changed to stripped media (20) and cells were incubated for 5-6 h. Then the cells were incubated either in the absence or presence of 50 mmol/l troglitazone (kindly provided by Sankyo Co., Ltd., Tokyo, Japan) or rosiglitazone (kindly provided by Sankyo Co., Ltd.) for an additional 12 h. Their total RNAs were then extracted using an RNeasy mini kit (Quiagen, Valencia, USA) according to the manufacture's instructions. The extracted RNAs (1 μg) were then subjected to reverse transcription (RT)-polymerase chain reaction (PCR) using specific primers for rat TXR (18), rat AT1R (21), or rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (18) for 30 cycles.

Transient Transfection Study/Luciferase Assay

When cultured rat VSMCs reached 70% confluence, the media were changed to stripped media (20) and cells were incubated for 5-6 h. Then the cells were transfected using lipofectin according to the manufacturer’s instructions (Gibco BRL, Gaithersburg, USA). Briefly, 1.2 μg reporter plasmid (either TXR-luc or AT1R-luc) and 0.8 μg β-galactosidase control plasmid were mixed with 6 μl lipofectin per 3.5 cm plate. Twelve hours after transfection, the media were replaced with stripped media and the cells were incubated for an additional 12 h. The cells were then incubated with several concentrations of troglitazone, pioglitazone (kindly provided by Takeda Chemical Industries, Osaka, Japan), rosiglitazone, or RS-1455 (kindly provided by Sankyo Co., Ltd.) for 12 h, and then their luciferase activities were measured. After harvesting, the cell extracts were analyzed for both luciferase and β-galactosidase activities (20) to determine the expression of both genes. The transfection efficiency was normalized by the β-galactosidase expression.

In order to study the potency of troglitazone and RS-1455 for PPAR-γ activation, HepG2 cells were transfected with a GAL4-PPAR-γ ligand binding domain (LBD) chimeric expression construct pM-PPAR-γ and a GAL4-responsive reporter plasmid pFR-Luc (Stratagene, La Jolla, USA) using FuGENE6 Transfection Reagent (Boehringer Mannheim, Mannheim, Germany) as previously reported (22). The transfected cells were incubated with several concentrations of either troglitazone or RS-1455 for 48 h, and then their luciferase activities were measured.

Statistical Analyses

Statistical analyses of the transient transfection studies were performed by one-factor ANOVA using StatView 4.0 software (ABACUS Concepts, Berkeley, USA).
Fig. 2. Effects of TZDs on TXR and AT1R mRNA expression. Total RNAs extracted from rat VSMCs incubated in the absence or presence of 50 μmol/l troglitazone or rosiglitazone were subjected to RT-PCR using specific primers either for rat TXR, rat AT1R, or rat GAPDH. The bar graph represents the mean±SE of RT-PCR products from TXR (A) and AT1R (B) mRNA normalized by those from GAPDH mRNA expressed as a percentage of the control value (n=4). *p<0.01 as compared with controls.

Results

Effects of TZDs on TXR and AT1R mRNA Expression

We first compared the effects of troglitazone and rosiglitazone on the endogenous TXR and AT1R mRNA expression in rat VSMCs. As seen in Fig. 2A and B, 50 μmol/l troglitazone significantly decreased the mRNA expression of both TXR (to 42.0% of the basal level) and AT1R (to 45.8% of the basal level), while 50 μmol/l rosiglitazone was less potent (reducing TXR mRNA to 77.2% and AT1R to 70.0% of the basal level).

Effects of TZDs on TXR Promoter Activity

We next compared the effects of troglitazone, pioglitazone, and rosiglitazone on transcription of the TXR gene. As seen in Fig. 3, troglitazone significantly suppressed TXR-luc activity in a dose-dependent manner (to 65.5% of the basal level at 10 μmol/l, and to 10.6% of the basal level at 50 μmol/l). Pioglitazone (86.6% of the basal level at 10 μmol/l, and 75.6% of the basal level at 50 μmol/l) and rosiglitazone (93.2% of the basal level at 10 μmol/l, and 55.7% of the basal level at 50 μmol/l) also suppressed TXR-luc activity in a dose-dependent manner, although their suppressive effects were much less potent than that of troglitazone. When we treated the transfected cells with RS-1455, a derivative of troglitazone which does not contain the hindered phenol, a similar dose-dependent suppression of TXR-luc activity that was as potent as those of pioglitazone and rosiglitazone was observed (to 93.0% of the basal level at 10 μmol/l, and to 66.3% of the basal level at 50 μmol/l).

Effects of TZDs on AT1R Promoter Activity

We next compared the effects of troglitazone, pioglitazone, and rosiglitazone on transcription of the AT1R gene. As seen in Fig. 4, troglitazone significantly suppressed AT1R-luc activity in a dose-dependent manner (to 44.3% of the basal level at 10 μmol/l, and to 7.4% of the basal level at 50 μmol/l). Pioglitazone (70.9% of the basal level at 10 μmol/l, and 50.0% of the basal level at 50 μmol/l) and rosiglitazone (69.5% of the basal level at 10 μmol/l, and 45.6% of the basal level at 50 μmol/l) weakly suppressed AT1R-luc activity in a dose-dependent manner. When the transfected cells were treated with RS-1455, a weak suppression of AT1R-luc activity (to 46.1% of the basal level at 50 μmol/l) was seen, much like those by pioglitazone and rosiglitazone.

Effects of Troglitazone and RS-1455 on PPAR-γ Activation

We next compared the effects of troglitazone and RS-1455 on PPAR-γ activation. As shown in Fig. 5, both
troglitazone and RS-1455 induced similar levels of GAL4-responsive reporter transactivation in a dose-dependent manner, most likely by activating PPAR-γ LBD of the GAL4-PPAR-γ chimera. These data suggest that the potency for activating PPAR-γ is almost identical between troglitazone and RS-1455.

**Discussion**

We have first confirmed our previous findings about the significant expression suppression of both TXR and AT1R genes by troglitazone at mRNA as well as transcriptional levels (7, 8). It is suggested that troglitazone inhibited the expression of TXR and AT1R at the gene transcription level. Pioglitazone and rosiglitazone also weakly suppressed the transcription of both genes. Since pioglitazone and rosiglitazone bind to and activate PPAR-γ as potently as troglitazone (3, 15, 16), we speculate that troglitazone may exert some additional effects other than activating PPAR-γ for the suppression of the TXR and AT1R genes comparing with other TZDs.

The chemical structure of troglitazone is unique among TZDs, since only troglitazone contains a hindered phenol structurally related to α-tocopherol (14). Recently, both basic (10, 11) and clinical (12, 13) studies have shown that troglitazone can function as an antioxidant to inhibit LDL oxidation, and the above-described hindered phenol has been shown to scavenge reactive oxygen species (14). RS-1455 is a derivative of troglitazone which does not contain the hindered phenol but still maintains the blood glucose-lowering effect of troglitazone (17). Therefore, in order to study the role played by the hindered phenol of troglitazone in suppression of the TXR and AT1R genes, we have compared between the effects of troglitazone and RS-1455 on the transcription of these genes. Interestingly, RS-1455 induced a weaker transcriptional suppression of both the TXR and AT1R genes than troglitazone, and its potency was almost the same as those of pioglitazone and rosiglitazone. Moreover, we observed that troglitazone and RS-1455 showed almost the same potency for activating PPAR-γ. These data strongly suggest that the antioxidative effect of the hindered phenol of troglitazone may partly contribute to the transcriptional suppression of both the TXR and AT1R genes in addition to activating PPAR-γ. The mechanisms by which the antioxidative effect of troglitazone suppresses transcription of these genes remain unclear. It is possible that the inhibition of oxidative stress by troglitazone may affect the redox state in VSMCs, which may in turn result in the transcriptional suppression of these genes either directly or indirectly.

In summary, we have observed that troglitazone suppresses transcription of both TXR and AT1R genes significantly more than other TZDs such as pioglitazone and rosiglitazone. The comparative study between troglitazone and RS-1455 strongly suggested that the antioxidative...
effect mediated by the hindered phenol of troglitazone may in part cause the transcriptional suppression of these genes. Since the suppression of TXR and AT1R expression may possibly inhibit the progression of hypertension and atherosclerosis, we speculate that TZDs containing the hindered phenol, such as troglitazone, may have a more beneficial therapeutic effect on hypertension and atherosclerosis, and particularly with diabetes mellitus, than other TZDs. The innovation of new TZDs containing a hindered phenol is thus desirable for the treatment of diabetic patients whose cases are complicated by hypertension or atherosclerosis.

References

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