Review

Metabolic and Non-Metabolic Factors Determining Troglitazone Hepatotoxicity: A Review

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Summary: Troglitazone (TGZ), a thiazolidinedione class of antidiabetic agent, causes serious idiosyncratic hepatotoxicity. TGZ is metabolized into reactive metabolites that covalently bind to cellular macromolecules, one of which is oxidation at the chromane ring, a unique structure of TGZ, and another involves oxidative cleavage of the thiazolidinedione ring, a structure common to less hepatotoxic antidiabetics, rosiglitazone and pioglitazone. TGZ is cytotoxic to HepG2 cells and rat and human hepatocytes. However, the role of the reactive metabolite on the TGZ toxicity is controversial, because there was no correlation of the generation of the reactive metabolites with susceptibility to the TGZ cytotoxicity, and chemical inhibitors of drug metabolizing enzymes could not protect the cells against the toxicity. Mitochondrial dysfunction, especially mitochondrial permeability transition, may be a pathophysiological event, which is mediated by TGZ itself and is a major non-metabolic factor. Other events such as apoptosis and PPARy-dependent steatosis could be also meditated by TGZ, while inhibition of bile salt export pump, a cause of TGZ-induced cholestasis, may be caused by the TGZ sulfate. In conclusion, although the TGZ is biotransformed into chemically reactive metabolites, there is currently no potential evidence for involvement of the reactive metabolite in the TGZ-induced liver injury.

Key words: troglitazone; thiazolidinediones; hepatotoxicity; reactive metabolites; CYP3A4; rosiglitazone; pioglitazone; hepatocytes; mitochondria; peroxisome proliferator-activated receptor y

Introduction

Troglitazone, a 2,4-thiazolidinedione, was the first of new class of drug for type two diabetes. It acts as an agonist of peroxisome proliferator-activated receptor y (PPARy) and improves insulin resistance. However, clinical trials indicated that 1.9% of patients with the troglitazone therapy experienced elevated serum alanine aminotransferase more than 3 times the upper limit of normal, revealing significant frequency of mild and moderate hepatotoxicity.1) Furthermore, it was also reported that rare but severe liver injury was developed, and this serious idiosyncratic hepatotoxicity of troglitazone led to its withdrawal from the market.2,3) Clinical feature of the troglitazone hepatotoxicity suggests that its mechanism is categorized into metabolic idiosyncrasy.4) Although such hepatotoxicity was not reproduced in experimental animals, extensive studies including in vitro experiments with hepatocytes have been attempted to elucidate mechanisms underlying the troglitazone hepatotoxicity.5,6) As well as other hepatotoxic drugs, troglitazone is metabolized to a reactive metabolite that covalently binds to cellular macromolecules, whereas its role in the hepatotoxicity is controversial. In addition, recent studies suggested newer and safer thiazolidinediones, rosiglitazone and pioglitazone shared common metabolic properties to troglitazone.7) This review focuses roles of reactive metabolites and importance of non-metabolic factors in pathogenesis of hepatotoxicity of troglitazone and other thiazolidinediones.

Metabolism of Troglitazone into Stable Metabolites

TGZ is a drug with an oral bioavailability of 40–50% and a high plasma protein binding (>99%).8) There is no evidence that TGZ accumulates specifically in the liver. The major metabolite of TGZ is the sulfate at phenolic hydroxy moiety (Fig. 1) in experimental animals and humans,8,9) which may be involved in cholestatic liver injury through inhibition of bile acid
transport as described below. A phenol sulfotransferase isozyme ST1A3 was identified as a major enzyme responsible for the sulfation of TGZ in human.\textsuperscript{9} The phenolic hydroxy group of TGZ is also conjugated to a glucuronide (Fig. 1), but to a lesser extent than the sulfate. Major UDP-glucuronosyltransferase (UGT) isozymes involved in the glucuronidation of TGZ were UGT1A1 in human livers and UGT2B2 in rat livers.\textsuperscript{11,12} TGZ is also subjected to oxidation, yielding a quinone metabolite (Fig. 1).\textsuperscript{9} The quinone metabolite is relatively stable, whereas it may be reduced to a quinol because a quinol glucuronide was obtained from bile in the TGZ-treated rats.\textsuperscript{13} CYP3A4 and CYP2C8 were reported to be major cytochrome P450 isoforms involved in the quinone formation in human.\textsuperscript{14} The reaction mechanism on quinone formation has been suggested to be atypical of CYP3A4 consistent with a one-electron oxidation mechanism where an intermediate phenoxy radical combines with ferryl oxygen to subsequently form the quinone metabolite.\textsuperscript{15,16} These conjugates were also obtained from incubation of human hepatocytes with TGZ\textsuperscript{9} and from bile of the TGZ-treated rats.\textsuperscript{16} A GSH conjugate directly substituted in the TZD ring (ML3) was also isolated from bile of the TGZ-treated rats (Fig. 2).\textsuperscript{13} Treatment of rats with TZD quinone also yielded an analogue of one of the GSH conjugates of TGZ and a GSH conjugate of TGZ hydroquinone as biliary metabolites, indicating that TGZ quinone acts as a precursor of reactive metabolites.\textsuperscript{13} A quinone epoxide was identified from the incubation of HepG2 cells and human primary hepatocytes with TGZ, while it may not be a product by epoxidation of TGZ quinone.\textsuperscript{16}

The GSH adduct formation by the TGZ oxidation is proposed to involve the potential of TGZ to covalently modify hepatic proteins and to cause oxidative stress through redox cycling processes, either of which may play a role in the drug-induced liver injury. It is based on the assumption that TGZ reactive metabolites appear relatively stable and are able to diffuse from the active site of P450 to react with cellular target and GSH. On the other hand, TGZ has been shown to behave as a mechanism-based inactivator of CYP3A4,\textsuperscript{16,21} suggesting a highly reactive metabolite that may not diffuse from active site of P450 is also generated through the TGZ metabolism. The modification of hepatic proteins with reactive metabolites of TGZ was recently demonstrated by using \textsuperscript{14}C-labeled TGZ. Incubation of liver microsomes from rats treated with various P450 inducers or Supersomes (cDNA-expressed human P450s) with \textsuperscript{14}C-TGZ indicated that CYP3A enzymes in rats and CYP3A4 were mainly responsible for the oxidation of TGZ into reactive metabolites that covalently bind to microsomal proteins.\textsuperscript{22}

\textbf{Roles of Reactive Metabolites in Cytotoxicity of Troglitazone to Hepatocytes}

In relation to the formation of reactive metabolite through the oxidation of TGZ, the cytotoxicity of TGZ
and its metabolites has been examined in various cell systems including HepG2 cells and hepatocytes from experimental animals and human. It should be noted that the cytotoxicity in most of the following in vitro studies was observed when the cells were exposed to TGZ at the concentration of nearly 50 μM, while concentrations of 3.6 and 6.3 μM were reported as the maximum plasma concentration in humans taking TGZ at therapeutic doses of 400 and 600 mg/day, respectively. In addition, it was pointed out that the absence of albumin in the culture media increased TGZ bioavailability. Indeed, the TGZ toxicity was markedly reduced when albumin was added to the media. In general, the cells have been cultured in the media with fetal calf (bovine) serum, while serum-free conditions or low concentrations of the serum were also shown to augment cellular responses. Therefore, data on in vitro cytotoxicity studies should be assessed with caution, because they may commonly overestimate the toxicity.

As described above, TGZ is metabolized to a reactive metabolite that covalently binds to cellular macromolecules, whereas the role of the covalent adduct to the cells is controversial. Primary cultured rat hepatocytes, which possess active drug-metabolizing enzymes, were less sensitive than HepG2 cells, which express lower P450 enzymes. Studies with cryopreserved human hepatocytes with various enzyme activities for P450s, UGT and PST revealed that there was no correlation between the TGZ cytotoxicity and any specific enzyme activities, whereas a negative correlation was made between the TGZ-induced cytotoxicity to hepatocytes and sum of CYP3A4 and UGT activities relative to PST activities, suggesting that TGZ sulfate in addition to TGZ may act as toxicants. On the other hand, porcine hepatocytes, which is deficient in TGZ sulfation, were resistant to TGZ toxicity at concentrations toxic to human hepatocytes, while it is suggested that porcine cells compensate the sulfation with glucuronidation, and the resistance to the toxicity may be explained by a higher capacity of pig cells to total conjugation. Chemical inhibitors of drug metabolizing enzymes responsible for the TGZ metabolism have not succeeded in protection against the TGZ-induced cytotoxicity. Inhibition of TGZ sulfation by 2,4-dichloro-4-nitrophenol and pentachlorophenol resulted in exaggeration of the TGZ toxicity to human hepatocytes, whereas the former did not affect TGZ toxicity to HepG2 cells. SKF-525A, a general inhibitor of P450 enzymes, and ketoconazole, a potent inhibitor of CYP3A4, were ineffective in attenuating the TGZ cytotoxicity. However, these inhibition experiments were carried out with HepG2 cells, which possess extremely low enzyme activities, and the data on effects of P450 inhibitors on the TGZ toxicity to rat or human hepatocytes has not been available. On the other hand, in a recent study, HepG2 cells together with microsomes containing cDNA-expressed CYP3A4 or HepG2 cells transfected with CYP3A4 were capable to metabolize the test compounds leading to increased toxicity, compared to their respective control systems. The
co-incubation with the CYP3A4 inhibitor ketoconazole protected the cells against the toxicity, confirming that the increased toxicity is mediated by CYP3A4-dependent formation of reactive metabolites. 29) A quinone metabolite of TGZ, a postulated precursor of the reactive metabolite, was less cytotoxic than TGZ both in rat hepatocytes and HepG2 cells. 31, 32) Similar results were obtained in rat hepatoma (N1S1) cells. 30) It would be difficult to evaluate of toxicity of the quinone metabolite that generated from TGZ in hepatocytes by the data for exogenously added one, whereas the above-described study, which used HepG2 cells together with microsomes containing cDNA-expressed CYP3A4, detected the toxicity initiated by extracellular metabolites. 29) It is thus suggested that some cytotoxic metabolites, which may not be derived from the TGZ quinone, could be generated by CYP3A4, but their contribution to overall toxicity is relatively low not only in HepG2 cells but also in normal human hepatocytes because these metabolites would not generate and accumulate in these cells much enough to exert their toxic effects.

**Difference from other Relatively Safer Thiazolidinediones**

Clinical studies have indicated that relatively newer TDZ antidiabetics, rosiglitazone and pioglitazone are less hepatotoxic than TGZ. 31, 32) To validate the above-described in vitro models, the models have been applied for comparison of toxicity of rosiglitazone and pioglitazone with TGZ. In isolated rat hepatocytes, rosiglitazone did not cause any enzyme leakage from the hepatocytes under the conditions where TGZ caused significant leakage. 24) It was also reported that TGZ induced apoptosis of rat hepatocytes but rosiglitazone or pioglitazone did not. 23) Cytotoxicity of TGZ and rosiglitazone evaluated in cryopreserved human hepatocytes from multiple donors indicated that rosiglitazone, albeit cytotoxic to hepatocytes in some donors, was less toxic in the study population. 33) In a single donor study using freshly isolated human hepatocytes, TGZ appeared to be more toxic than rosiglitazone by all endpoints, i.e., ATP content, MTT metabolism, depletion of intracellular glutathione, etc. 33) Toxicity of rosiglitazone or pioglitazone could not be detected in SV40 immortalized normal human hepatocytes that were transfected to overexpress CYP 3A4, while TGZ was toxic in this system. 24)

As described above, cytotoxicity of TGZ seems not to be mediated by a reactive metabolite unless the activating enzyme was overexpressed. However, the generation of reactive metabolites has been discussed as a factor that accounts for difference between compounds, namely only TGZ has the chromane ring that can convert to a reactive quinone methide, whereas the other TDZ drugs do not. 37) (Table 1). On the other hand, they commonly possess the TDZ ring, and ring opening of this part of the molecule to reactive intermediates is unlikely to explain the higher toxicity of TGZ. Indeed, GSH adducts were detected after incubation of human liver microsomes with rosiglitazone and pioglitazone in the presence of NADPH and GSH or GSH ethyl ester. 35) The GSH adducts of pioglitazone obtained in rat and human liver microsomes and in freshly isolated rat but not human hepatocytes were identified as TDZ ring-opened products. 36)

The difference in cytotoxicity between the compounds was also observed in the following cell lines, which may possess very low enzyme activities. TGZ exhibited dose-dependent cytotoxicity, i.e., cell viability and LDH leakage and apoptosis, to HepG2 cells, but rosiglitazone or pioglitazone did not. 25, 37) Similar results along with G1 cell cycle arrest by TGZ but not by rosiglitazone were obtained in rat hepatoma cell lines as well as HepG2 cells. 38) TDZ phenoxyacetic acid, a model compound, which possesses TDZ ring but not chromane ring like rosiglitazone, did not exhibit cytotoxicity to rat hepatoma cell lines under the condition where TGZ exhibited cytotoxicity. 30) Since the in vitro studies reproduced relative toxicity of TDZ drugs similar to those observed in the clinical studies, these tests may provide some mechanistic information relevant to the clinically observed hepatotoxicity. However, determinants other than metabolic activation for the relative toxicity remain to be elucidated. A comparison of the effects of TDZ drugs on taxologically relevant gene expression in primary culture hepatocytes by using microarrays showed that substantially higher numbers of genes were affected by treatment with TGZ than rosiglitazone and pioglitazone. 39) A proteomics study with two-dimensional electrophoresis also showed that TGZ but not rosiglitazone highly induced chaperone proteins in HepG2 cells. 40) As shown in the following sections, recent studies have focused on mitochondrial effects of TGZ as mechanisms underlying TGZ-specific hepatotoxicity.

**Enzymatic Detoxification of Reactive Metabolites**

Although GSH-adducts of the reactive metabolites of TGZ were isolated from biological fluids, little information is available about enzymatic detoxification of the reactive metabolites. A clinical study in Japanese

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<th>Table 1. Pathophysiological events in livers treated with troglitazone but not with rosiglitazone or pioglitazone</th>
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<td>• Metabolic activation at the chromane ring</td>
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<td>• Mitochondrial permeability transition</td>
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<td>• Apoptosis</td>
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<td>• Down-regulation of proinflammatory cytokine in Kupffer cells</td>
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patients with type 2 diabetes mellitus suggested that the double null mutation of GSTT1 and GSTM1 might influence troglitazone-associated abnormal increases of liver enzyme levels.\textsuperscript{[30]} However there is currently no experimental data supporting the hypothesis that GSTT1 and/or GSTM1 are responsible for detoxification of the postulated reactive metabolites or play other beneficial role against the TGZ-induced liver injury.

**Mitochondria-Mediated Toxicity**

During the appearance of hepatocyte toxicities by TGZ, mitochondrial dysfunction was often observed (Fig. 3).\textsuperscript{[24,28,30,42,43]} Moreover, in some other studies, the cytotoxicity was assessed by decrease in cellular ATP contents or MTT assay,\textsuperscript{[25-27,29,33,38]} both of which revealed mitochondrial injury. Exposure of rat and human hepatocytes to TGZ caused a decline in mitochondrial transmembrane potential ($\Delta \Psi$) as an initiating event and subsequent ATP depletion.\textsuperscript{[24]} Rosiglitazone had similar effects, but it required much higher concentration than TGZ.\textsuperscript{[24]} Because the mitochondrial effects preceded detection of metabolic biotransformation products of TGZ and were also observed in peripheral blood mononuclear cells, which may not have P450 activity, the decreased $\Delta \Psi$ is unlikely to be accounted for by covalent adduct of the TGZ metabolite. Uncoupling of mitochondrial oxidative phosphorylation probably induced by TGZ itself was proposed as a trigger to initiate mitochondrial dysfunction.\textsuperscript{[24]}

Similar mitochondrial effects, where decreases in cellular ATP levels and mitochondrial $\Delta \Psi$ were observed with HepG2 Cells, which have low P450 activity.\textsuperscript{[28,43]} Furthermore, preincubation of mitochondria with cyclosporin A, an inhibitor of mitochondrial permeability transition (MPT) provided a protection against the TGZ-induced cell death.\textsuperscript{[28]} MPT is characterized by a progressive permeabilization of the inner mitochondrial membrane dependent on the excessive amount of intramitochondrial Ca\textsuperscript{2+} and results in mitochondrial swelling, decrease in mitochondrial $\Delta \Psi$ and release of accumulated Ca\textsuperscript{2+}.\textsuperscript{[44,45]} Recent studies have suggested a pathogenetic role of MPT in the mitochondria-mediated hepatocyte injury by drugs, which is sometimes initiated by the drug itself,\textsuperscript{[46,47]} and sometimes by reactive metabolites of the drug, which are targeted to mitochondrial protein or alter mitochondrial redox status.\textsuperscript{[47]}

It is proposed that TGZ-induced mitochondrial depolarization subsequently trigger MPT, leading to depletion of cellular ATP.\textsuperscript{[28]} TGZ may directly affect the electron transport chain or modulate the MPT pore.\textsuperscript{[43]} It is also suggested that mitochondrial dysfunction leads to the production of reactive oxygen species (ROS) and further results in increase in intracellular calcium.\textsuperscript{[43]} Multiparameter flow cytometric evaluation of rat hepatoma N1S1 cells actually demonstrated that TGZ induced intracellular oxidative stress, which was proposed to be mediated by metabolic intermediates of TGZ, although N1S1 cells have low P450 activities.\textsuperscript{[30]} Alternatively, mitochondria are also known to the source of ROS, suggesting that a direct effect of TGZ on mitochondrial physiology may play a role in TGZ hepatotoxicity.\textsuperscript{[30]} Another study with similar methods indicated that expose of an immortalized human hepatocyte cell line OUMS-29 to TGZ caused a marked enlargement of the mitochondria and a decline of mitochondrial $\Delta \Psi$.\textsuperscript{[42]} The treatment of the cells also caused overproduction of hydrogen peroxide but not superoxide, and the production of hydrogen peroxide along with the mitochondrial abnormality was attenuated by an antioxidant, N-acetyl-L-cysteine, suggesting that ROS generated probably in the mitochondria deteriorated both mitochondrial membrane structures and mitochondrial function, leading to a possible priming for the severe hepatocyte toxicity.\textsuperscript{[43]}

TGZ shares a common structure to vitamin E and has
potent antioxidant properties, which may explain pharmacological activities of TGZ at least partially and may be mediated presumably through PPAR\(\gamma\) signaling.\(^6\) It is consistent with the facts that the antioxidant vitamin E also activates PPAR\(\gamma\).\(^6\) Therefore, it cannot be concluded that oxidative stress-related mitochondrial abnormalities is responsible for TGZ hepatotoxicity, although it is proposed that ROS is involved in mitochondrial dysfunction as described above.

In conclusion, mitochondrial dysfunction by TGZ provides mechanisms for the metabolism-independent toxicity of TGZ. However, the possibility has been also suggested that the postulated reactive metabolites are also involved in the events in mitochondria, because all of the data were derived from the experiments on cells, even though it is likely that the TGZ reactive metabolites are hardly produced in these systems. Recently, effects of troglitazone and other thiazolidinediones on isolated mitochondria have been investigated, and have indicated that TGZ but not rosiglitazone or pioglitazone induces MPT (Table 1) and a decrease in \(\Delta\Psi\), demonstrating that interaction of TGZ with mitochondria does not require metabolism of TGZ.\(^{48}\)

**Troglitazone-Induced Apoptosis in Hepatocytes and Cancer Cells**

The release of cytochrome c into the cytoplasm, which is caused by TGZ-induced MPT, can be a trigger of apoptosis. Indeed, apoptotic cell death was observed in rat hepatocytes and HepG2 cells treated with TGZ.\(^{23,25,37,38}\) TGZ induced apoptosis in HepG2 cells that preceded by activation of c-Jun N-terminal protein kinase (JNK) and p38 mitogen-activated protein kinase (p38 kinase) and increased levels of proapoptotic proteins, Bad, Bax, release of cytochrome c, and cleavage of Bid.\(^{37}\) Furthermore, pretreatment of HepG2 cells with a selective JNK inhibitor, anthra[1,9-cd]pyrazol-6(2H)-one, reduced the rate of TGZ-induced cell death and prevented the TGZ-mediated changes in Bad, Bax, Bid cleavage, and cytochrome c release, whereas an inhibitor of p38 kinase had little effect on apoptosis, suggesting that TGZ causes apoptosis by activating the JNK-dependent cell death pathway accompanied by increased Bid cleavage and elevation of proapoptotic proteins.\(^{37}\) However, exact role of mitochondria in the TGZ-induced apoptosis of HepG2 cells needs to be investigated further. Cytosolic ATP content is a determinant of necrotic and apoptotic cell death, since ATP is required for apoptosis.\(^{49,50}\) During the appearance of TGZ-induced hepatocyte toxicities, a decline of cellular ATP was observed.\(^{24,26,28,33,43}\) Therefore, mitochondria could determine potentially to induce apoptosis and necrosis not only by releasing the apoptosis signals but also by changing the availability of ATP.

TGZ caused cell death in various cancer cell lines, but did not affect corresponding normal primary cells, suggesting that cancer cells is more sensitive to the TGZ-induced apoptosis than normal cells, probably because of abnormalities of cancer cells such as mitochondrial morphology.\(^6\) Instead, in recent reports from different viewpoints, apoptosis of cancer cells by TGZ is proposed as a target of cancer chemotherapy.\(^{51,52}\) TGZ-induced apoptosis in hepatocellular carcinoma cell is suggested to be mediated by PPAR\(\gamma\) activation,\(^{52}\) whereas those in prostate cancer cells seems to be caused not through PPAR\(\gamma\)-dependent mechanism, but through change in mitochondrial integrity, which is based on data that rosiglitazone and pioglitazone, potent PPAR\(\gamma\)-agonists, had less effective than TGZ.\(^{51}\) Conversely, it was reported that rosiglitazone caused apoptosis in vascular smooth muscle cell, whose potency was 10-fold more effective than TGZ.\(^{53}\)

In HepG2 cells, rosiglitazone did not induce apoptosis like prostate cancer cells, suggesting the binding of TGZ to PPAR\(\gamma\) may not be involved in the apoptosis in HepG2 cells.\(^{25,37,38}\) PPAR\(\gamma\)-independent mechanism is also possible in human hepatocytes, because the expression of PPAR\(\gamma\) in normal human liver cells was very low\(^{54}\) and lack of apoptosis by rosiglitazone was also shown in rat hepatocytes.\(^{23}\) Therefore, it seems that there are some targets other than PPAR\(\gamma\) in normal liver, some of which may located in mitochondria and lead to hepatocyte apoptosis. Because apoptosis of liver cells, like other toxicity parameters, is induced by TGZ but not by other TDZs (Table 1), it is suggested that the TGZ-induced apoptosis is involved in the pathogenesis of the liver toxicity, while actual role of apoptosis in the TGZ-induced hepatotoxicity remains to be elucidated.

Furthermore, apoptosis in cancer cells including HepG2 cells requires careful consideration because of their abnormalities as compared with normal hepatocytes.

**Possible Roles of PPAR\(\gamma\) in the Troglitazone Toxicity**

Increased expression in PPAR\(\gamma\) levels in the liver was observed in diabetic or obese mouse models as compared with lean control mice.\(^{55}\) Moreover, TGZ and rosiglitazone induced the hepatic PPAR\(\gamma\) levels in these models, leading to possible disruption of lipid homeostasis.\(^{56}\) In fact, treatment of diabetic KKA\(^\text{m}\) mice with TGZ or rosiglitazone resulted in severe microvesicular periacinar steatosis, whereas lean control mice did not develop any pathological liver changes.\(^{55,56}\) Although the steatosis did not result in liver injury in these mouse models, hepatic steatosis can lead to more serious consequences, such as lipid peroxidation and development of fibrosis. Thus, the mouse livers with obesity-associated upregulated PPAR\(\gamma\) expression become sensitized to PPAR\(\gamma\) ligands and may produce hepatic steatosis, implying similar profound effects of
TDZs on the liver in diabetics and obese individuals, which can explain idiosyncratic hepatotoxicity of TGZ in human. However, such of the PPARγ-dependent mechanism cannot explain the specificity of TGZ to induce hepatotoxicity.

Possible Regulation of Hepatotoxicity of Thiazolidinediones by Their Own Anti-Inflammatory Properties

TDZs are commonly pleiotropic drugs with potent anti-inflammatory properties for tissue protection, suggesting their potential for treating a number of degenerative inflammatory diseases, which include non-alcoholic steatohepatitis (NASH).\(^5\) PPARγ is expressed abundantly expressed in Kupffer cells and can be a target of TDZs. Beneficial effects of pioglitazone, which may be accounted for by its anti-inflammatory properties, have been observed in rat models of liver injury. Pioglitazone prevented alcoholic liver injury through abrogation of Kupffer cell sensitization to LPS.\(^5\) Similar protective action of pioglitazone was observed against acute liver injury induced by ethanol and LPS through the suppression of TNF-α.\(^5\) It was also reported that pioglitazone attenuated ethanol-induced hepatic steatosis by activation of c-Met and very low-density lipoprotein (VLDL)-dependent lipid retrieval and suppression of triglyceride synthesis.\(^5\) It could be conceivable that the beneficial effects of pioglitazone on livers, which have not been observed for TGZ, are involved in its lower risk of hepatotoxicity than TGZ.

However, it was also shown that TGZ down-regulated proinflammatory cytokines, TNF-α and IL-6, in livers of LPS-stimulated mice, which was not observed by the treatment with rosiglitazone (Table 1), suggesting PPARγ-independent inhibition of Kupffer cell function.\(^4\) The toxicological consequences of the down-regulation of these cytokines by TGZ, which often leads to beneficial outcome against the liver injury in the case of pioglitazone as described above, remains to be elucidated. While obviously speculative, suppression of IL-6 can contribute to exaggerate the toxicity, since IL-6 was shown to be protective against drug-induced liver injury in mouse models.\(^2\)

Troglitazone Sulfate as a Potent Inhibitor of BSEP

Induction of intrahepatic cholestasis is proposed to contribute liver injury induced by TGZ. It results from accumulation of toxic bile salts in hepatocytes by inhibition of canalicular bile salt export pump (BSEP/ABCB11), a major protein that excretes bile salts from hepatocytes by utilizing the energy of ATP hydrolysis. TGZ impaired the hepatobiliary export of bile acid in isolated perfused rat liver, which was blunted by addition of albumin.\(^4\) An increase in plasma bile acid was also observed after intravenous administration of TGZ in rats.\(^4\) TGZ inhibited the ATP-dependent taurocholate transport mediated by BSEP in isolated canalicular rat liver plasma membrane preparations, while TGZ sulfate, a major metabolite of TGZ (Fig. 1) was 10 times more potent in the inhibition than the parent TGZ.\(^4\) The high BSEP inhibition potential in addition to accumulation in liver tissues suggest that TGZ sulfate is mainly responsible for the interaction with the hepatobiliary export of bile acids at the level of the canalicular BSEP in rats.

Recent studies suggested that TGZ sulfate could be uptaken into hepatocytes by a human basolateral organic anion transporting polypeptide-C (OATP-C, \(\text{SLCO1B1}\),\(^6\)) and be excreted from hepatocytes into bile duct by multidrug resistance-associated protein 2 (Mrp2/ABCC2),\(^6\) both of which may be involved in accumulation of TGZ sulfate in hepatocytes. In addition, TGZ sulfate and TGZ with a lesser extent inhibit OATP-C more potently than rosiglitazone and pioglitazone.\(^6\) Because these proteins are involved in the hepatic handling of bile acids, bilirubin, and other endogenous anionic compounds, role of OATPs as well as BSEP in the TGZ-mediated indirect toxicity should be elucidated in future studies.

Higher intracellular bile salts could induce cell death through various mechanisms such as mitochondria dysfunction and apoptosis,\(^5\) while impaired ability of mitochondria to generate ATP would lead to further dysfunction of BSEP. However, an animal model of TGZ-induced cholestasis has not been established, namely, an increase in serum alkaline phosphatase, a diagnostic marker of cholestasis, has not been observed in experimental animals as well as the low incidence of cholestasis in humans.\(^6\) Therefore, the actual contribution of this mechanism to the TGZ hepatotoxicity remains to be determined.

Conclusions

Clinically observed hepatotoxicity by TGZ has been apparently reproduced as the \(\text{in vitro}\) hepatocyte toxicity, which was not initiated by rosiglitazone or pioglitazone. Although the TGZ is biotransformed into chemically reactive metabolites, it could be concluded that TGZ itself rather than the reactive metabolites was responsible for the pathogenesis of the TGZ-induced liver injury. Mitochondria are the major targets in the toxicity and MPT is suggested as one of the important events leading to the mitochondrial dysfunction. Role of PPARγ in the pathogenesis of the TGZ hepatotoxicity is controversial, probably because it may be involved both in progression and suppression of the toxicity. Although it is not known whether the mechanism underlying the hepatocyte toxicity obtained in the \(\text{in vitro}\) studies is the same as those observed in the clinical settings, some cellular events induced specifically by
TGZ (Table 1) may contribute also to the clinically observed TGZ hepatotoxicity. Both the metabolic and non-metabolic factors focused in this review could also explain why TGZ hepatotoxicity occurs only in limited patients, and further studies are required to identify determinants of the idiosyncrasy in TGZ hepatotoxicity.

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