

FIGURE LEGENDS

FIGURE 1. Correlation between the PXR mRNA and protein levels in 25 human livers. The PXR mRNA level was determined by real-time RT-PCR and normalized with the GAPDH mRNA level. The PXR protein level was determined by Western blot analysis and normalized with the GAPDH protein level.

FIGURE 2. Repressive regulation of human PXR by miR-148a. *A*, Complementarity of miR-148a to the predicted target sequence of human PXR. The potential miR-148a recognition element (PXR_{MRE148}) is located on +3359 to +3386 in the 3' UTR of human PXR mRNA, where the numbering refers to the 5' end of mRNA as 1. *B*, The mature miR-148a levels in HepG2, HuH7, HLE, Caco-2, Caco-2/D (differentiated), LS180, HEK293, MCF-7 cells were determined by real-time RT-PCR analysis using NCode miRNA First-Strand cDNA Synthesis Kit. The values were the mature miR-148a levels normalized with the U6 snRNA levels relative to that in HLE cells. *C, D*, Luciferase assays were performed to investigate whether PXR_{MRE148} is functional in the regulation by miR-148a. The reporter constructs were transiently transfected with 4 pmol of the precursors for miR-148a or control into HEK293 cells (*C*) or 10 pmol of the AsO for miR-148a or control into HepG2 cells (*D*). The data were the firefly luciferase activities normalized with the *Renilla* luciferase activities relative to that of pGL3p plasmid. Each column represents the mean \pm SD of three independent experiments. $**P < 0.01$, compared with pGL3p; $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$, compared with AsO for control.

FIGURE 3. Effects of overexpression or inhibition of miR-148a on the PXR protein level in HepG2 cells. The precursors for miR-148a or control (50 nM, *A*) or AsOs for miR-148a or control (50 nM, *B*) were transfected into HepG2 cells. After 72 h, the cells were harvested and total RNA and nuclear extracts were isolated. *A, B*, The mature miR-148a levels were determined by real-time RT-PCR analyses. The values were the mature miR-148a levels normalized with the U6 snRNA levels relative to control. The PXR and RXR α protein levels in nuclear extracts were determined by Western blot analyses. The values are the mean \pm SD for three independent experiments ($*P < 0.05$, compared with control). *C, D*, Schemes represent the principle of the reporter gene assay to evaluate the changes in the endogenous PXR protein level by the overexpression (*C*) or inhibition (*D*) of miR-148a. The pCYP3A4-362-7.7K plasmid contains the PXR responsive elements, ER6 (-362 to +11) and DR3 (-7836 to -7200), of the *CYP3A4* gene upstream of the *luciferase* gene. *E, F*, The cells were transfected with the reporter plasmid and the precursors or AsOs. After 48 h, the cells were

treated with 10 μ M rifampicin (square symbols) or 0.1% DMSO (circle symbols) for 24 h. The data were the firefly luciferase activities normalized with the *Renilla* luciferase activities relative to that of pGL3p plasmid without the precursors or AsOs. Each point represents the mean \pm SD of three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001, compared with control.

FIGURE 4. Effects of overexpression of miR-148a on the induction of endogenous CYP3A4 mRNA in LS180 cells. **A, B,** The precursors for miR-148a or control (50 nM) were transfected into LS180 cells. **A,** After 72 h, the cells were harvested and total RNA and nuclear extracts were isolated. The mature miR-148a level was determined by real-time RT-PCR analysis. The values are the mature miR-148a levels normalized with the U6 snRNA levels relative to control. The PXR and RXR α protein levels were determined by Western blot analysis. The values are the mean \pm SD of three independent experiments (** P < 0.01, compared with control). **B,** After 24, 48, 72, 96 hr, the cells were harvested and total RNA was isolated. The PXR mRNA level was determined by real-time RT-PCR analysis. The values are the PXR mRNA levels normalized with the GAPDH mRNA levels relative to control. Each point represents the mean \pm SD of three independent experiments. **C,** Precursor-transfected LS180 cells were treated with 50 μ M rifampicin or 0.1% DMSO for 24 h and then total RNA was isolated. The CYP3A4 mRNA levels were determined by real-time RT-PCR and normalized with the GAPDH mRNA level. The data are relative to that with the precursor for the control without rifampicin. Each column represents the mean \pm SD of three independent experiments. *** P < 0.001; NS: Not significant.

FIGURE 5. Relationship between the expression levels of miR-148a, PXR, and CYP3A4 in human liver tissue. **A,** The CYP3A4 mRNA level was significantly correlated with the CYP3A4 protein levels. **B,** The miR-148a level was inversely correlated with the translational efficiency of PXR (PXR protein/mRNA ratio). **C, D,** The PXR protein level was significantly correlated with the CYP3A4 mRNA (**C**) and protein level (**D**). **E,** Summary of the correlation analyses (* P < 0.05, ** P < 0.01, *** P < 0.001).

Fig. 1. Takagi *et al.*

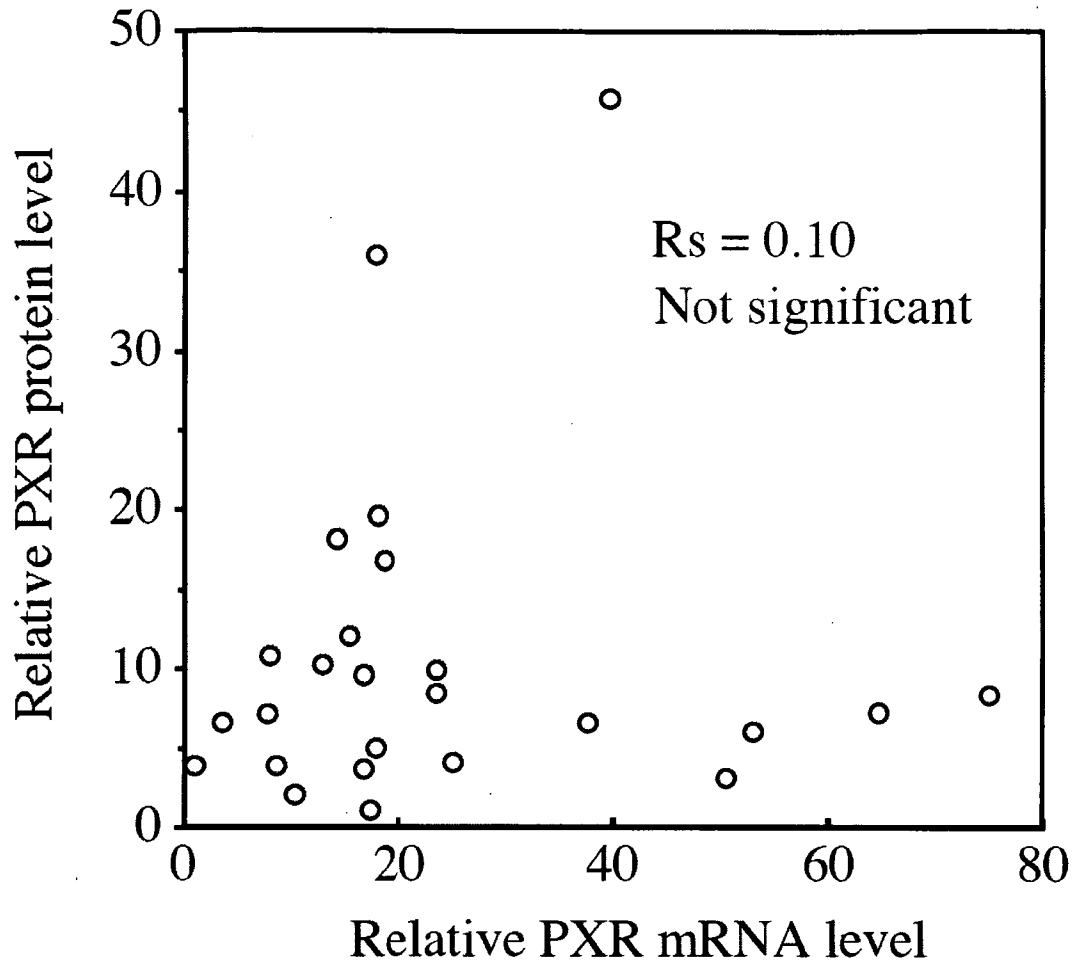


Fig. 2. Takagi et al.

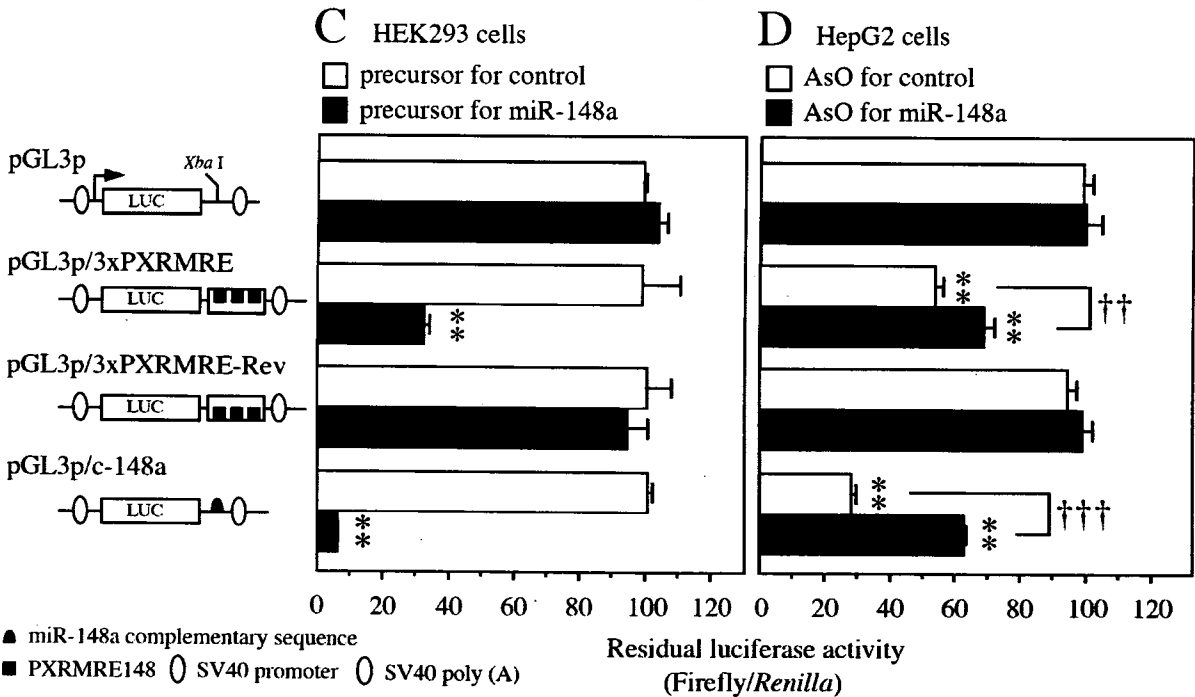
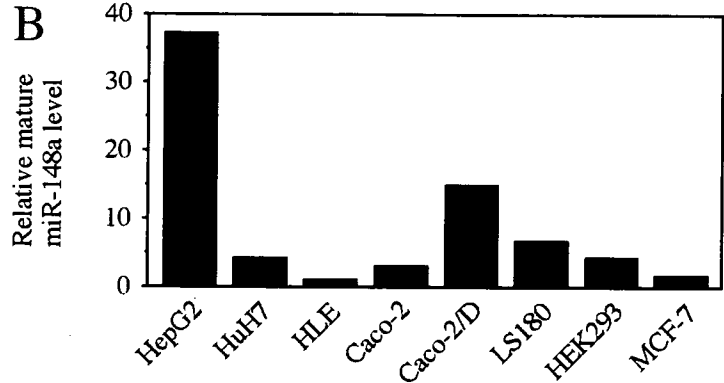
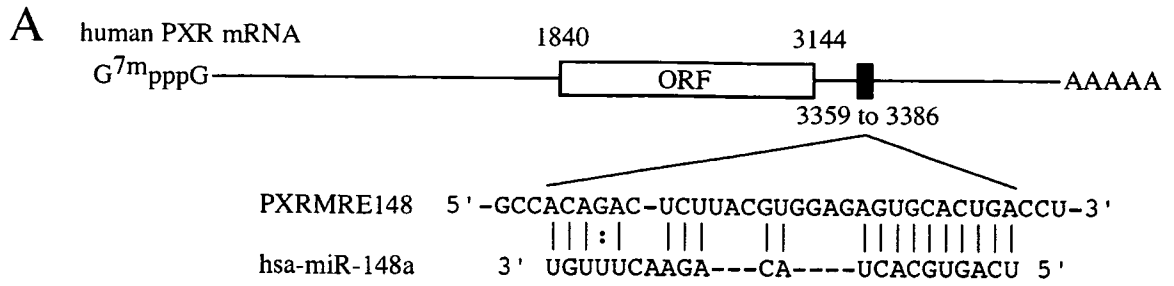


Fig. 3. Takagi et al.

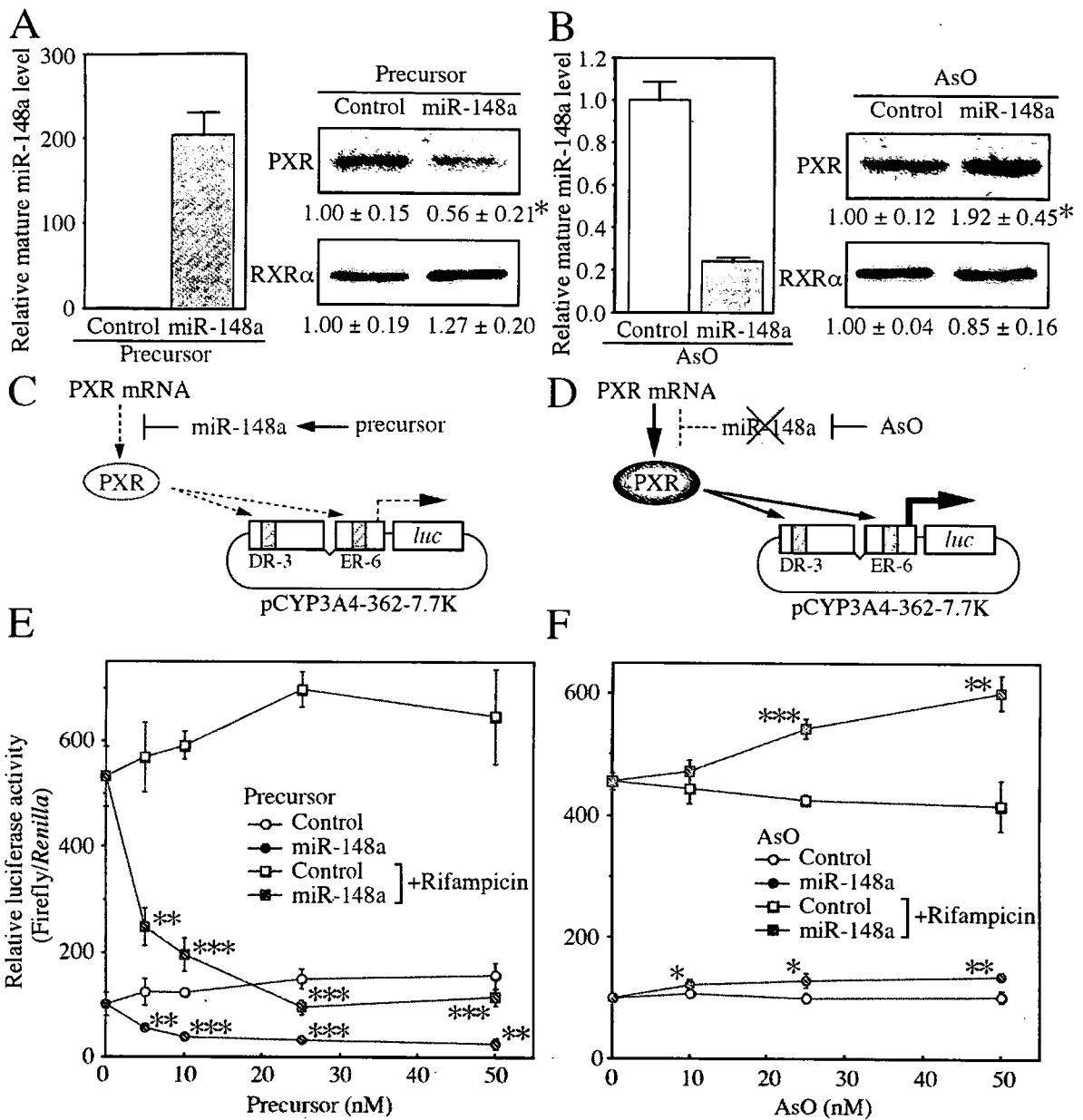


Fig. 4. Takagi et al.

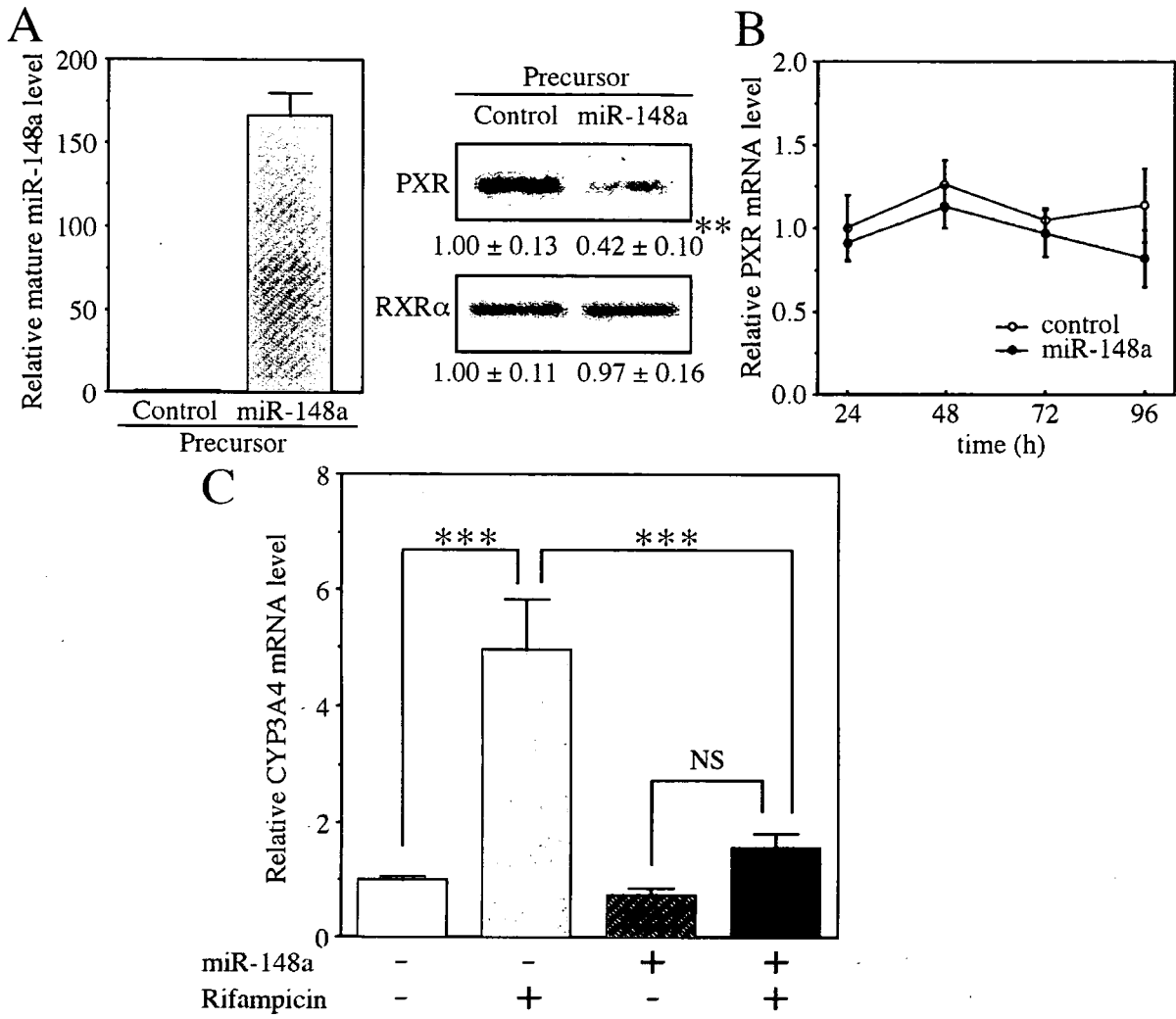
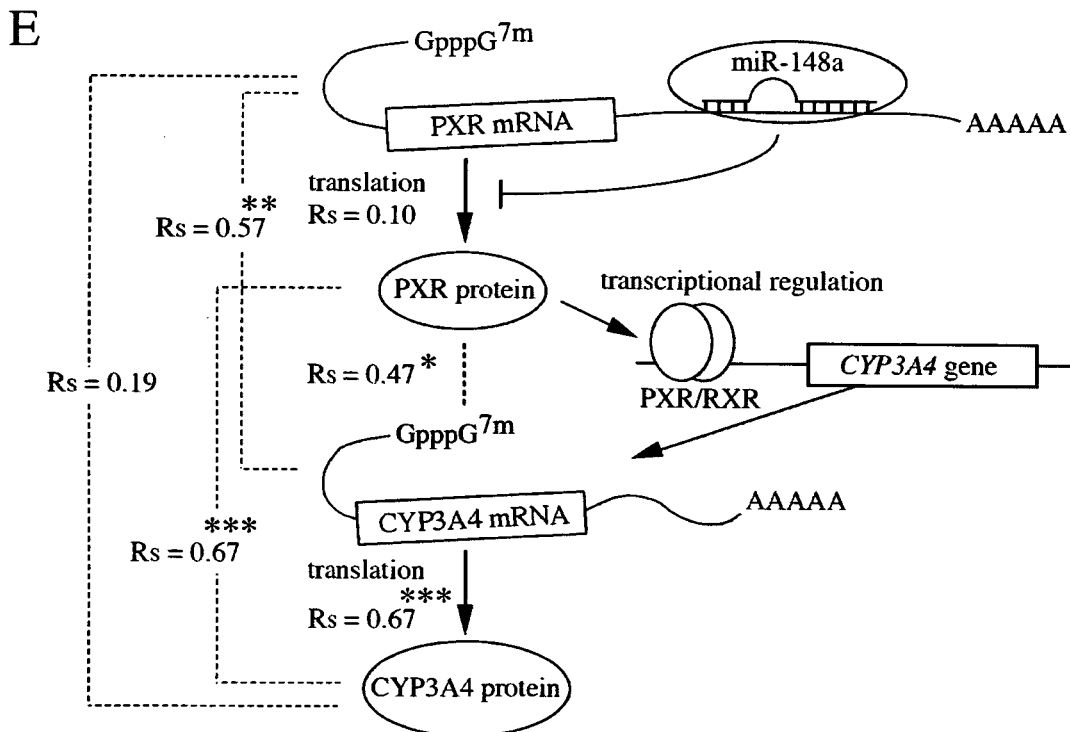
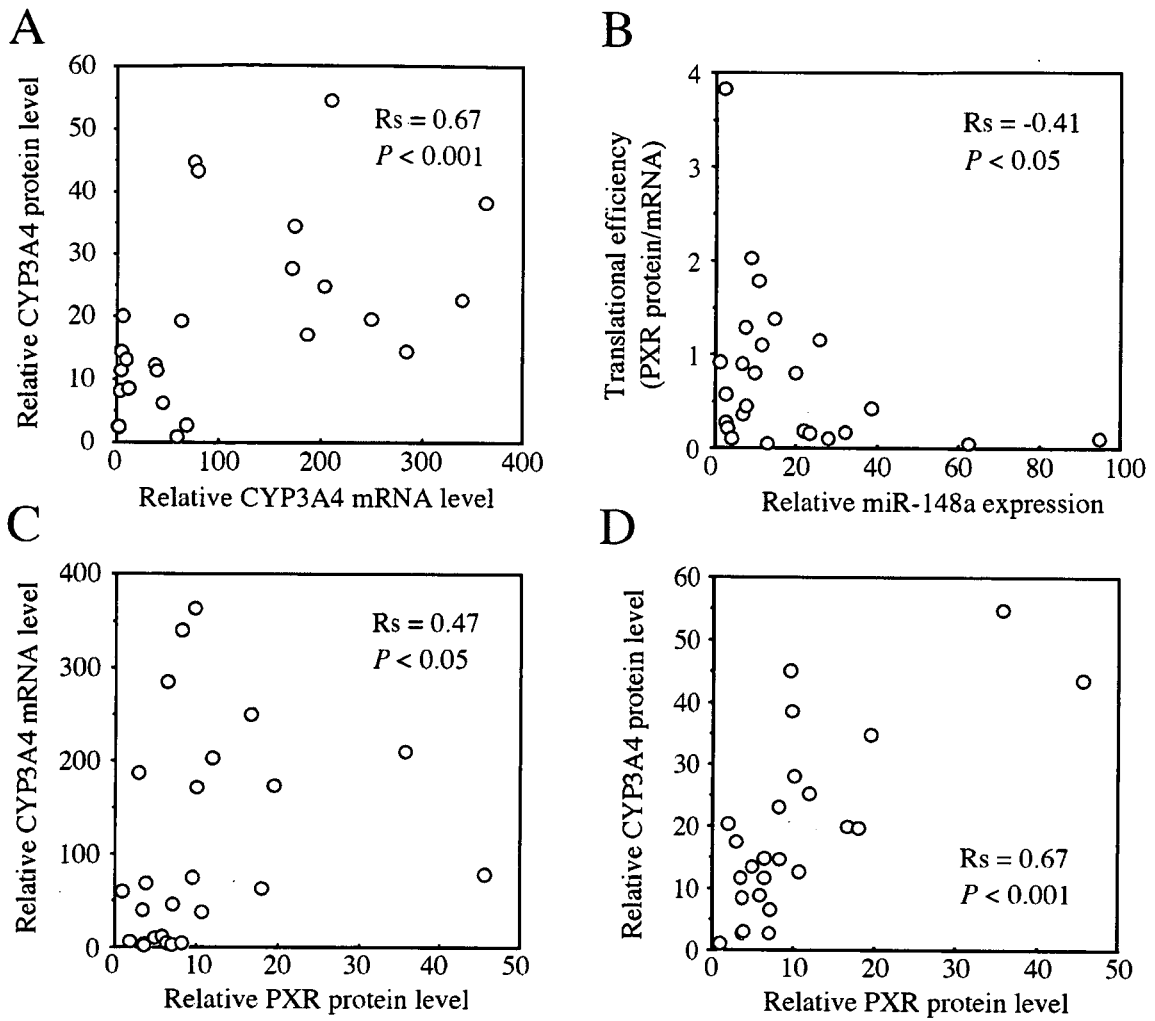


Fig. 5. Takagi et al.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

11

A Mechanistic View of Troglitazone Hepatotoxicity

Rawiwan Maniratananachote and Tsuyoshi Yokoi

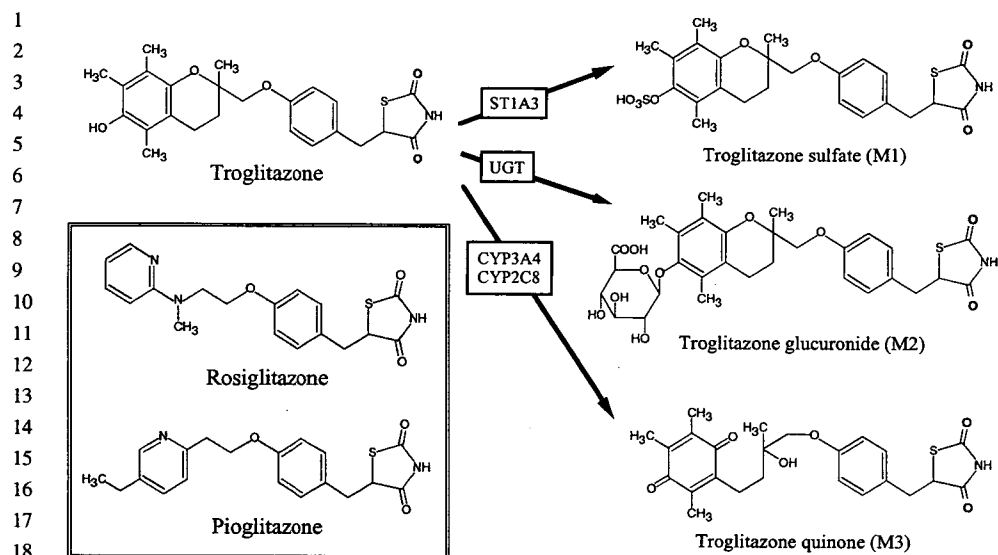
11.1 Introduction

Thiazolidinediones (Figure 11.1) are a class of oral antidiabetic agents and are the synthetic ligands for the peroxisome proliferator-activated receptor γ (PPAR γ) (Lehmann *et al.*, 1995). Troglitazone (Rezulin[®], (\pm)-5-[[4-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]-2,4-thiazolidinedione) was the first thiazolidinedione antidiabetic agent approved for clinical use by the US Food and Drug Administration in 1997. Troglitazone lowers the blood glucose levels through increased glucose uptake by skeletal muscle, decreased hepatic glucose production and increased insulin sensitivity of the target tissue in animal models of metabolic impairment (Ciaraldi *et al.*, 1990; Fujiwara *et al.*, 1988, 1995). These pharmacological effects are exerted through PPAR γ -dependent transcription of genes involved in glucose and lipid metabolism and energy homeostasis (Lehmann *et al.*, 1995; Saltiel and Olefsky, 1996; Spiegelman, 1998). Based on the pharmacological advantages and the apparent absence of severe toxic effects, troglitazone was thought likely to become a promising treatment for type II diabetes mellitus in patients with insulin resistance.

However, in the combined North American clinical trials, elevations of serum alanine aminotransferase more than three times the upper limit of normal were observed in 48 out of 2510 patients (1.9%) treated with troglitazone. Liver biopsies from two patients confirmed the hepatocellular nature of the injury as an idiosyncratic drug reaction (Watkins and Whitcomb, 1998). Meanwhile, troglitazone had been concomitantly reported to be associated with idiosyncratic hepatotoxicity, with some patients showing severe or fatal

Hepatotoxicity: From Genomics to in vitro and in vivo Models Edited by S. C. Sahu
© 2007 John Wiley & Sons, Ltd

300 Hepatotoxicity



20 **Figure 11.1** Structures of thiazolidinediones and pathways of troglitazone metabolism

21
22 liver damage (Gitlin *et al.*, 1998; Neuschwander-Tetri *et al.*, 1998; Shibuya *et al.*, 1998).
 23 Consequently, it was withdrawn from the market in the USA and Japan in March 2000. The
 24 hepatotoxic effects of troglitazone were not predicted from conventional animal models
 25 (Watanabe *et al.*, 1999) or in cynomolgus monkeys (300–1200 mg/kg/day for 52 weeks),
 26 a primate model having similar metabolic profiles to humans (Rothwell *et al.*, 2002). Two
 27 other thiazolidinediones, which are now on the market, rosiglitazone and pioglitazone, have
 28 been introduced in 1999 and are unlikely to share the hepatotoxic effects of troglitazone
 29 (Freid *et al.*, 2000; Isley and Oki, 2000; Lebovitz *et al.*, 2002). It should also be noted that the
 30 clinical dosage regimen for improvement of fasting glucose is distinguishable among these
 31 thiazolidinediones (Table 11.1). The recommended dose for troglitazone was 200 to 600
 32 mg/day, for rosiglitazone 4 to 8 mg/day and for pioglitazone 15 to 45 mg/day (Hanefeld,
 33 2001; Loi *et al.*, 1999; PDR, 1999, 2005a,b). The dosage requirement for their efficacy
 34 might have reflected their hepatotoxic potential.

35 This review summarizes the molecular mechanism of troglitazone hepatotoxicity from
 36 studies both *in vivo* and *in vitro*. Even though, there is no direct evidence to indicate the
 37 precise mechanism of the toxicity so far. Many points of view, however, have been proposed
 38 to contribute to the toxic effects of troglitazone.

39
40
41 **11.2 Potential of Troglitazone Metabolites for Hepatotoxicity**

42
43 In humans, troglitazone is predominantly metabolized by three pathways: sulfation, glu-
 44 curonidation and oxidation to form a sulfate conjugate (M1), a glucuronide conjugate (M2)
 45 and a quinone metabolite (M3), respectively (Figure 11.1). M1 and M3 are the major
 46 metabolites in plasma, while M2 is a minor metabolite (Izumi *et al.*, 1997a,b; Kawai *et al.*,

1 **Table 11.1** Pharmacokinetic parameters of thiazolidinediones

2

3 Parameter	Troglitazone (1997–2000) ^a	Rosiglitazone (1999–present) ^a	Pioglitazone (1999–present) ^a
5 Oral dosage (mg/day)	200–600	1–8	15–45
6 Plasma protein binding (%)	>99	99.8	>99
7 Absolute bioavailability (%)	40–50	99	83
8 C _{max} (µg/ml)	0.90–2.82	0.076–0.598	1.4 ± 0.2 ^b
9 AUC (µg-h/ml)	7.4–22.1	0.358–2.971	11.6 ± 2.2 ^b
10 t _{max} (h)	<2–3	1.75	<2
11 Plasma elimination half-life (h)	16–34	3–4	3–7
12 Biliary excretion (%)	85	23	NA ^c
12 Urinary excretion (%)	3	64	15–30
13 Effects of food	Increases the extent of absorption by 30 to 80 %	Decrease in C _{max} by 28 % and delay in t _{max}	Slight delay in t _{max} to 3–4 h

14

15

16 ^a Year on clinical application.

17 ^b For 30 mg/day oral dosage (data from the product leaflet of Actos[®], Takeda Pharmaceutical Company Ltd, Japan).

18 ^c Not applicable – most of the oral dose is excreted into the bile.

19

20

21 1998; Loi *et al.*, 1999). The main metabolite, troglitazone sulfate (M1), is formed by the
 22 action of phenol sulfotransferase, ST1A3 (Honma *et al.*, 2002). It accounts for about 70 %
 23 of the metabolites detected in human plasma (Loi *et al.*, 1999).

24 Differing from other thiazolidinediones, troglitazone contains a 6-hydroxy-5,7,8-
 25 trimethylchromane moiety (a chroman ring of vitamin E). This structure accounts for
 26 the effective antioxidant property of troglitazone and suggests an advantage in preventing
 27 diabetic vascular complications in addition to its hypoglycemic and hypolipidemic effects
 28 (Inoue *et al.*, 1997). This structure, however, has the potential to undergo metabolic activa-
 29 tion to form troglitazone quinone or metabolite M3 by cytochrome P450s (CYPs) 3A4 and
 30 2C8 (Yamazaki *et al.*, 1999). In humans, it is likely that CYP3A4 is primarily responsible for
 31 this reaction (He *et al.*, 2001). In addition, troglitazone has been shown to induce CYP3A in
 32 human and rat hepatocytes, which stimulates the formation of the quinone (Ramachandran
 33 *et al.*, 1999; Sahi *et al.*, 2000). By the action of CYP3A, troglitazone yields several reactive
 34 intermediates in rats (Kassahun *et al.*, 2001; Tettey *et al.*, 2001) (Figure 11.2). *In vitro*,
 35 the formation of an epoxide of troglitazone quinone was also identified (Yamamoto *et al.*,
 36 2002). It is known that quinones represent a class of toxicological intermediates, which can
 37 result in acute cytotoxicity and immunotoxicity as well as carcinogenesis (Bolton *et al.*,
 38 2000). The maximum plasma concentrations in patients taking troglitazone at the dosage
 39 of 600 mg/day reached to only about 2.82 µg/ml or 6.3 µM (Loi *et al.*, 1999). However, a
 40 study in rats demonstrated that the concentration of troglitazone in liver tissues was 10–12
 41 fold higher than that in the plasma (Sahi *et al.*, 2000). Therefore, the troglitazone levels in
 42 human liver might allow the formation of these putative reactive intermediates and their
 43 accumulation may lead to the hepatotoxicity (see next section).

44 A relatively minor metabolite, troglitazone glucuronide (M2), is catalyzed by UGT
 45 (Yoshigae *et al.*, 2000). The glucuronidation of troglitazone in human intestine is 3-fold
 46 higher than that in human liver. In the liver, the reaction is likely mediated by UGT1A1,

302 Hepatotoxicity

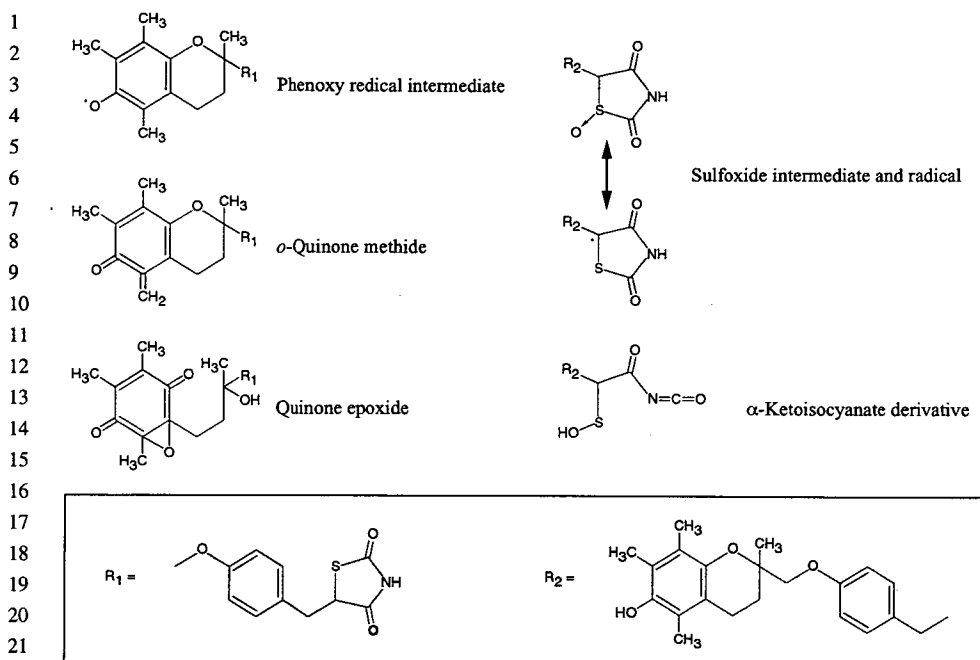


Figure 11.2 Reactive metabolites of troglitazone catalyzed by CYP3A

23
 24
 25 while in the intestine it is mediated by UGT1A8 and UGT1A10 (Watanabe *et al.*, 2002).
 26 A polar, partially β -glucuronidase-sensitive metabolite with retention properties similar
 27 to M2 was found in the profiling of urine samples (Loi *et al.*, 1999). There has been no
 28 reported evidence that M2 is responsible for the hepatotoxic effects.

29
 30
 31 **11.3 Susceptible Genetic Factors Associated**
 32 **with Troglitazone Hepatotoxicity**
 33

34 As mentioned above, troglitazone can undergo metabolic biotransformation by CYP3A4
 35 to form a quinone metabolite (M3) and epoxide specie (Izumi *et al.*, 1997a,b; Kawai *et al.*,
 36 1998; Loi *et al.*, 1999; Yamamoto *et al.*, 2002). Quinones can react readily with sulfur nu-
 37 cleophiles such as glutathione (GSH) or cysteine residues on proteins (Bolton *et al.*, 2000).
 38 The toxic effects of troglitazone have been thought to be mediated by the depletion of GSH,
 39 covalent binding to cellular macromolecules or oxidative stress. In cryopreserved-human
 40 hepatocytes, large variations in the sensitivity to troglitazone were observed and sensitive
 41 donors were demonstrated to form significantly lower amounts of GSH conjugates and glu-
 42 curonides than resistant donors (Kostrubsky *et al.*, 2000; Prabhu *et al.*, 2002). It is known
 43 that the GSH conjugation is formed by the action of glutathione *S*-transferase (GST). A
 44 study in rats has shown that GSH adducts of troglitazone are formed and the reaction is
 45 enhanced by CYP3A (Tetty *et al.*, 2001). An epoxide of troglitazone quinone catalyzed
 46 by CYP3A4 might also be eliminated by GSTs and epoxide hydrolase (Yamamoto *et al.*,

1 2002). These findings indicate an association between metabolic activation by CYP and
2 detoxification by GSTs. In a key report concerning this aspect, Watanabe *et al.* (2003)
3 investigated the genetic factors responsible for troglitazone hepatotoxicity *in vivo*, in hu-
4 mans. Among one hundred and ten patients prescribed troglitazone, 25 case patients had
5 an abnormal increase in ALT or AST levels to at least 9 times or 5 times the upper limit of
6 the normal range, respectively, while 85 control patients showed no significant increase in
7 the ALT levels during more than 6 months of treatment. Interestingly, they found that this
8 abnormal elevation of liver enzymes caused by troglitazone treatment was highly associ-
9 ated with the double null genotype of *GSTM1* and *GSTT1* (Watanabe *et al.*, 2003). Hence,
10 interindividual differences in the detoxification ability might contribute to the susceptibility
11 and individual risk for troglitazone hepatotoxicity. However, the complete mechanism is
12 still largely unknown.

13
14
15
16
17

11.4 Implications of Canalicular Bile Salt Export Pump and Drug Transporters

18 The main metabolite of troglitazone, troglitazone sulfate, undergoes biliary excretion and
19 accounts for up to 60% of the dose in rats (Kawai *et al.*, 1997). In patients with hepatic
20 impairment, troglitazone sulfate was found to accumulate about 4-fold in plasma with a
21 3-fold increased half-life (Ott *et al.*, 1998). This metabolite also showed an inhibition effect
22 on the canalicular bile salt export pump (Bsep) as well as drug transporters, suggesting it
23 contributes to the liver toxicity.

24 Funk *et al.* (2001a,b) reported that troglitazone sulfate inhibits the ATP-dependent tauro-
25 cholate transport mediated by Bsep in isolated canalicular rat liver plasma membrane (IC₅₀
26 0.4–0.6 μM) about 10 times more strongly than the parent compound (IC₅₀ 3.9 μM). The
27 inhibition of Bsep suggests it is one of the possible factors contributing to the hepatotoxicity
28 since the subsequent accumulation of bile salts may lead to intrahepatic cholestasis in hu-
29 mans. Previously, cholestatic signs have also been described in a patient with troglitazone
30 hepatotoxicity (Gitlin *et al.*, 1998).

31 Another group of researchers reported that troglitazone sulfate is transported by organic
32 anion transporting polypeptide (OATP) transporters with higher affinity to OATP-C than
33 OATP8, and showed a strong inhibitory effect on estrone-3-sulfate transport by these trans-
34 porters (Nozawa *et al.*, 2004). Both OATP-C and OATP8 are members of the organic anion
35 transporting polypeptides, which are expressed in the basolateral membrane of hepato-
36 cytes (Hagenbuch and Meier, 2003; Krebs, 2006). They play important roles in the hepatic
37 handling of endogenous compounds and xenobiotics. Therefore, any factors that affect or
38 impair the OATP-C levels or activity, for example, genetic polymorphisms (Krebs, 2006),
39 may cause an accumulation of M1, leading to troglitazone hepatotoxicity.

40
41

11.5 Hypersensitivity Reaction in Troglitazone Hepatotoxicity

42 Idiosyncratic toxicity is generally considered to be host-dependent, dose-independent,
43 difficult to reproduce in experimental animals and relatively uncommon. Some idiosyn-
44 cratic drug reactions are due to a metabolic abnormality of the host, but many have an
45
46

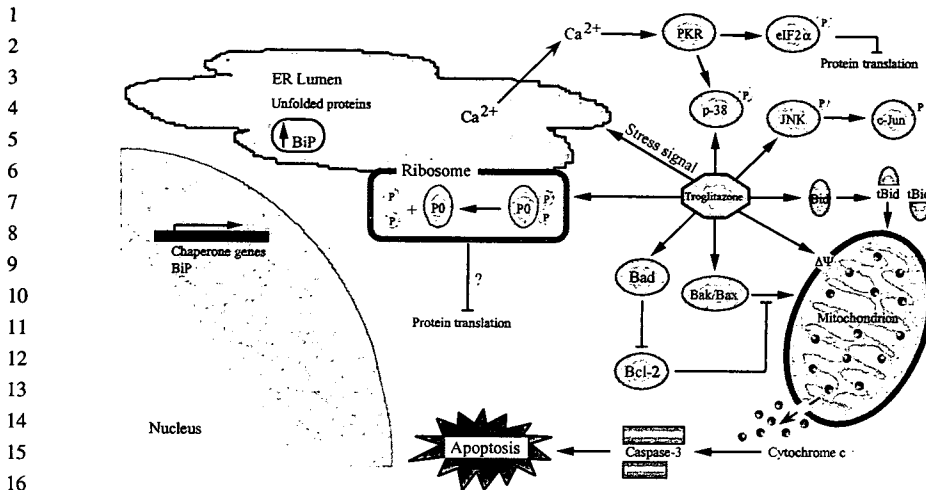
304 Hepatotoxicity

1 immunological basis or result from immune-mediated hypersensitivity (Pohl *et al.*, 1988).
2 A well-characterized example is the idiosyncratic hepatitis induced by halothane. Sera of
3 these patients contain autoantibodies directed against some trifluoroacetyl (TFA)-protein
4 adducts, including protein disulfide isomerase, microsomal carboxyesterase, calreticulin,
5 ERp-72, GRP-78 and GRP-94 (review in Gut *et al.*, 1993), as well as CYP2E1 (Bourdi
6 *et al.*, 1996). Our recent report described that aldolase B, which is an enzyme predominantly
7 localized in the liver and kidney (Penhoet *et al.*, 1966; Rutter, 1964), was detected as an
8 autoantigen that reacted with antibodies in the sera from two patients with type II diabetes
9 mellitus with troglitazone-induced liver dysfunction (Maniratanachote *et al.*, 2005b). The
10 titer of anti-aldolase B remained high for several weeks after stopping troglitazone ad-
11 ministration. This finding supported the idea that troglitazone hepatotoxicity may have an
12 immunological basis. However, autoantibodies to aldolase B were also detected in the sera
13 of patients with chronic hepatitis as well as liver cirrhosis (Brown *et al.*, 1987; Manirata-
14 nachote *et al.*, 2005b). At present, a definitive explanation for the occurrence of aldolase B
15 autoantibodies, whether it is the cause or consequence of the progression of hepatotoxic-
16 ity, is still lacking. There are several reactive metabolites generated by troglitazone (Figure
17 11.2) (Kassahun *et al.*, 2001; Tettey *et al.*, 2001; Yamamoto *et al.*, 2002). Aldolase B, which
18 is an enzyme predominantly localized in the liver (Penhoet *et al.*, 1966; Rutter, 1964), may
19 be one of the target proteins that interact with those reactive species and trigger the immune
20 response. Further investigation may provide better understanding of this mechanism.

21
22
23 **11.6 Molecular Mechanism of Troglitazone-Induced Liver Toxicity**

24
25 Troglitazone has been shown to induce apoptosis in various hepatic (Bae and Song, 2003;
26 Tirmenstein *et al.*, 2002; Yamamoto *et al.*, 2001) and non-hepatic (Shiau *et al.*, 2005) cell
27 types depending on the concentration and time of exposure. Unlike its pharmacological
28 effects, the toxicity of troglitazone seems to be a PPAR γ -independent mechanism and the
29 higher affinity PPAR γ agonists such as rosiglitazone possess much lower toxic effects
30 (Lehmann *et al.*, 1995; Shiau *et al.*, 2005). In addition, Shiau *et al.* (2005) demonstrated
31 that a synthetic counterpart of troglitazone, which lacks PPAR γ activation activity, was also
32 able to induce apoptosis in cultured cells. As mentioned above, troglitazone can generate
33 the main metabolites M1 and M3 via the action of ST1 and CYP2C8/CYP3A4, respec-
34 tively. In *in vivo* experiments in rats, M1 showed the potential to inhibit Bsep, suggesting
35 it is one of the factors contributing to cholestasis in humans (Funk *et al.*, 2001a,b). In
36 addition, the troglitazone quinone metabolite M3 has been suggested to be associated with
37 troglitazone hepatotoxicity in humans (Neuschwander-Tetri *et al.*, 1998). However, these
38 metabolites showed fewer toxic effects compared to the parent compound, troglitazone,
39 when mammalian hepatocytes and hepatoma cell lines were treated directly (Honma *et al.*,
40 2002; Kostrubsky *et al.*, 2000; Tettey *et al.*, 2001; Tirmenstein *et al.*, 2002; Yamamoto
41 *et al.*, 2001, 2002).

42 It is most likely that troglitazone causes hepatic cell death via apoptosis. Apoptosis is a
43 normal physiologic form of cell death and plays a prominent role in liver pathogenesis such
44 as autoimmune liver diseases, viral hepatitis, and drug-induced hepatitis. In this regard, we
45 summarize below the molecular responses to troglitazone toxicity in the cells (Figure 11.3).
46



Au: Pls. check hard copy and msp mismatch.

Figure 11.3 Schematic representation of the effects of troglitazone in liver cells

11.6.1 MAPK-Mediated Cell Death Pathway

The three well-characterized subfamilies of mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (Erk), c-Jun N-terminal kinase (JNK) and p38, are regulated by phosphorylation and play important roles in a variety of cellular process including growth, differentiation and apoptosis (Johnson and Lapadat, 2002). Erk is generally activated by mitogens, while JNK and p38 are preferentially activated by stress and inflammatory cytokines. The most obvious effect of troglitazone on apoptosis is likely via the promotion of JNK, which in turn activates c-Jun by phosphorylation as well as by activation of p38 (Bae and Song, 2003). Gardner *et al.* (2005) reported that calcium/calmodulin-dependent kinase II (CaMKII) is a critical upstream activator of p38 phosphorylation in GN4 cells. In addition, troglitazone also causes the induction of Bax, Bad, the cleavage of Bid and release of cytochrome c. However, troglitazone showed a negligible effect on Erk (Bae and Song, 2003; Gardner *et al.*, 2003, 2005). JNK is characterized as a stress-activated protein kinase based on its activation in response to the inhibition of protein synthesis. We will provide the additional information supporting this point in a later section.

11.6.2 Impairment of Mitochondrial Functions

Another obvious mechanism of troglitazone-induced toxicity in liver cells is by causing a reduction of the mitochondrial membrane potential with a concomitant depletion of the cellular ATP concentration (Bova *et al.*, 2005; Tirmenstein *et al.*, 2002). Subsequently, it increases the plasma membrane permeability and calcium ion (Ca²⁺) efflux. The result of these effects on mitochondria is the release of cytochrome c into the cytoplasm and activation of the caspases leading to apoptosis (Bova *et al.*, 2005).

1 **11.6.3 Induction of Cell Cycle Arrest**

2
3 Cyclin-dependent kinases (CDKs) are serine–threonine protein kinases that regulate the
4 cell cycle progression. These kinases are activated by various cyclins, inhibited by natural
5 inhibitors such as p21, p27 and p18 and are tightly controlled by transcriptional and post-
6 transcriptional modifications (Sherr and Roberts, 1999). Bae *et al.* (2003) reported that
7 troglitazone-induced cell cycle arrest by this pathway and that apoptosis of hepatoma cell
8 lines was caused an elevation of the levels of p53 and its downstream proteins, Gadd45,
9 p21 and p27, as well as by a reduction in the levels of cyclin D1 and phospho-Rb.

10
11
12 **11.7 Effect of Troglitazone on the Inhibition of Protein Translation**

13
14 The endoplasmic reticulum (ER) is a major site of protein synthesis and its inside, or lumen,
15 is a major site of protein folding (Gething and Sambrook, 1992). In mammalian cells,
16 naturally the rate of protein synthesis is rapidly down-regulated following the induction of
17 apoptosis. The phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) is important in
18 the regulation of the selective translation during ER stress and the unfolded protein response
19 (Holcik and Sonenberg, 2005). Troglitazone was shown to promote Ca²⁺ release from the
20 ER leading to PERK and PKR activation, phosphorylation of eukaryotic initiation factor
21 2 α (eIF2 α), translation inhibition and growth arrest (Figure 11.3) (Fan *et al.*, 2004; Gardner
22 *et al.*, 2005; Palakurthi *et al.*, 2001). A study using PPAR γ ^{-/-} and PPAR γ ^{+/+} mouse
23 embryonic stem cells suggested that these effects were PPAR γ -independent (Palakurthi
24 *et al.*, 2001).

25 It is known that the ER is a major cellular storage site of Ca²⁺ in the cell, and ER
26 chaperones play important roles in Ca²⁺ accumulation and release. Any disturbance in
27 the ER homeostasis causes a release of Ca²⁺, which in turn blocks ER protein processing,
28 resulting in the accumulation of incompletely folded proteins, and activates the transcription
29 of ER chaperone genes (Liu *et al.*, 1998; Lodish and Kong, 1990). We found that the
30 immunoglobulin heavy-chain binding protein (BiP), an abundant chaperone protein in the
31 ER, was overexpressed in hepatoma cell lines by troglitazone treatment (Maniratanachote
32 *et al.*, 2005a). The important role of this chaperone protein was indicated by the phenotypic
33 change in cell viability when BiP expression was inhibited by small interference RNA
34 (Maniratanachote *et al.*, 2005a). This condition rendered cells more susceptible to the
35 toxic effects of troglitazone. Collectively, it might be postulated that troglitazone acts as a
36 chemical stress signal that causes the release of Ca²⁺ from the ER, and that BiP expression
37 is one of the cellular defense mechanisms of the ER in response to troglitazone-induced
38 toxicity (Figure 11.3).

39 With respect to the inhibition of translation by troglitazone toxicity, we recently found
40 that the ribosomal protein P0 (P0) is also one of the targets of troglitazone cytotoxicity in
41 HepG2 cells (Maniratanachote *et al.*, ••••). P0 is known as a phosphoprotein that functions
42 in some processes of protein translation (Gonzalo *et al.*, 2001). It was found that, rather than
43 its overexpression, dephosphorylation of P0 occurred in troglitazone-induced cytotoxicity
44 (Maniratanachote *et al.*, ••••). Therefore, dephosphorylation of P0 may play a role in the
45 regulation of protein translation in response to the toxic effects of troglitazone (Figure 11.3).

46

1 **11.8 Conclusions and Perspectives**

2
3 Troglitazone is a drug that can cause idiosyncratic hepatotoxicity in human. This kind of
4 toxicity is usually unpredictable, pharmacologically independent, rare and not reproducible
5 in experimental animal models, which makes it difficult to study (Lee, 2003). A number
6 of toxicological tests, both *in vivo* and *in vitro*, have been performed. So far, no direct
7 mechanism has been found that can explain why troglitazone hepatotoxicity occurred in
8 only some individuals. The failure to develop such toxicity in animal models is still inexplic-
9 cable. However, we have learned from previous reports that the mechanism of troglitazone
10 hepatotoxicity is PPAR γ -independent, the molecular mechanisms of apoptotic cell death
11 are most likely involved in the hepatotoxicity and the idiosyncratic hepatotoxicity might
12 be a consequence of a genetic basis in susceptible individuals.

13 Recent findings concerning the miRNA functions in specific tissues has enabled better
14 understanding of the molecular mechanisms of various pathologies and diseases (review in
15 Bartel, 2004). Among several hundreds miRNAs, miR-122 is the most abundant and liver-
16 specific (Lagos-Quintana *et al.*, 2002; Baskerville and Bartel, 2005). It has been shown to
17 have various roles, for example, in hepatitis C viral infection (Jopling *et al.*, 2005) and in
18 lipid metabolism (Esau *et al.*, 2006). Therefore, studies on miRNAs and their targets might
19 reveal clues concerning troglitazone idiosyncratic hepatotoxicity.

21
22 **Acknowledgments**

23
24 We thank Mr Brent Bell for reviewing the manuscript.

25
26
27 **References**

- 28
29 Bae, M. A. and Song, B. J. (2003). Critical role of c-Jun N-terminal protein kinase activation in
30 troglitazone-induced apoptosis of human HepG2 hepatoma cells. *Mol. Pharmacol.* **63**: 401–408.
31 Bae, M. A., Rhee, H. and Song, B. J. (2003). Troglitazone but not rosiglitazone induces G1 cell cycle
32 arrest and apoptosis in human and rat hepatoma cell lines. *Toxicol. Lett.* **139**: 67–75.
33 Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281–297.
34 Baskerville S. and Bartel, D. P. (2005). Microarray profiling of microRNAs with neighboring miRNAs
35 and host genes. *RNA* **11**: 241–247.
36 Bolton, J. L., Trush, M. A., Penning, T. M., Dryhurst, G. and Monks, T. J. (2000). Role of quinone
37 in toxicology. *Chem. Res. Toxicol.* **13**: 135–160.
38 Bourdi, M., Chen, W., Peter, R. M., Martin, J. L., Buters, J. T. M., Nelson, S. D. and Pohl, L. R.
39 (1996). Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis.
40 *Chem. Res. Toxicol.* **9**: 1159–1166.
41 Bova, M. P., Tam, D., McMahon, G. and Mattson, M. N. (2005). Troglitazone induces a rapid drop
42 of mitochondrial membrane potential in liver HepG2 cells. *Toxicol. Lett.* **155**: 41–50.
43 Brown, C., Toh, B. H., Pedersen, J. S., Clarke, F. M., Mackay, I. R. and Gust, I. (1987). Autoantibody
44 to aldolase in acute and chronic hepatitis. *Pathology* **19**: 347–350.
45 Ciaraldi, T. P., Gilmore, A., Olefsky, J. M., Goldberg, M. and Heidenreich, K. A. (1990). *In vitro*
46 studies on the action of CS-045, a new antidiabetic agent. *Metabolism* **39**: 1056–1062.

308 Hepatotoxicity

- 1 Esau, C., Davis, S., Murray, S. F., Yu, X. X., Pandey, S. K., Pear, M., Watts, L., Booten, S. L., Graham,
2 M., McKay, R., Subramaniam, A., Propp, S., Lollo, B. A., Freier, S., Bennett, C. F., Bhanot, S.
3 and Monia, B. P. (2006). miR-122 regulation of lipid metabolism revealed by *in vivo* antisense
4 targeting. *Cell Metab.* **3**: 87–98.
- 5 Fan, Y. H., Chen, H., Natarajan, A., Guo, Y., Harbinski, F., Iyasere, J., Christ, W., Aktas, H. and
6 Halperin, J. A. (2004). Structure–activity requirements for the antiproliferative effect of troglita-
7 zone derivatives mediated by depletion of intracellular calcium. *Bio. Med. Chem. Lett.* **44**: 2547–
8 2550.
- 9 Freid, J., Everitt, D. and Boscia, J. (2000). Rosiglitazone and hepatic failure. *Ann. Inter. Med.* **132**:
10 164.
- 11 Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I. and Horikoshi, H. (1988). Characterization of
12 new oral antidiabetic agent CS-045: Studies in KK and ob/ob mice and Zucker fatty rats. *Diabetes*
13 **37**: 1549–1558.
- 14 Fujiwara, T., Okuno, A., Yoshioka, T. and Horikoshi, H. (1995). Suppression of hepatic gluconeoge-
15 nesis in long-term troglitazone treated diabetic KK and C57BL/KsJ-db/db mice. *Metabolism* **44**:
16 486–490.
- 17 Funk, C., Pantze, M., Jehle, L., Ponelle, C., Scheuermann, G., Lazendic, M. and Gasser, Rodolfo.
18 (2001a). Troglitazone-induced intrahepatic cholestasis by an interference with the hepatobiliary
19 export of the bile acids in male and female rats. Correlation with the gender difference in troglita-
20 zone sulfate formation and the inhibition of the canalicular bile salt export pump (Bsep) by
21 troglitazone and troglitazone sulfate. *Toxicology* **167**: 83–98.
- 22 Funk, C., Ponelle, C., Scheuermann, G. and Pantze, M. (2001b). Cholestatic potential of troglita-
23 zone as a possible factor contributing to troglitazone-induced hepatotoxicity: *In vivo* and *in vitro*
24 interaction at the canalicular bile salt export pump (Bsep) in the rat. *Mol. Pharmacol.* **59**: 627–
25 635.
- 26 Gardner, O. S., Dewar, B. J., Earp, S., Samet, J. M. and Graves, L. M. (2003). Dependence of per-
27 oxisome proliferator-activated receptor ligand-induced mitogen activated protein kinase signaling
28 on epidermal growth factor transactivation. *J. Biol. Chem.* **278**: 46261–46269.
- 29 Gardner, O. S., Shiau, C. W., Chen, C. H. and Graves, L. M. (2005). Peroxisome proliferator-activated
30 receptor-independent activation of p38 MAPK by troglitazone involves calcium/calmodulin-
31 dependent protein kinase II and protein kinase R: Correlation with endoplasmic reticulum stress.
32 *J. Biol. Chem.* **280**: 10109–10118.
- 33 Gething, M. J. and Sambrook, J. (1992). Protein folding in the cell. *Nature* **355**: 33–45.
- 34 Gitlin N, Julie, N. L., Spurr, C. L., Lim, K. N. and Juarbe, H. M. (1998). Two cases of severe
35 clinical and histologic hepatotoxicity associated with troglitazone. *Ann. Intern. Med.* **129**: 36–
36 38.
- 37 Gonzalo, P., Lavergne, J. P. and Reboud, J. P. (2001). Pivotal role of the P1 N-terminal domain in
38 the assembly of the mammalian ribosomal stalk and in the proteosynthetic activity. *J. Biol. Chem.*
39 **276**: 19762–19769.
- 40 Gut, J., Christen, U. and Huwyler, J. (1993). Mechanism of halothane toxicity: Novel insights.
41 *Pharmac. Ther.* **58**: 133–155.
- 42 Hagenbuch, B. and Meier, P. J. (2003). The superfamily of organic anion transporting polypeptides.
43 *Biochim. Biophys. Acta* **1609**: 1–18.
- 44 Hanefeld, M. (2001). Pharmacokinetics and clinical efficacy of pioglitazone. *Int. J. Clin. Pract.*
45 (*Suppl.*) **121**: 19–25.
- 46 He, K., Woolf, T. F., Kindt, E. K., Fielder, A. E. and Talaat, R. E. (2001). Troglitazone quinone
formation catalyzed by human and rat CYP3A: An atypical CYP oxidation reaction. *Biochem.*
Pharmacol. **62**: 191–198.
- Holcik, M. and Sonenberg, N. (2005). Translational control in stress and apoptosis. *Nature Rev. Mol.*
Cell Biol. **6**: 318–327.

Mechanistic View of Troglitazone Hepatotoxicity 309

- 1 Honma, W., Shimada, M., Sasano, H., Ozawa, S., Miyata, M., Nagata, K., Ikeda, T. and Yamazoe, Y.
2 (2002). Phenol sulfotransferase, ST1A3, as the main enzyme catalyzing sulfation of troglitazone
3 in human liver. *Drug Metab. Dispos.* **30**: 944–952.
- 4 Inoue, I., Katayama, S., Takahashi, K., Negishi, K., Miyazaki, T., Sonoda, M. and Komoda, T. (1997).
5 Troglitazone has a scavenging effect on reactive oxygen species. *Biochem. Biophys. Res. Commu.*
6 **235**: 113–116.
- 7 Isley, W. L. and Oki, J. C. (2000). Rosiglitazone and liver failure. *Ann. Intern. Med.* **133**: 393.
- 8 Izumi, T., Enomoto, S., Hoshiyama, K., Sasahara, K. and Sugiyama, Y. (1997a). Pharmacokinetic
9 stereoselectivity of troglitazone, an antidiabetic agent, in the KK mouse. *Biopharm. Drug Dispos.*
10 **18**: 305–324.
- 11 Izumi, T., Hoshiyama, K., Enomoto, S., Sasahara, K. and Sugiyama, Y. (1997b). Pharmacokinetics of
12 troglitazone, an antidiabetic agent: Prediction of *in vivo* stereoselective sulfation and glucuronida-
13 tion from *in vitro* data. *J. Pharmacol. Exp. Ther.* **280**: 1392–1400.
- 14 Johnson, G. L. and Lapadat, R. (2002). Mitogen-activated protein kinase pathways mediated by ERK,
15 JNK and p38 protein kinases. *Science* **298**: 1911–1912.
- 16 Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M. and Sarnow, P. (2005). Modulation of hepatitis
17 C virus RNA abundance by a liver-specific microRNA. *Science* **309**: 1577–1581.
- 18 Kassahun, K., Pearson, P. G., Tang, W., McIntosh, I., Leung, K., Elmore, C., Dean, D., Wang, R., Doss,
19 G. and Baille, T. A. (2001). Studies on the mechanism of troglitazone to reactive intermediates
20 *in vitro* and *in vivo*. Evidence for novel biotransformation pathways involving quinone methide
21 formation and thiazolidinedione ring scission. *Chem. Res. Toxicol.* **14**: 62–70.
- 22 Kawai, K., Kawasaki-Tokui, Y., Odaka, T., Tsuruta, F., Kazui, M., Iwabuchi, H., Nakamura, T.,
23 Kinoshita, T., Ikeda, T., Yoshioka, T., Komai, T. and Nakamura, K. (1997). Disposition and
24 metabolism of the new oral antidiabetic drug troglitazone in rats, mice and dogs. *Arzneimittelforschung*
25 **47**: 356–368.
- 26 Kawai, K., Odaka, T., Tsuruta, F., Tokui, T., Ikeda, T. and Nakamura, K. (1998). Stereoselective
27 metabolism of new oral anti-diabetic agent troglitazone stereoisomers in liver. *Xenobio. Metab.*
28 *Dispos.* **13**: 362–368.
- 29 Kostrubsky, V. E., Sinclair, J. F., Ramachandran, V., Venkataramanan, Wen, Y. H., Kindt, E., Galchev,
30 V., Rose, K., Sinz, M. and Strom, S. C. (2000). The role of conjugation in hepatotoxicity of
31 troglitazone in human and porcine hepatocyte cultures. *Drug Metab. Dispos.* **28**: 1192–1197.
- 32 Krebs, R. (2006). Implications of genetic polymorphism in drug transporters for pharmacotherapy.
33 *Cancer Lett.* **234**: 4–33.
- 34 Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W. and Tuschl, T. (2002). Identifi-
35 cation of tissue-specific microRNAs from mouse. *Current Biol.* **12**: 735–739.
- 36 Lebovitz, H. E., Kreider, M. and Freed, M. I. (2002). Evaluation of liver function in type 2 diabetic
37 patients during clinical trials. *Diabetes Care* **25**: 815–821.
- 38 Lee, W. M. (2003). Drug-induced hepatotoxicity. *New Engl. J. Med.* **349**: 474–485.
- 39 Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. and Kliewer, S. A.
40 (1995). An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome-activated receptor
41 γ (PPAR γ). *J. Biol. Chem.* **270**: 12953–12956.
- 42 Liu, H., Miller, E., van de Water, B. and Stevens, J. L. (1998). Endoplasmic reticulum stress proteins
43 block oxidant-induced Ca²⁺ increases and cell death. *J. Biol. Chem.* **273**: 12858–12862.
- 44 Lodish, H. F. and Kong, N. (1990). Perturbation of cellular calcium blocks exit of secretory proteins
45 from the rough endoplasmic reticulum. *J. Biol. Chem.* **265**: 10893–10899.
- 46 Loi, C. M., Young, M., Randinitis, E., Vassos, A. and Koup, J. R. (1999). Clinical pharmacokinetics
of troglitazone. *Clin. Pharmacokinet.* **37**: 91–104.
- Maniratanachote, R., Minami, K., Katoh, M., Nakajima, M. and Yokoi, T. (2005a). Chaperone proteins
involved in troglitazone-induced toxicity in human hepatoma cell lines. *Toxicol. Sci.* **83**: 293–
302.

310 Hepatotoxicity

- 1 Maniratanachote, R., Shibata, A., Kaneko, S., Yamamori, I., Wakasugi, T., Sawazaki, T., Katoh, K.,
2 Tokudome, S., Nakajima, M. and Yokoi, T. (2005b). Detection of autoantibody to aldolase B in
3 sera from patients with troglitazone-induced liver dysfunction. *Toxicology* **216**: 15–23.
- 4 Maniratanachote, R., Minami, K., Katoh, M., Nakajima, M. and Yokoi, T. (2006). Dephosphorylation
5 of ribosomal protein P0 in response to troglitazone-induced cytotoxicity. *Toxicol. Lett.* **166**: 189–
6 199.
- 7 Neuschwander-Tetri, B. A., Isley, W. L., Oki, J. C., Ramrakhiani, S., Quiason, S. G., Phillips, N. J.
8 and Brunt, E. M. (1998). Troglitazone-induced hepatic failure leading to liver transplantation. *Ann.*
9 *Intern. Med.* **129**: 38–41.
- 10 Nozawa, T., Sugiura, S., Nakajima, M., Goto, A., Yokoi, T., Nezu, J., Tsuji, A. and Tamai, I. (2004).
11 Involvement of organic anion transporting polypeptides in the transport of troglitazone sulfate:
12 Implications for understanding troglitazone hepatotoxicity. *Drug Metab. Dispos.* **32**: 291–294.
- 13 Ott, P., Ranek, L. and Young, M. A. (1998). Pharmacokinetics of troglitazone, a PPAR-gamma agonist,
14 in patients with hepatic insufficiency. *Eur. J. Clin. Pharmacol.* **54**: 567–571.
- 15 Palakurthi, S. S., Aktas, H., Grubisich, L. M., Mortensen, R. M. and Halperin, J. A. (2001). Anticancer
16 effects of thiazolidinediones are independent of peroxisome proliferators-activated receptor γ and
17 mediated by inhibition of translation initiation. *Cancer Res.* **61**: 6213–6218.
- 18 PDR (1999). Rezulin[®], in *Physician's Desk Reference*, 52nd Edition, Medical Economics Company,
19 Inc., Montvale, NJ, USA, pp. 2310–2314.
- 20 PDR (2005a). Actos[®], in *Physician's Desk Reference*, 59th Edition, Thomson PDR, Montvale, NJ,
21 USA, pp. 3181–3185.
- 22 PDR (2005b). Avandia[®], in *Physician's Desk Reference*, 59th Edition, Thomson PDR, Montvale,
23 NJ, USA, pp. 1438–1443.
- 24 Penhoet, E., Rajkumar, T. and Rutter, W. J. (1966). Multiple forms of fructose diphosphate aldolase
25 in mammalian tissues. *Proc. Natl. Acad. Sci. USA* **56**: 1275–1282.
- 26 Pohl, L. R., Satoh, H., Christ, D. D. and Kenna, J. G. (1988). The immunologic and metabolic basis
27 of drug hypersensitivity. *Ann. Rev. Pharmacol.* **28**: 367–387.
- 28 Prabhu, S., Fackett, A., Lloyd, S., McClellan, H. A., Terrell, C. M., Silber, P. M. and Li, A. P. (2002).
29 Identification of glutathione conjugates of troglitazone in human hepatocytes. *Chem. Biol. Interact.*
30 **142**: 83–97.
- 31 Ramachandran, V., Kostubsky, V. E., Komoroski, B. J., Zhang, S., Dorko, K., Esplen, J. E., Strom,
32 S. C. and Venkataraman, R. (1999). Troglitazone increases cytochrome P-450 3A protein and
33 activity in primary cultures of human hepatocytes. *Drug Metab. Dispos.* **27**: 1194–1199.
- 34 Rothwell, C., McGuire, E. J., Altrogge, D. M., Masuda, H. and de la Iglesia, F. A. (2002). Chronic
35 toxicity in monkeys with the thiazolidinedione antidiabetic agent troglitazone. *J. Toxicol. Sci.* **27**:
36 35–47.
- 37 Rutter, W. J. (1964). Evolution of aldolase. *Fed. Proc.* **23**: 1248–1257.
- 38 Sahi, J., Hamilton, G., Sinz, M., Barros, S., Huang, S. M., Lesko, L. J. and LeCluyse, E. L. (2000).
39 Effects of troglitazone on cytochrome P450 enzymes in primary cultures of human and rat hepa-
40 tocytes. *Xenobiotica* **30**: 273–284.
- 41 Saltiel, A. R. and Olefsky, J. M. (1996). Thiazolidinediones in the treatment of insulin resistance and
42 type II diabetes. *Diabetes* **45**: 1661–1669.
- 43 Sherr, C. J. and Roberts, J. M. (1999). CDK inhibitors: Positive and negative regulators of G1-phase
44 progression. *Genes Dev.* **13**: 1501–1512.
- 45 Shiao, C. W., Yang, C. C., Kulp, S. K., Chen, K. F., Chen, C.S., Huang, J. W. and Chen, C. H.
46 (2005). Thiazolidinediones mediate apoptosis in prostate cancer cells in part through inhibition of
Bcl-xl/Bcl-2 functions independently of PPAR γ . *Cancer Res.* **65**: 1561–1569.
- Shibuya, A., Watanabe, M., Fujita, Y., Saigenji, K., Kuwano, S., Takahashi, H. and Takeuchi, H. (1998).
An autopsy case of troglitazone-induced fulminant hepatitis. *Diabetes Care* **21**: 2140–2143.

Mechanistic View of Troglitazone Hepatotoxicity 311

1 Spiegelman, B. M. (1998). PPAR- γ : Adipogenic regulator and thiazolidinedione receptor. *Diabetes*
2 47: 507–514.

3 Tettey, J. N., Maggs, J. L., Rapeport, W. G., Pirmohamed, M. and Park, B. K. (2001). Enzyme
4 induction dependent bioactivation of troglitazone and troglitazone quinone *in vivo*. *Chem. Res.*
5 *Toxicol.* 14: 965–974.

6 Tirmenstein, M. A., Hu, C. X., Gales, T. L., Maleeff, B. E., Narayanan, P. K., Kurali, E., Hart,
7 T. K., Thomas, H. C. and Schwartz, L. W. (2002). Effects of troglitazone on HepG2 viability and
8 mitochondrial function. *Toxicol. Sci.* 69: 131–138.

9 Watanabe, T., Ohashi, Y., Yasuda, M., Takaoka, M., Furukawa, T., Yamoto, T., Sanbuissho, A. and
10 Manabe, S. (1999). Was it possible to predict liver dysfunction caused by troglitazone during the
11 nonclinical safety studies? *Iyakuhin Kenkyu* 30: 537–546.

12 Watanabe, Y., Nakajima, M. and Yokoi, T. (2002). Troglitazone glucuronidation in human liver and
13 intestine microsomes: high catalytic activity of UGT1A8 and UGT1A10. *Drug Metab. Dispos.* 30:
14 1462–1469.

15 Watanabe, I., Tomita, A., Shimizu, M., Sugawara, M., Yasumo, H., Koishi, R., Takahashi, T., Miyoshi,
16 K., Nakamura, K., Izumi, T., Matsushita, Y., Furukawa, H., Haruyama, H. and Koga, T. (2003). A
17 study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity
18 in Japanese patients with type 2 diabetes mellitus. *Clin. Pharmacol. Ther.* 73: 435–455.

19 Watkins, P. B. and Whitcomb, R. W. (1998). Hepatic dysfunction associated with troglitazone. *New*
20 *Engl. J. Med.* 338: 916–917.

21 Yamamoto, Y., Nakajima, M., Yamazaki, H. and Yokoi, T. (2001). Cytotoxicity and apoptosis pro-
22 duced by troglitazone in human hepatoma cells. *Life Sci.* 70: 471–482.

23 Yamamoto, Y., Yamazaki, H., Ikeda, T., Watanabe, T., Iwabuchi, H., Nakajima, M. and Yokoi, T.
24 (2002). Formation of a quinone epoxide metabolite of troglitazone with cytotoxic to HepG2 cells.
25 *Drug Metab. Dispos.* 30: 155–160.

26 Yamazaki, H., Shibata, A., Suzuki, M., Nakajima, M., Shimada N., Guengerich, F. P. and Yokoi, T.
27 (1999). Oxidation of troglitazone to a quinone-type metabolite catalyzed by cytochrome P-450
28 2C8 and P-450 3A4 in human liver microsomes. *Drug Metab. Dispos.* 27: 1260–1266.

29 Yoshigae, Y., Konno, K., Takasaki, W. and Ikeda, T. (2000). Characterization of UDP-
30 glucuronosyltransferases (UGTS) involved in the metabolism of troglitazone in rats and humans.
31 *J. Toxicol. Sci.* 25: 433–441.

32
33
34
35
36
37
38
39
40
41
42
43
44
45
46