

Co-administration of cyclosporine A alleviates thioacetamide-induced liver injury

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Abstract

AIM: To investigate the effects of cyclosporine A (CsA) on thioacetamide (TAA)-induced liver injury.

METHODS: CsA was co-administrated (7.5 µg/kg body weight per day, i.p.) into rat to investigate the role of CsA on TAA-(200 mg/kg body weight per 3 d for 30 d, i.p.) induced liver injury.

RESULTS: The data show that TAA caused liver fibrosis in rat after 30 d of treatment. CsA alleviates the morphological changes of TAA-induced fibrosis in rat liver. The blood glutamyl oxaloacetic transaminase (GOT)/glutamyl pyruvic transaminase (GPT) in the TAA-injury group is elevated compared to that of the normal rat. Compared with the TAA-injury group, the blood GOT/GPT and TGFβ1 (by RT-PCR analysis) are reduced in the CsA plus TAA-treated rat. The level of the transforming growth factor receptor I (TGFβ-R1) in the CsA plus TAA-treated group shows higher than that in the TAA only group, but shows a lower level of the fibroblast growth factor receptor 4 (FGFR4) in the CsA plus TAA-treated group, when using the Western blot analysis. After immunostaining of the frozen section, TGFβ-R1 and FGFR4 are more concentrated in rat liver after CsA plus TAA injury.

CONCLUSION: This result suggests that CsA has an alleviated effect on TAA-induced liver injury by increasing the multidrug resistance P-glycoprotein and could be through the regulation of TGFβ-R1 and FGFR4.

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Key words: Cyclosporine A; Thioacetamide; Liver injury; P-glycoprotein; TGFβ1; TGFβ-R1; FGFR4

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INTRODUCTION

Thioacetamide [TAA, CH₃-C(S)NH₂], a hepatotoxin, was first used to control the decay of oranges and then as a fungicide^[1]. In the liver, TAA is S-oxidized at the thioamide group to TAA sulfoxide [CH₃-C(SO)NH₂] and subsequently to di-S-oxide [CH₃-C(SO₂)NH₂]. Reactive intermediates in this pathway covalently bind to hepatic macromolecules and eventually cause liver injury^[2,3]. TAA undergoes an extensive metabolism to acetamide shortly after administration and to the hepatotoxic metabolite TAA-S-oxide by the mixed function oxidase system^[2-4]. Free radical-mediated lipid peroxidation contributes to the development of TAA-induced liver fibrosis^[5,6]. In chronic TAA intoxication, substantial liver fibrosis and prominent regenerative nodules develop after 3 mo of TAA administration and are associated with portal hypertension and the hyperdynamic circulation characteristic of liver cirrhosis^[7]. Concerning biochemical and morphological observations, the TAA-induced rat liver cirrhosis has been shown to resemble the human disease and serves as a suitable animal model for studying the causes of human liver fibrosis and cirrhosis^[8]. The transforming growth factor beta-1 (TGFβ1) is synthesized in non-parenchymal cells such as hepatic stellate cell (HSC) and inhibits hepatocellular DNA synthesis, both in culture and *in vivo*. Picomolar concentrations of TGFβ1 suppress hepatocyte DNA synthesis in culture. Moreover, injection of TGFβ1 into partially hepatectomized rats significantly delays the onset of DNA synthesis. TGFβ1 mediates the transformation of quiescent HSCs into myofibroblast-like cells with an increased production of extracellular matrix (ECM) proteins, including type I collagen^[9-13]. In addition, TGFβ1 increases the synthesis and deposition of ECM proteins such as fibronectin by HSC, and is closely associated with the progression of hepatic fibrosis.

Cyclosporine A (CsA), a fungal cyclic polypeptide and used clinically as an immunosuppressive agent^[14,15] following renal, cardiac, pancreatic, bone marrow, and hepatic transplantation, has a number of adverse effects including renal, hepatic, cardiovascular, alimentary, skin and neural toxicity^[16,17]. The alterations of dilatation of the endoplasmic reticulum, loss of ribosomes, centrilobular fatty infiltration and focal hepatocyte necrosis have been observed microscopically in livers from CsA-treated animals^[16,18]. Inhibition of the ATP-dependent bile salt export carrier in the canalicular membrane and the P-glycoprotein (P-gp) transporter is probably involved in cholestasis caused by CsA^[19,20]. Despite extensive research on the sideeffects of CsA including hypertension, hepatotoxicity and nephrotoxicity, the exact mechanisms of CsA-induced hepatotoxicity remain

obscure. Fibroblast growth factors (FGFs) comprise a growing family of structurally related polypeptide growth factors, currently consisting of 23 members^[21]. They transduce their signals through four high-affinity transmembrane protein tyrosine kinases, FGF receptors 1-4 (FGFR1-4)^[21-24], which bind the different FGFs with different affinities. Of the four FGFR isotypes, only FGFR4 is expressed in mature hepatocytes^[25]. It has become increasingly clear that the ubiquitous and extremely diverse members of the FGF family of activating FGF polypeptides, FGFR transmembrane kinases and heparan sulfate oligosaccharide chains combine in a tissue-specific mode to sense perturbation and maintain homeostasis, both in diverse developing and adult tissues^[22,25,26]. Recently, an agonist role for FGF1 and FGF2 is seen in specifically insult-induced liver matrix deposition and hepatic fibrogenesis and as a potential target for the prevention of hepatic fibrosis by acute CCl₄ exposure^[27]. The aim of this study was to investigate whether co-administration of CsA with liver fibrotic agent TAA had any beneficial or deleterious effects on TAA-induced liver injury.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250-300 g) were obtained from the National Laboratory Animal Center, Taipei, Taiwan, and were kept in a temperature-controlled environment (22 °C) and fed ad libitum with standard rat chow. Rats were randomly allotted into TAA-, CsA-, CsA plus TAA- and placebo-treated groups ($n = 5$ in each group). Liver cirrhosis in rats was induced by intraperitoneal (i.p.) injection of TAA (200 mg/kg) every 3 d for 30 d is previously described. Animals assigned to the CsA (Neoral[®]; Sandimmune[®]) group were given a daily dose of CsA (7.5 µg/kg body weight, i.p.) dissolved in olive oil. The control group received the vehicle (normal saline solution) only. Treatments were carried out for 30 d. Rats were bled for blood test and sacrifices were treated with carbon dioxide gas anesthesia in closed chamber after the end of the experiment. One part of the liver was sampled for immunohistology. The remainder of the liver was rapidly removed and stored at -80 °C for RT-PCR and Western blot analysis.

GOT/GPT

The level of glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT) in the blood, as a hepatic index for determining the status of liver function, was measured by using the Johnson and Johnson assay on the Vitros 750 (J and J/Vitros 750), a kinetic enzymatic assay in which the rate of formation of the final oxidized leuco dye is monitored at 670 nm. All assays were performed according to the procedures described by the manufacturers.

Semi-quantitative RT-PCR

The liver was weighed before being homogenized with a motorized Teflon pestle at 1 000 r/min for 2 min on ice, in cold sterile water (W:V = 1:2), or in a Trizol reagent (Invitrogen, USA) in order to isolate total RNA. After centrifugation (12 902 g for 30 min at 4 °C), the supernatant was kept at -70 °C until the assay. Total RNA was extracted, and 5 µg of each RNA was soon thereafter reverse transcribed to

first strand cDNA in 20 µL of reaction mixture using a SuperScript[™] First-Strand Synthesis System for RT-PCR kit (Invitrogen). Sets of PCR primers for selected genes and β-actin were designed based on the NCBI database of conserved coding regions (Table 1). One microliter of cDNA solution and two sets (β-actin and selected gene) of primers were used in 25 µL of PCR reaction samples. The parameters of the β-actin PCR reaction were 25 cycles at 95 °C for 45 s, 50 °C for 45 s and 72 °C for 1 min. After 25 cycles of amplification and sampling, 10 additional cycles under the same condition for the selected gene were amplified with a single cycle at 72 °C for 10 min. PCR products were separated in 12 g/L agarose gel. After ethidium bromide (EtBr, 0.5 µg/mL) staining and photographing, the data were analyzed by phoretix ID standard software, after β-actin (as an internal control) internalization.

Table 1 Primer sequence and expected lengths of fragments in RT-PCR analysis of rat liver selected genes and β-actin

Gene		Primer sequence	Expected lengths of fragments
β-actin	Forward	5'-GTCTCCCTCCATCGTG-3'	992 bp
	Reverse	5'-TGCTTGCTGATCCACATCTG-3'	
FGFR2	Forward	5'-GGACAGACCCAAGGAGGCAG-3'	667 bp
	Reverse	5'-GCCAGCAGTCCCTCATCATC-3'	
FGFR4	Forward	5'-GGAGGTGCTGTATCTGAGGAACGTG-3'	599 bp
	Reverse	5'-TGTCGGAGGCATTGTCTTTCAG-3'	
TGFβ R1	Forward	5'-CGTCGCTGCTGCTTCTCATC-3'	646 bp
	Reverse	5'-CCGCCATTTCCTCGCC-3'	
TGFβ R2	Forward	5'-CGACAACCTGCGCCATCATCC-3'	649 bp
	Reverse	5'-GGCCATGTATCTCGCTGTCCCC-3'	
TGFβ R3	Forward	5'-GGTGTGGCATCTGAAGACGGAG-3'	787 bp
	Reverse	5'-GCTCAGGAGGAATGGTGTGGACT-3'	
Collagen1	Forward	5'-GCGAAGCAACAGTCGATTC-3'	69 bp
	Reverse	5'-CCCAAGTTCGGGTGTGACTC-3'	
Collagen3	Forward	5'-CAGCTGGCCTTCTCAGACTT-3'	70 bp
	Reverse	5'-GCTGTTTTTGCAGTGGTATGTAATG-3'	
Abcb1	Forward	5'-GGACCCACAGCGGAGG-3'	634 bp
	Reverse	5'-GCAGGGTTGTGTAGGGCTCA-3'	

Sequence analysis

To confirm the nucleotide sequence of the PCR-amplified product selected, the product was cloned into plasmid following a cloning procedure as previously described. Plasmid DNA of the recombinant colonies was isolated and purified, using a Qiagen Plasmid Mini Kit. Fifty vectors were sequenced at once, from the 5' end using a Dye Terminator Cycle Sequence FS Ready Kit and a T7 primer from Applied Biosystems (Applied Biosystems ABI 377 sequencer). Sequence files were processed electronically to remove vector sequences, and were then analyzed using an automatic BLAST algorithm, to screen the public nucleotide databases.

Western blotting

The liver was homogenized in a homogenization solution (137 mmol/L NaCl, 1 mmol/L CaCl₂, 1 mmol/L MgCl₂, 0.1 mmol/L sodium ortho-vanadate, 20 mmol/L Tris-HCl pH 7.4, 10 g/L NP-40, 1 mmol/L PMSF) with a motorized Teflon pestle at 600 r/min for 20 strokes, on ice. After centrifugation (at 12 902 g for 30 min at 4 °C), the supernatant

was kept at -70°C until the assay. Total protein was determined using the Bradford protein assay kit (Bio-Rad, Hercules, CA) and calculated using bovine serum albumin (Sigma, St. Louis, MO) as a standard. The whole liver homogenate (total protein $40\ \mu\text{g}$) was mixed with $6\times$ electrophoresis sample buffer, containing 1,4-dithiothreitol (DTT, Sigma). The proteins were separated by electrophoresis on a 40–120 g/L gradient polyacrylamide slab gel, and then electrophoretically transferred to a PVDF membrane (NENTM Life Science Products, Boston, MA). The blots were incubated for 2 h in blocking buffer (30 g/L BSA in TBST buffer) and were washed twice in TBST buffer (8 g/L NaCl, 0.2 g/L KCl, 3 g/L Tris-base, pH 7.5, 0.24 g/L KH_2PO_4 and 2 g/L Tween-20). Membranes were incubated overnight at 4°C with the first antibody as shown in Table 2. After incubation with a secondary antibody (alkaline phosphatase-conjugated goat anti-rabbit/sheep antibody, 1:4 000, ZYMED, San Francisco), the protein bands were analyzed by ECL autoradiography.

Table 2 Antibodies used in current investigation

Antibody ¹	Dilution	Protein size (ku)
β -actin, mouse polyclone	1:500	41
FGFR-2, rabbit polyclone, sc-122	1:500	119
FGFR-4, rabbit polyclone, sc-124	1:500	93
TGF β R1, rabbit polyclone, sc-402	1:1 000	53

¹All antibodies were purchased from Santa Cruz Biotechnology (CA, USA).

Immunohistochemistry

Fresh tissues were carefully embedded in OCT (Optimal Cutting Temperature, 4583, Tissue-Tek, SAKURA) in a plastic mold, without air bubbles surrounding the tissue. The mold was then put on top of liquid nitrogen until 70–80% of the block turned white, and then the block was put on top of dry ice. Prior to being sectioned, the frozen block was equilibrated in the cryostat chamber for about 5 min. Sections, 10- μm thick, were mounted on glass slides, dried at room temperature (RT) for at least 30 min. After washing with PBS twice, the tissue slide was incubated with 30 mL/L H_2O_2 for 10 min to cut down the endogenous peroxidase. The slide was blocked with 70–100 g/L BSA for 20 min after washing. The primary antibodies (diluted in 0.05 mol/L Tris-saline, pH 7.4, 250 mL/L serum) as shown in Table 2 were directly added onto the sections and incubated overnight at 4°C . The antibodies were removed, and the tissue slide was washed thrice, for 10 min each time, with PBS buffer, at RT. The secondary antibody, diluted in Tris-saline/25 mL/L serum, was added and incubated for an additional 45 min. After washing, an ABC (avidin and biotinylated horseradish peroxidase macromolecular complex, PK-6105, VECTOR) reagent was added for 30 min of incubation. Methyl green was counterstained for less than 5 min after washing. The slide was sequentially fixed with 700, 850, 950 and 1 000 mL/L ethanol and xylene. The slide was then washed with deionized water for 5 min and left on a bench to air dry. The stained slide was mounted with VectaMountTM, P0505, (VECTOR, CA, USA), and microscopic examinations were carried out.

Sirius red staining (collagen)

The tissue slide was stained with Weigert's hematoxylin for cell nuclear staining prior to the Sirius red staining. After a 10-min wash, the slide was incubated with Pico Sirius red at RT for 1 h. The slide was then washed twice with acidified water before dehydration with ethanol absolute anhydrous. Afterwards, the tissue slide was washed with xylene and fixed in a permanent mounting medium (VECTOR).

Statistical analysis

All data are expressed as the mean \pm SE. Differences among the groups were determined by one-way analysis of variance (ANOVA) using SuperANOVA, statistical software from MICROSOFT (Abacus Concepts Inc., Berkeley, CA), and the means among them were compared with control values by the Student's *t* test. *P* values <0.05 were chosen to be significant.

RESULTS

Morphology/blood GOT/GPT

After carbon dioxide anesthetic, the internal organ was removed immediately and a photograph was taken. The data showed that TAA obviously induced morphological injury in the liver after 30-d treatment. CsA alleviated the morphological changes of TAA-induced fibrosis in rat liver. The GOT/GPT of the blood in the TAA-injury group was elevated compared to that of the normal rat. Compared with the TAA-injury group, the blood GOT/GPT declined in the CsA plus TAA-treated rat (Figures 1A, B).

Collagen

After Sirius red staining of the frozen sections, the collagen in the CsA plus TAA group was lower than that in the TAA group (Figure 2A). After RT-PCR of collagen I and III, the data showed that TAA induced collagen I and III in the RNA level compared to that of the control group. The collagen I expression of the CsA plus TAA group was lower than that in the TAA group (Figure 2B). However, the collagen III level was not significantly different between the TAA and the CsA plus TAA groups (Figure 2C).

TGF β 1 and receptor

TGF β 1 appears to be an important regulator in both normal and pathological conditions in the liver. In the TAA or CsA group, the expression of the TGF β 1 level was elevated compared to that of the control group. Conversely, the TGF β 1 in the CsA plus TAA group was lower than that in CsA or TAA alone (Figure 3A). The expression levels of TGF β -R2 and TGF β -R3 are not significantly different among treatments (Figures 3B, C). The protein level of TGF β -R1 in CsA plus TAA-treated liver is higher than that of the TAA group (Figure 4B), but not significantly different in RNA level among treatments (Figure 4A). The aggregations of TGF β -R1 immunostaining in liver caused by the TAA or the CsA group were sparse and scattered compared with those by the CsA plus TAA group (Figure 4C). This result implies that CsA might have a protective effect on TAA-induced liver injury through the regulation of the TGF β 1 receptor.

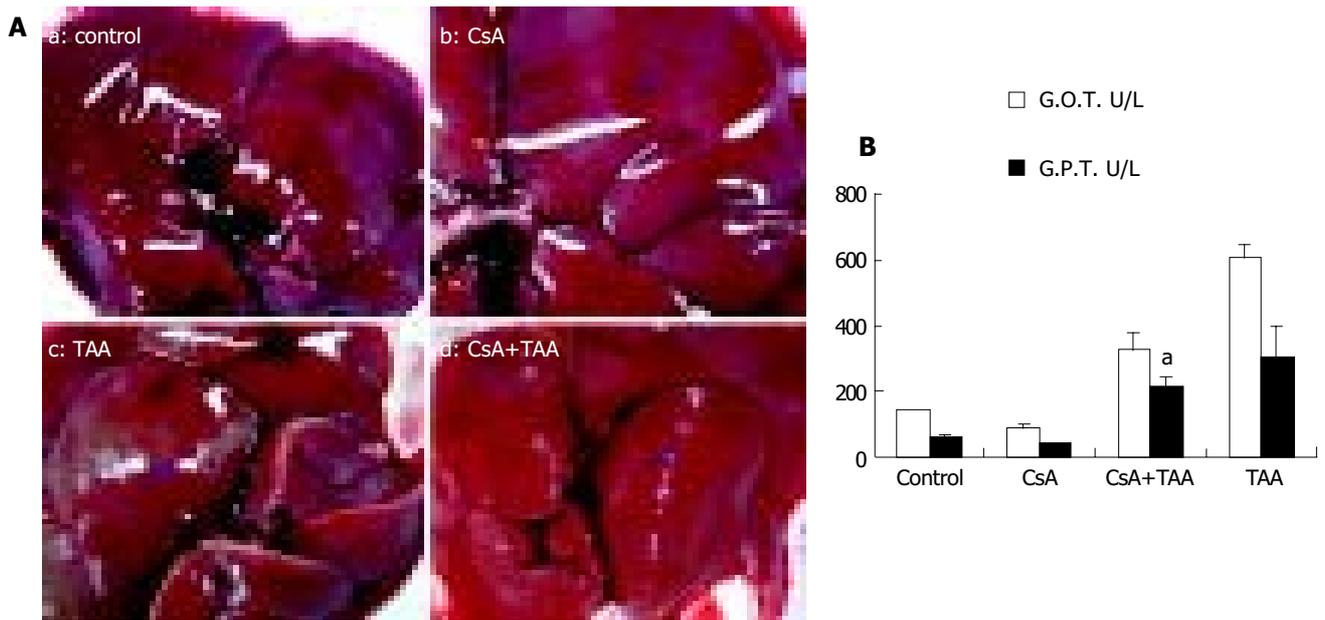


Figure 1 (A) morphology of liver, and the (B) alterations of blood GOT/GPT in (a) control, (b) CsA treatment, (c) TAA-induced, (d) CsA plus TAA treatment rats. The regimens of CsA, TAA or CsA plus TAA treatment were as described in Materials and Methods. ^aP<0.05 between TAA and TAA plus CsA treatments.

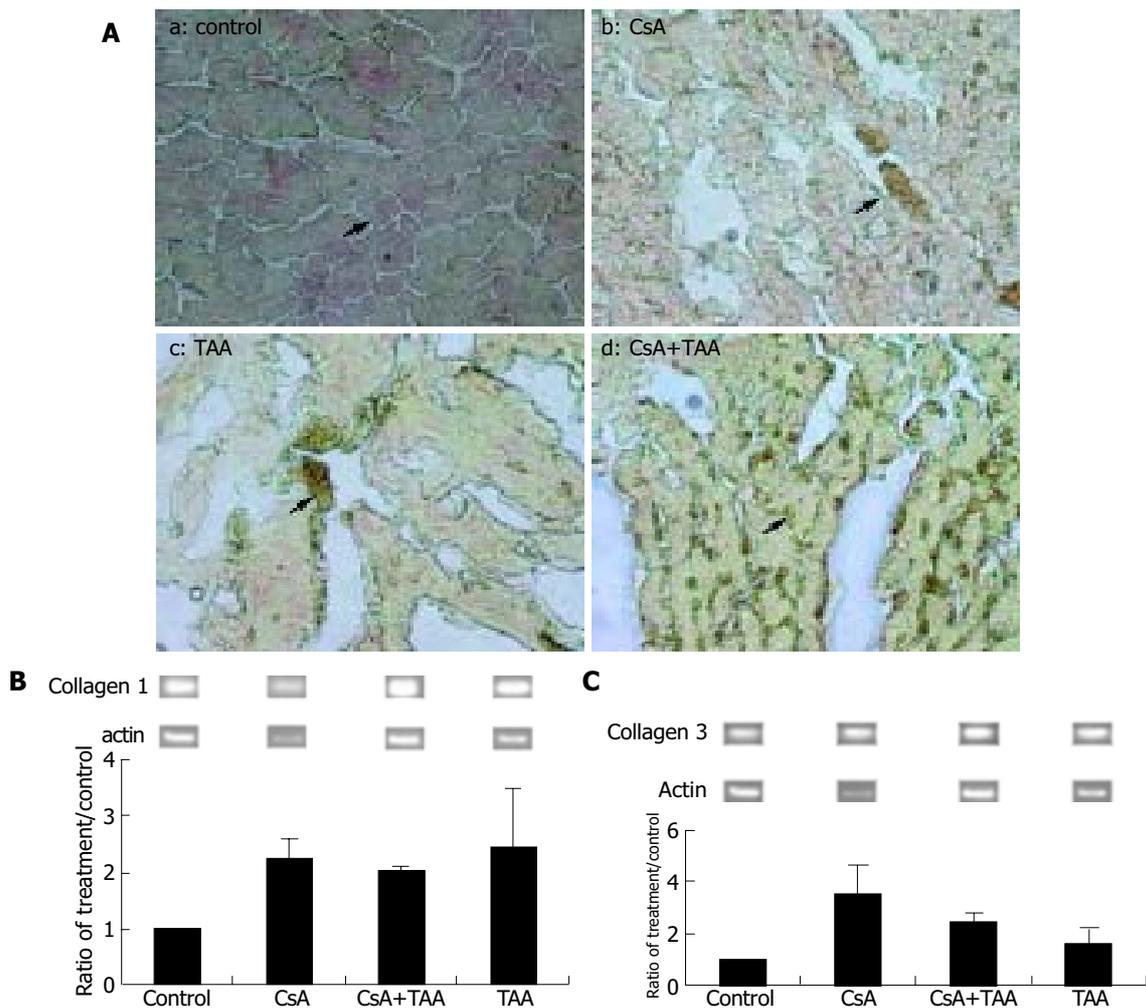


Figure 2 Levels of (A) collagen staining, (B) collagen 1 RNA expression, and (C) collagen 3 RNA expression in rat liver after various treatments. Immunocytochemistry of collagen (red) was stained by using Sirius red. Magnification = ×200. The expression levels of collagen 1 and 3 were measured by using semi-quantitative PCR.

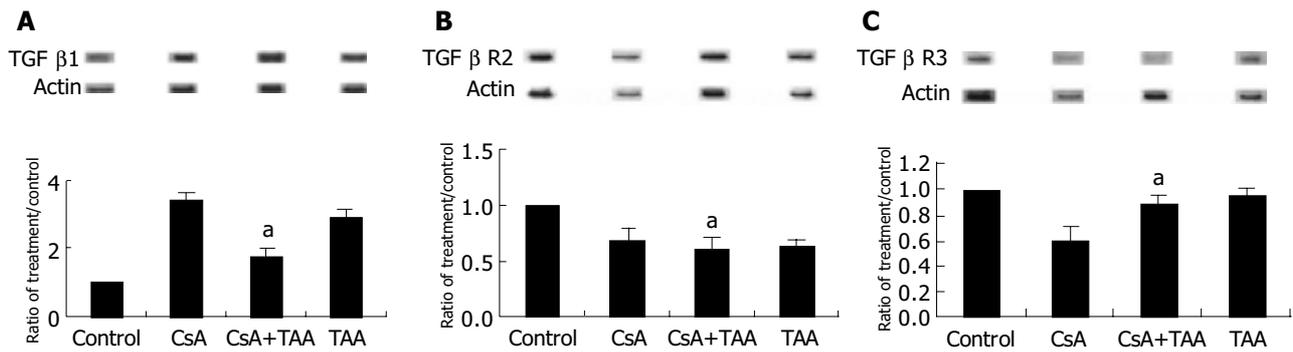


Figure 3 The RNA expression levels of (A) TGFβ1, (B) TGFβ2 and (C) TGFβ3 in rat liver after various treatments by using semi-quantitative PCR. 1 represents the significant difference ^aP<0.05 between TAA and TAA plus CsA treatments.

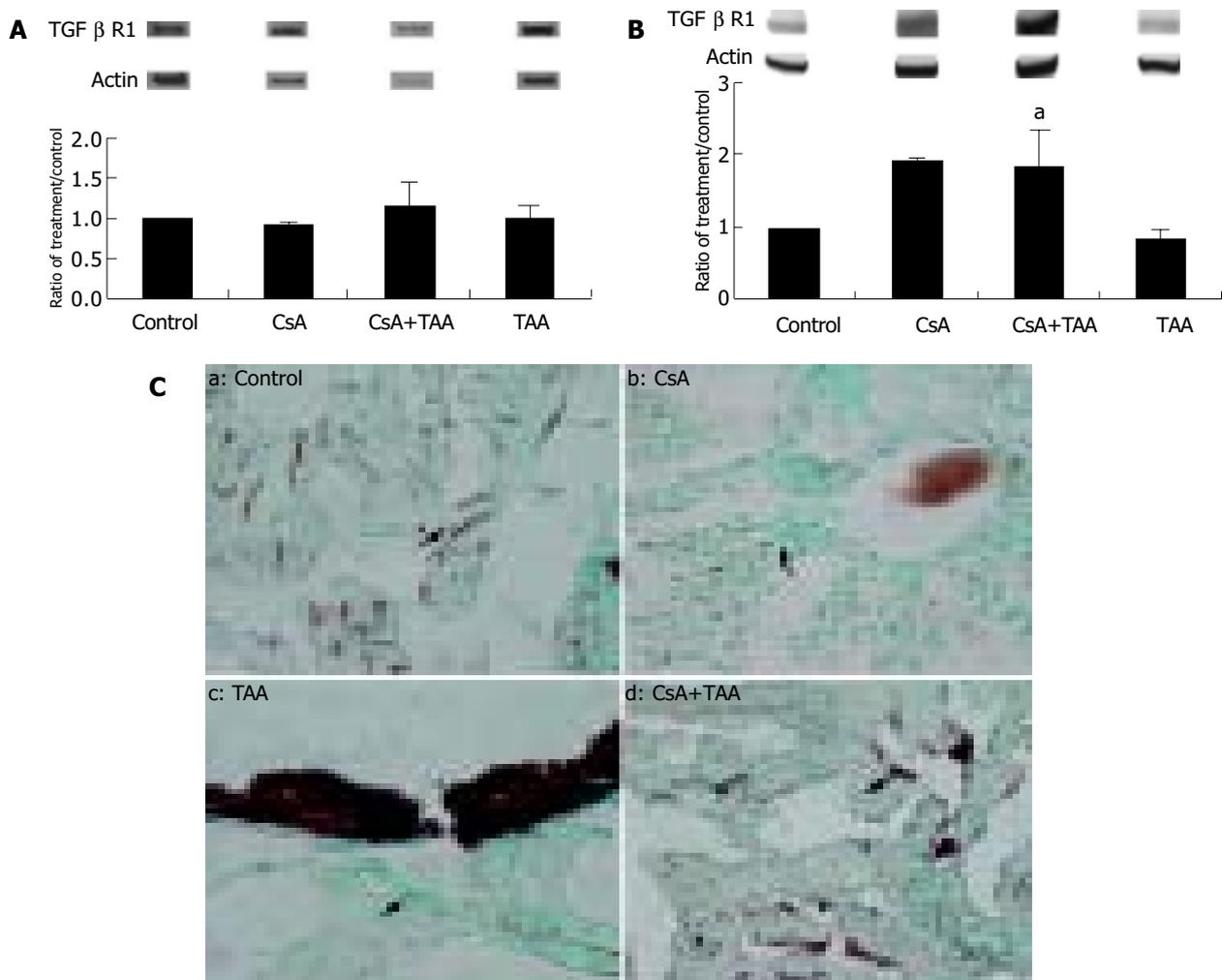


Figure 4 The levels of (A) semi-quantitative PCR for TGFβR1 RNA expression, (B) Western blot for TGFβR1 protein expression and (C) immunostaining for TGFβR1 in rat liver after various treatments. ^aP<0.05 between TAA and TAA plus CsA treatments. Blue arrows indicate the positive staining and methyl green (green) for cell nuclear stained. Magnification = ×200.

Fibroblast growth factor receptor (FGFR2, 4)

RT-PCR, Western blot and immunostaining of FGFR2 analysis showed that the FGFR2 was not significantly different among treatments (Figures 5A, B). The aggregations of FGFR2 in liver, caused by TAA or CsA treatment, are reduced when treated with CsA plus TAA (Figure 5C). CsA or TAA treatment increased the FGFR4 in the RNA and

protein levels, but this trend was reduced in the CsA plus TAA group (Figures 6A, B). Interestingly, the FGFR4 expression of liver was scattered in the CsA plus TAA treatment after immunostaining (Figure 6C). The RNA level of P-gp ATP binding cassette (ABC) transporter in CsA plus TAA-treated liver is higher than that of the TAA or CsA group (Figure 7).

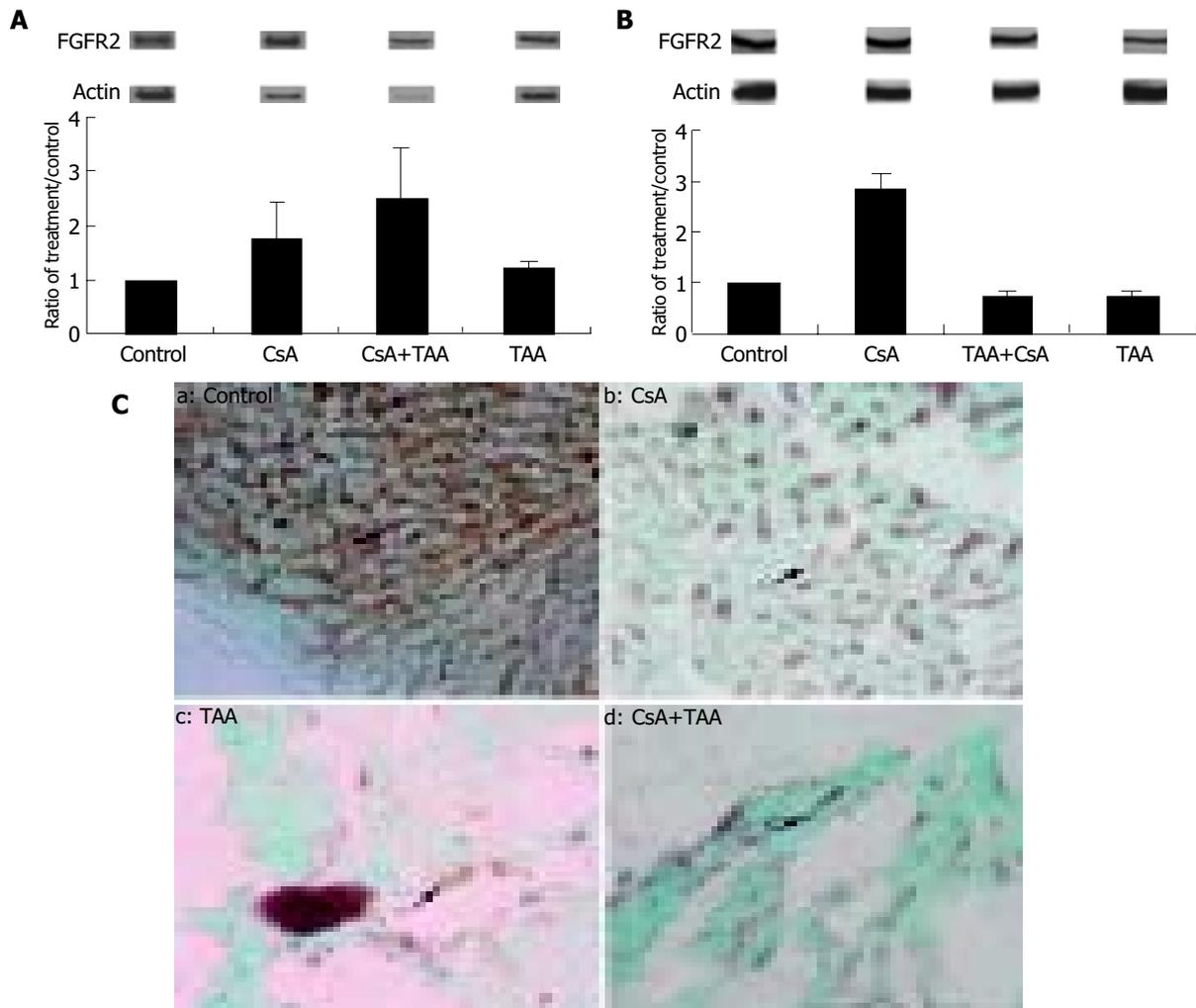


Figure 5 Levels of (A) semi-quantitative PCR for FGFR2 RNA expression, (B) Western blot for FGFR2 protein expression and (C) immunostaining for FGFR2 in rat liver after various treatments. Blue arrows indicate the positive staining and methyl green (green) for cell nuclear stained. Magnification = $\times 200$.

DISCUSSION

After CsA plus TAA treatment, the blood GOT/GPT of the liver function index is shown to have improved significantly, and the collagen of liver is scattered after being stained with Sirius red. In the present study, the fact that TAA caused the increment of blood GOT/GPT, agrees with previous studies in TAA administration^[28-32] and in CsA treatment^[33]. The collagen I level in liver of rat is reduced after the CsA plus TAA treatment in this study. CsA can alleviate the collagen formation in liver of TAA-induced rat. Collagens are the major components of liver ECM^[34]. In the normal liver, interstitial collagen I and III are present in approximately equal quantities, constituting about 80% of the total^[35] and are mainly located within the portal areas^[34,36]. In a normal liver, HSCs are non-parenchymal, quiescent cells whose main function is to store vitamin A. When liver injury associated with the activation of HSCs, undergoes an activation process in which they lose vitamin A, they become highly, proliferative and synthesize a fibrotic matrix rich in type I collagen, exhibiting features of myofibroblasts. HSCs are the key matrix-producing cells of a normal or fibrotic liver and are intimately regulated by

TGF- β ^[37]. HSC produces and secretes TGF- β , and responds to this cytokine with an increased production of type I collagen, the predominant ECM protein in liver fibrosis^[38,39]. In chronic injury, stellate cell activation and the consequent secretion of the matrix by activated stellate cells result in liver fibrosis and ultimately cirrhosis.

TGF- β ^[1] is a cytokine that plays a pivotal role in liver fibrosis by regulating the matrix synthesis and deposition^[40]. The hepatic expression of TGF- β in liver fibrosis is markedly increased in animal models, and in human patients with chronic liver disease^[9,40-47]. Recent investigations have shown that TGF- β is one of the most powerful profibrogenic mediators playing a major role in the development of liver cirrhosis^[48]. Although TGF- β is also an important negative regulator of proliferation and an inducer of hepatocyte apoptosis, TGF- β up regulates the expression of collagens I, II and IV, fibronectin and laminin in HSCs, and accelerates the transformation of quiescent HSCs into myofibroblasts. In addition to accelerating activation and stimulating matrix synthesis, TGF- β down regulates the degradation of ECM proteins by matrix metalloproteinases through the up-regulation of tissue inhibitors of metalloproteinases in activated HSCs. TGF- β ^[1] is considered to be the most potent

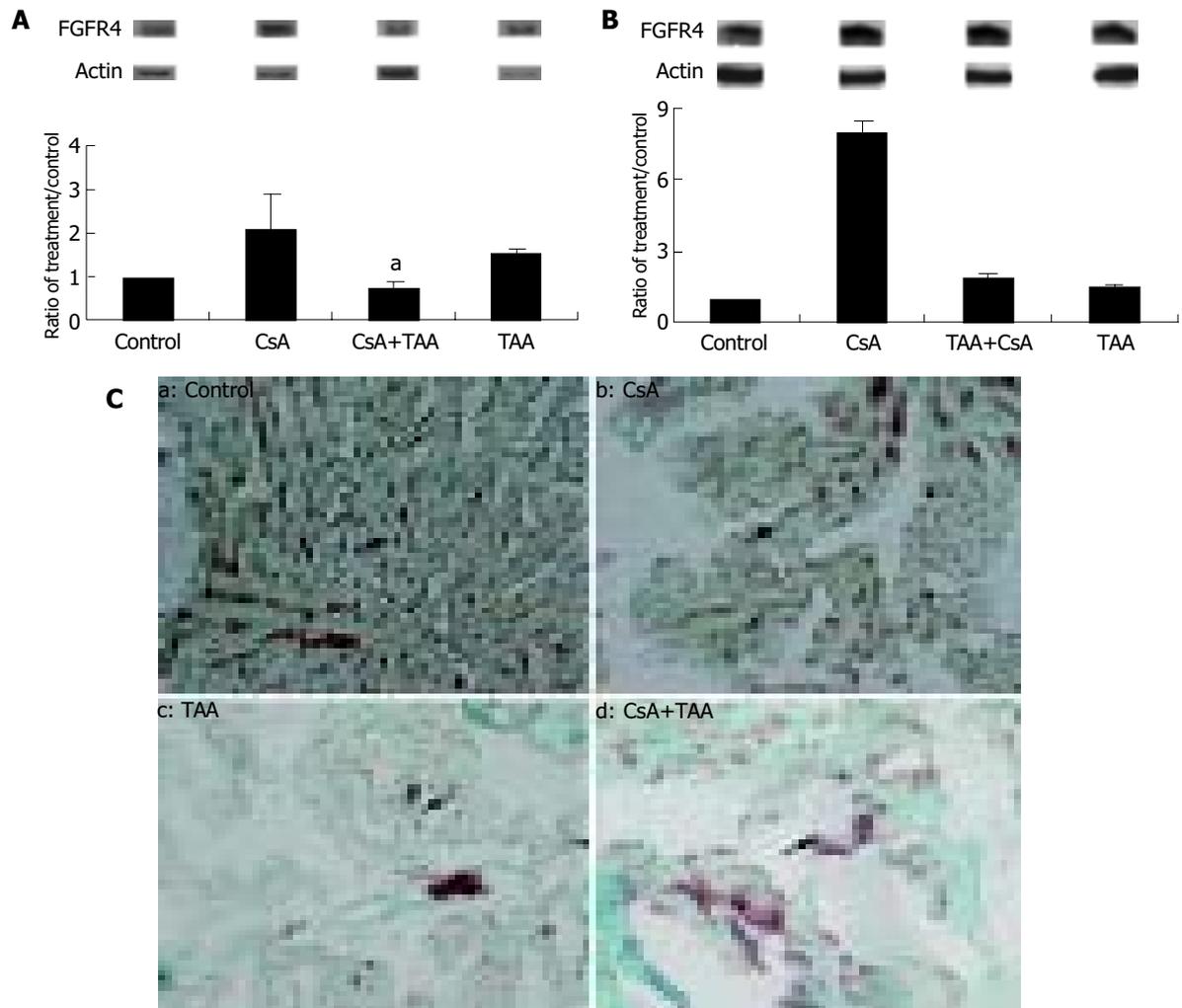


Figure 6 Levels of (A) semi-quantitative PCR for FGFR4 RNA expression, (B) Western blot for FGFR4 protein expression and (C) immunostaining for FGFR4 in rat liver after various treatments. ^a*P*<0.05 between TAA and TAA plus CsA treatments. Blue arrows indicate the positive staining and methyl green (green) for cell nuclear stained. Magnification ×200.

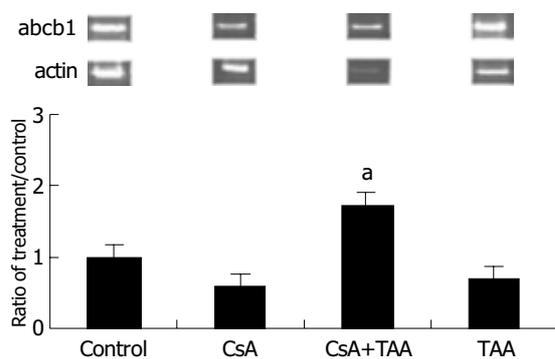


Figure 7 Levels of semi-quantitative PCR for P-gp RNA expression in rat liver after various treatments. ^a*P*<0.05 between TAA and TAA plus CsA treatments.

profibrogenic cytokine in liver fibrosis through activation of HSCs that apparently includes the stimulation of an increase in cell numbers, migratory behavior, and the deposition of ECM components both *in vitro* and *in vivo*^[49-52]. The function of this cytokine is dependent on the interaction

with TGF surface receptor types 1, 2 and 3 (TGFβ-Rs 1-3). TGFβ-R3 is proposed to be involved in regulating the access of ligands to TGFβ-Rs 1 and 2. The activation of TGFβ-R2 leads to the phosphorylation of TGFβ-R1, which, in turn, phosphorylates Smad proteins that transduce the signal to the nucleus^[53-57]. TGFβ is reduced in the CsA plus TAA group, while TGFβ-R1 is increased. This implies that CsA might alleviate the collagen formation via the down-regulation of TGFβ and the up-regulation of TGFβ-R1. In CCl4-induced liver regeneration, the up-regulation of both TGFβ-1 and TGFβ-Rs 1 to 3 may ensure that cycling hepatocytes stop proliferation, thereby preventing overshoots in liver growth^[58]. The levels of TGFβ-R1 and TGFβ-R2 mRNA expression changes occurred in the hepatocytes of rat livers injured by CCl4 administration, while TGFβ-R3 mRNA decreased only in non-parenchymal cells^[59].

In this study, CsA possesses the stimulatory effects on the RNA level of P-gp in TAA-treated liver. The ABC superfamily of membrane transporters is one of the largest protein classes known, and counts numerous proteins involved in the trafficking of biological molecules across cell membranes. The first known human ABC transporter

was P-gp, which confers multidrug resistance (MDR) to anticancer drugs (reviewed in Ref.^[60]). Previous studies showed that the immunosuppressive agent CsA modulates the MDR protein P-gp and exerts a hepatotrophic influence in the regenerating liver^[61-63]. CsA has an additive effect on the expression of P-gp during liver regeneration in the rat. The induction of P-gp might be considered in patients receiving CsA after liver transplantation for hepatocellular carcinoma and chemotherapy as an adjuvant treatment for the prevention of tumor recurrence^[64]. Following acute CCl₄ exposure, FGFR4 acts promoting processes that restore hepatobular architecture rather than cellularity, while limiting the damage due to prolonged CYP2E1 activity. In the present study, the CsA or TAA treatment increased FGFR4 in RNA and in the protein levels, but this trend was decreased in the CsA plus TAA group. A dual role for hepatocyte FGFR4 occurs as a consequence of toxic insult, in limiting the product-induced damage during biochemical detoxification and by the reconstitution of normal architecture. The modulation of hepatocyte FGFR4 signaling may be a useful target for the therapeutic mediation of toxin-induced liver injury and fibrosis, while the FGFR4-deficient mice is a useful model for the study of both high-level acute and low-level chronic liver insult, including cirrhosis. These results suggest that the up-regulation of hepatic regeneration with CsA pre-treatment might be attributed in part to changes in the production of these mitogenic and mitoinhibitory cytokines. Furthermore, CsA stimulates rat liver cell proliferation *in vivo* without inducing liver cell necrosis. This effect may contribute to accelerate the development of hepatocellular carcinomas in rats fed a CsA diet. As previously observed with BR 931, a hypolipidemic peroxisome proliferator, stimulation of liver cell growth by CsA does not entail changes in the production of HGF, TGF α or TGF β ^[63].

In conclusion, immunosuppressive molecules such as CsA, when co-administrated with TAA-treated liver, inhibited TGF- β activation by a mechanism that may involve down regulation of transglutaminase and collagen expression. The augmentation of the MDR protein P-gp by CsA-induced could protect TAA injury in rat liver. CsA indeed alleviates the damage caused by TAA in the rat liver, and this might be because of the mediating expressions of the growth factor and its receptor.

REFERENCES

- 1 Childs JFL, Siegler EA. Uses of thioacetamide in agriculture. *Science* 1945; **102**: 68-72
- 2 Hunter AL, Holscher MA, Neal RA. Thioacetamide-induced hepatic necrosis. I. Involvement of the mixed-function oxidase enzyme system. *J Pharmacol Exp Ther* 1977; **200**: 439-448
- 3 Porter WR, Neal RA. Metabolism of thioacetamide and thioacetamide S-oxide by rat liver microsomes. *Drug Metab Dispos* 1978; **6**: 379-388
- 4 Chieli E, Malvaldi G. Role of the microsomal FAD-containing monooxygenase in the liver toxicity of thioacetamide S-oxide. *Toxicology* 1984; **31**: 41-52
- 5 Sanz N, Díez-Fernández C, Valverde AM, Lorenzo M, Benito M, Cascales M. Malic enzyme and glucose-6-phosphate dehydrogenase gene expression increases in rat liver cirrhogenesis. *Br J Cancer* 1997; **75**: 487-492
- 6 Bruck R, Aeed H, Shirin H, Matas Z, Zaidel L, Avni Y, Halpern Z. The hydroxyl radical scavengers dimethylsulfoxide and dimethylthiourea protect rats against thioacetamide-induced fulminant hepatic failure. *J Hepatol* 1999; **31**: 27-38
- 7 Hori N, Okanou T, Sawa Y, Mori T, Kashima K. Hemodynamic characterization in experimental liver cirrhosis induced by thioacetamide administration. *Dig Dis Sci* 1993; **38**: 2195-2202
- 8 Muller A, Machnik F, Zimmermann T, Schubert H. Thioacetamide-induced cirrhosis-like liver lesions in rats-usefulness and reliability of this animal model. *Exp Pathol* 1988; **34**: 229-236
- 9 Matsuoka M, Tsukamoto H. Stimulation of hepatic lipocyte collagen production by Kupffer cell-derived transforming growth factor beta: Implication for a pathogenetic role in alcoholic liver fibrogenesis. *Hepatology* 1990; **112**: 599-605
- 10 Wu J, Zern MA. Hepatic stellate cells as a target for the treatment of liver fibrosis. *J Gastroenterol* 2000; **135**: 665-672
- 11 Casini A, Pinzani M, Milani S, Grappone C, Galli G, Jezequel AM, Schuppan D, Rotella CM, Surrenti C. Regulation of extracellular matrix synthesis by transforming growth factor beta 1 in human fat-storing cells. *Gastroenterology* 1993; **105**: 245-253
- 12 Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: A pilot study. *Aliment Pharmacol Ther* 2001; **15**: 1667-1672
- 13 Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratzu V, Bedossa P. Expression of connective tissue growth factor in experimental rat and human liver fibrosis. *Hepatology* 1999; **30**: 968-976
- 14 Borel JF, Feurer C, Gubler HU, Stahelin H. Biological effects of cyclosporin A: A new antilymphocytic agent. 1976. *Agents Actions* 1994; **43**: 179-186
- 15 Margreiter R, Huber C, Spielberger M, Konig P. Cyclosporine in the treatment of acute cadaveric kidney graft rejection refractory to high-dose methylprednisolone. *Transplantation* 1983; **36**: 203-204
- 16 Farthing MJ, Clark ML. Nature of the toxicity of cyclosporin A in the rat. *Biochem Pharmacol* 1981; **24**: 3311-3316
- 17 Sibley RK, Rynasiewicz J, Ferguson RM, Fryd D, Sutherland DE, Simmons RL, Najarian JS. Morphology of cyclosporine nephrotoxicity and acute rejection in patients immunosuppressed with cyclosporine and prednisone. *Surgery* 1983; **94**: 225-234
- 18 Ryffel B, Donatsch P, Madorin M, Matter BE, Ruttimann G, Schon H, Stoll R, Wilson J. Toxicological evaluation of cyclosporine A. *Arch Toxicol* 1983; **53**: 107-141
- 19 Bohme M, Muller M, Leier I, Jedlitschky G, Keppler D. Cholestasis caused by inhibition of the adenosine triphosphate-dependent bile salt transport in rat liver. *Gastroenterology* 1994; **107**: 255-265
- 20 Thalhammer T, Stapf V, Gajdzik L, Graf J. Bile canalicular cationic dye secretion as a model for P-glycoprotein mediated transport. *Eur J Pharmacol* 1994; **270**: 213-220
- 21 Ornitz DM, Itoh N. Fibroblast growth factors. *Genome Biol* 2001; **2**: REVIEW3005
- 22 Johnson DE, Williams LT. Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 1993; **60**: 1-41
- 23 McKeehan WL, Wang F, Kan M. The heparan sulfate-fibroblast growth factor family: Diversity of structure and function. *Prog Nucleic Acid Res Mol Biol* 1998; **59**: 135-176
- 24 Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 2000; **7**: 165-197
- 25 Kan M, Wu X, Wang F, McKeehan WL. Specificity for fibroblast growth factors determined by heparin sulfate in a binary complex with the receptor kinase. *J Biol Chem* 1999; **274**: 15947-15952
- 26 Wang F, McKeehan WL. The fibroblast growth factor (FGF) signaling complex. Handbook of Cell Signaling Vol. I, Chap. 46 (R. Bradshaw and E. Dennis, eds.), Academic/Elsevier Press, 2003: 265-270
- 27 Yu C, Wang F, Jin C, Huang X, Miller DL, Basilico C, McKeehan WL. Role of fibroblast growth factor type 1 and 2 in carbon

- tetrachloride-induced hepatic injury and fibrogenesis. *Am J Pathol* 2003; **163**: 1653–1662
- 28 **Shakoori AR**, Cheema IA, Rani A, Ali SS. Evaluation of liver function after thioacetamide treatment of partially hepatectomized rabbits. *Acta Physiol Pharmacol Latinoam* 1984; **34**: 301–312
 - 29 **Younes M**, Albrecht M, Siegers CP. Interrelationship between *in vivo* lipid peroxidation, microsomal Ca²⁺-sequestration activity and hepatotoxicity in rats treated with carbon tetrachloride, cumene hydroperoxide or thioacetamide. *Res Commun Chem Pathol Pharmacol* 1983; **40**: 405–415
 - 30 **Akbay A**, Cinar K, Uzunalimoglu O, Eranil S, Yurdaydin C, Bozkaya H, Bozdayi M. Serum cytotoxin and oxidant stress markers in N-acetylcysteine treated thioacetamide hepatotoxicity of rats. *Hum Exp Toxicol* 1999; **18**: 669–676
 - 31 **Bergasa NV**, Borque MJ, Wahl LM, Rabin L, Jones EA. Modulation of thioacetamide-induced hepatocellular necrosis by prostaglandins is associated with novel histologic changes. *Liver* 1992; **12**: 168–174
 - 32 **Osada J**, Aylagas H, Sanchez-Vegazo I, Gea T, Millan I, Palacios-Alaiz E. Effect of S-adenosyl-L-methionine on thioacetamide-induced liver damage in rats. *Toxicol Lett* 1986; **32**: 97–106
 - 33 **Al-Nasser IA**. *In vivo* prevention of adriamycin cardiotoxicity by cyclosporin A or FK506. *Toxicology* 1998; **131**: 175–181
 - 34 **Martinez-Hernandez A**, Amenta PS. The hepatic extracellular matrix. II. Ontogenesis, regeneration and cirrhosis. *Virchows Arch A Pathol Anat* 1993; **423**: 77–84
 - 35 **Biagini G**, Ballardini G. Liver fibrosis and extracellular matrix. *J Hepatol* 1989; **8**: 115–124
 - 36 **Rojkind M**, Giambone MA, Biempica L. Collagen types in normal and cirrhotic liver. *Gastroenterology* 1979; **76**: 710–719
 - 37 **Knittel T**, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G. Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: Regulation by TNF-alpha and TGF-beta1. *J Hepatol* 1999; **30**: 48–60
 - 38 **Sanderson N**, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, Roberts AB, Sporn MB, Thorgeirsson SS. Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. *Proc Natl Acad Sci USA* 1995; **92**: 2572–2576
 - 39 **Clouthier DE**, Comerford SA, Hammer RE. Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-like syndrome in PEPCK-TGF-beta1 transgenic mice. *J Clin Invest* 1997; **100**: 2697–2713
 - 40 **Bedossa P**, Paradis V. Transforming growth factor-beta (TGF-beta): A key-role in liver fibrogenesis. *J Hepatol* 1995; **22**(Suppl 2): 37–42
 - 41 **Czaja MJ**, Weiner FR, Flanders KC, Giambone MA, Wind R, Biempica L, Zern MA. *In vitro* and *in vivo* association of transforming growth factor-beta 1 with hepatic fibrosis. *J Cell Biol* 1989; **108**: 2477–2482
 - 42 **Nakatsukasa H**, Evarts RP, Hsia CC, Thorgeirsson SS. Transforming growth factor-beta 1 and type I procollagen transcripts during regeneration and early fibrosis of rat liver. *Lab Invest* 1990; **63**: 171–180
 - 43 **Nakatsukasa H**, Nagy P, Evarts RP, Hsia CC, Marsden E, Thorgeirsson SS. Cellular distribution of transforming growth factor-beta1 and procollagen types I, III, and IV transcripts in carbon tetrachloride-induced rat liver fibrosis. *J Clin Invest* 1990; **85**: 1833–1843
 - 44 **Rieder H**, Armbrust T, Meyer zum Buschenfelde KH, Ramadori G. Contribution of sinusoidal endothelial liver cells to liver fibrosis: Expression of transforming growth factor-beta 1 receptors and modulation of plasmin-generating enzymes by transforming growth factor-beta 1. *Hepatology* 1993; **18**: 937–944
 - 45 **Friedman SL**, Yamasaki G, Wong L. Modulation of transforming growth factor beta receptors of rat lipocytes during the hepatic wound healing response. Enhanced binding and reduced gene expression accompany cellular activation in culture and *in vivo*. *J Biol Chem* 1994; **269**: 10551–10558
 - 46 **Knittel T**, Janneck T, Muller L, Fellmer G. Transforming growth factor beta 1-regulated gene expression of Ito cells. *Hepatology* 1996; **24**: 352–360
 - 47 **Castilla A**, Prieto J, Fausto N. Transforming growth factors beta 1 and alpha in chronic liver disease. Effects of interferon alfa therapy. *New Engl J Med* 1991; **324**: 933–940
 - 48 **Flisiak R**, Pytel-Krolczuk B, Prokopowicz D. Circulating transforming growth factor beta (1) as an indicator of hepatic function impairment in liver cirrhosis. *Cytokine* 2000; **12**: 677–681
 - 49 **Hellerbrand C**, Stefanovic B, Giordano F, Burchardt ER, Brenner DA. The role of TGFbeta1 in initiating hepatic stellate cell activation *in vivo*. *J Hepatol* 1999; **30**: 77–87
 - 50 **Gressner AM**, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF beta in hepatic fibrosis. *Front Biosci* 2002; **7**: 793–807
 - 51 **Win KM**, Charlotte F, Mallat A, Cherqui D, Martin N, Mavier P, Preaux AM, Dhumeaux D, Rosenbaum J. Mitogenic effect of transforming growth factor-beta 1 on human Ito cells in culture: Evidence for mediation by endogenous platelet-derived growth factor. *Hepatology* 1993; **18**: 137–145
 - 52 **Fibbi G**, Pucci M, D'Alessio S, Grappone C, Pellegrini G, Salzano R, Casini A, Milani S, Del Rosso M. Transforming growth factor-beta1 stimulates invasivity of hepatic stellate cells by engagement of the cell-associated fibrinolytic system. *Growth Factors* 2001; **19**: 87–100
 - 53 **Nakao A**, Roijer E, Imamura T, Souchelnytskyi S, Stenman G, Heldin CH, ten Dijke P. Identification of Smad2, a human Mad-related protein in the transforming growth factor beta signaling pathway. *J Biol Chem* 1997; **272**: 2896–2900
 - 54 **Inagaki M**, Moustakas A, Lin HY, Lodish HF, Carr BI. Growth inhibition by transforming growth factor beta (TGF-beta) type I is restored in TGF-beta-resistant hepatoma cells after expression of TGF-beta receptor type II cDNA. *Proc Natl Acad Sci USA* 1993; **90**: 5359–5363
 - 55 **Bassing CH**, Yingling JM, Howe DJ, Wang T, He WW, Gustafson ML, Shah P, Donahoe PK, Wang XF. A transforming growth factor beta type I receptor that signals to activate gene expression. *Science* 1994; **263**: 87–89
 - 56 **Tsuchida K**, Lewis KA, Mathews LS, Vale WW. Molecular characterization of rat transforming growth factor-beta type II receptor. *Biochem Biophys Res Comm* 1993; **191**: 790–795
 - 57 **Wang XF**, Lin HY, Ng-Eaton E, Downward J, Lodish HF, Weinberg RA. Expression cloning and characterization of the TGF-beta Type III receptor. *Cell* 1991; **67**: 797–805
 - 58 **Grasl-Kraupp B**, Rossmannith W, Ruttkay-Nedecky B, Mullauer L, Kammerer B, Bursch W, Schulte-Hermann R. Levels of transforming growth factor beta and transforming growth factor beta receptors in rat liver during growth, regression by apoptosis and neoplasia. *Hepatology* 1998; **28**: 717–726
 - 59 **Date M**, Matsuzaki K, Matsushita M, Sakitani K, Shibano K, Okajima A, Yamamoto C, Ogata N, Okumura T, Seki T, Kubota Y, Kan M, McKeegan WL, Inoue K. Differential expression of transforming growth factor-beta and its receptors in hepatocytes and nonparenchymal cells of rat liver after CCl4 administration. *J Hepatol* 1998; **28**: 572–581
 - 60 **Litman T**, Druley TE, Stein WD, Bates SE. From MDR to MXR: New understanding of multidrug resistance systems, their properties and clinical significance. *Cell Mol Life Sci* 2001; **58**: 931–959
 - 61 **Kahn D**, Makowka L, Lai H, Eagon PK, Dindzans V, Starzl TE, Van Thiel DH. Cyclosporine augments hepatic regenerative response in rats. *Dig Dis Sci* 1990; **35**: 392–398
 - 62 **Delle Monache MD**, Gigliozzi A, Benedetti A, Marucci L, Bini A, Francia C, Papa E, Di Cosimo E, Fraioli F, Jezequel AM, Alvaro D. Effect of pharmacological modulation of liver P-glycoproteins on cyclosporin A biliary excretion and cholestasis: A study in isolated perfused rat liver. *Dig Dis Sci* 1999; **44**: 2196–2204
 - 63 **Morii Y**, Kawano K, Kim YI, Aramaki M, Yoshida T, Kitano S. Augmentative effect of cyclosporin A on rat liver regeneration: Influence on hepatocyte growth factor and transforming growth factor-beta(1). *Eur Surg Res* 1999; **31**: 399–405
 - 64 **Daoudaki M**, Fouzas I, Stapf V, Ekmekcioglu C, Invrios G, Andoniadis A, Demetriadou A, Thalhammer T. Cyclosporine augments P-glycoprotein expression in the regenerating rat liver. *Biol Pharm Bull* 2003; **26**: 303–307