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The protective effects of ursodeoxycholic acid on isoniazid plus rifampicin induced liver injury in mice

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ABSTRACT

Antitubercular drugs have been known to be potentially hepatotoxic and may lead to drug-induced liver injury. In this study, we aimed to investigate the protective effects of ursodeoxycholic acid (UDCA) on liver injury caused by co-administration with isoniazid and rifampicin, two famous antitubercular drugs. Liver injury was induced by co-treatment with isoniazid (75 mg/kg) and rifampicin (150 mg/kg) for one week. Mice were orally administered with UDCA (15, 50 and 150 mg/kg) 30 min before isoniazid and rifampicin. We show that serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly increased in mice treated with isoniazid plus rifampicin. An obvious fatty accumulation, accompanied by mild necrosis and inflammation, was observed in liver of mice treated with rifampicin plus isoniazid. In addition, isoniazid plus rifampicin resulted in hepatic apoptosis, as determined by terminal dUTP nick-end labeling (TUNEL) staining and caspase-3 activation. Additional experiment showed that isoniazid plus rifampicin significantly increased the level of hepatic malondialdehyde (MDA) and caused glutathione (GSH) depletion and 3-nitrotyrosine (3-NT) residues in liver. UDCA pretreatment significantly attenuated isoniazid plus rifampicin induced oxidative stress in liver. Importantly, UDCA pretreatment significantly alleviated isoniazid plus rifampicin induced hepatic apoptosis. Moreover, UDCA-mediated anti-apoptotic effect seemed to be associated with its regulation of Bcl-2 and Bax gene expression in liver. These findings suggest that UDCA might protect against isoniazid and rifampicin induced liver injury through its anti-oxidative and anti-apoptotic effects.

1. Introduction

Isoniazid and rifampicin, two front-line drugs used in antituberculosis therapy, have been known to be potentially hepatotoxic and may lead to drug-induced liver injury (Hwang et al., 1997). A meta-analysis of studies involving the use of a multiplicity of anti-tuberculosis drug regimens predominantly in adults has shown an incidence of toxic hepatitis of 1.6% in patients with isoniazid alone, 1.1% in patients with RPF alone and 2.55% in patients with isoniazid plus rifampicin (Steele et al., 1991). Isoniazid is thought to be initiated by cytochrome P450 mediated metabolism of isoniazid to acetylhydrazine and hydrazine that is hepatotoxic (Preziosi, 2007; Yue et al., 2004; Bhadauria et al., 2007). Rifampicin, which is generally co-administered with isoniazid in treatment of tuberculosis, is toxic to hepatocytes (Christiane and Peter, 2006). In addition, as a powerful inducer of drug metabolizing enzymes in man and rats, rifampicin aggravates isoniazid-induced hepatotoxicity by enhancing the production of toxic metabolites (Tasdug et al., 2007).

Reactive oxygen species play an important role in isoniazid and rifampicin induced liver injury (Chowdhury et al., 2006). According to several earlier studies, hepatic glutathione-S transferase activity and glutathione (GSH) level were significantly decreased in rats treated with isoniazid or hydrazine (Sodhi et al., 1996, 1997). Clinic data showed that an increased level of GSH and a decreased level of malondialdehyde (MDA) were measured in plasma of patient treated with isoniazid and rifampicin, suggesting that isoniazid and rifampicin could result in oxidative stress (Chowdhury et al., 2001). Since oxidative stress has been regarded as the major mechanism of antituberculosis drug-induced hepatotoxicity, antioxidants might be used as potential antihepatotoxic drugs against isoniazid and rifampicin caused liver injury (Sano et al., 2004).

Ursodeoxycholic acid (UDCA, 3α, 7β-dihydroxy-5β-cholanic acid) is a hydrophilic dihydroxy bile acid which was found in the bile of black bear (Hagey et al., 1993). UDCA has been used in Chinese traditional medicine for the treatment of liver diseases for centuries and was also wildly used for the treatment of various cholestatic disorders (Paumgartner and Beuers, 2002). UDCA exerts its actions in

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Table 1
Effect of UDCA on serum parameters and the relative liver weight in isoniazid and rifampicin treated mice.

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)	Liver/body eight (%)
Control	30.00 ± 6.43	113.50 ± 9.14	74.70 ± 9.63	4.5 ± 0.5
Isoniazid + rifampicin	38.70 ± 6.51[a]	124.20 ± 12.04	90.70 ± 12.91[a]	5.6 ± 0.3[a]
UDCA (150 mg/kg)	28.89 ± 5.13	108.11 ± 10.82	66.22 ± 10.34	4.1 ± 0.6
Isoniazid + rifampicin + UDCA (15 mg/kg)	28.90 ± 6.45[b]	120.30 ± 12.29	69.60 ± 10.79[b]	5.5 ± 0.4
Isoniazid + rifampicin + UDCA (50 mg/kg)	26.90 ± 5.89[b]	99.50 ± 12.51	80.50 ± 8.69[b]	5.2 ± 0.5[b]
Isoniazid + rifampicin + UDCA (150 mg/kg)	29.60 ± 6.96[b]	126.00 ± 13.29	68.50 ± 11.02[b]	5.1 ± 0.5[c]

[a] $P<0.01$ vs control group.
[b] $P<0.05$ vs isoniazid + rifampicin group.
[c] $P<0.01$ vs isoniazid + rifampicin group.

liver through multiple possibly interrelated pathways including alterations of bile acid pool, choleresis, immune modulation and cytoprotective effects (Lazaridis et al., 2001). UDCA is an antioxidant. UDCA protects against secondary biliary cirrhosis in rats by preventing mitochondrial oxidative stress (Serviddio et al., 2004). A recent study showed that UDCA protected against hepatotoxicity caused by amoxicillin-clavulanic acid in rats though its antioxidant properties (El-Sherbiny et al., 2009). In addition, UDCA is also an antiapoptotic agent. An earlier study showed that UDCA pretreatment prevented fetal rat liver from apoptosis induced by maternal cholestasis (Perez et al., 2005). In the present study, we aimed to investigate the protective effect of UDCA on isoniazid and rifampicin induced liver injury in mice. Our results indicate that UDCA pretreatment could protect isoniazid and RPF induced liver injury not only through its antioxidant effect but also its antiapoptotic ability.

2. Materials and methods

2.1. Reagents

Isoniazid and rifampicin were from Sigma Chemical Co. (St. Louis, MO). 3-NT, β-actin, Bax, Bcl-2 and active caspase-3 antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). TUNEL detection kit was from Promega Corporation (Madison, WI). Chemiluminescence (ECL) detection kit was from Pierce Biotechnology (Rockford, IL). All the other reagents were from Sigma or indicated in the specified methods.

2.2. Animals and treatments

Female CD-1 mice (6–8 week-old, 24–26 g) were purchased from Beijing Vital River (Beijing, China). The animals were allowed free access to food and water at all times and were maintained on a 12 h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. In isoniazid plus rifampicin group, mice were administered with isoniazid (75 mg/kg) plus rifampicin (150 mg/kg) by gavage daily for one week. The doses of isoniazid and rifampicin used in the present study referred to the results from preliminary study. Preliminary study showed that administration with 75 mg/kg isoniazid plus 150 mg/kg rifampicin daily for a week resulted in obvious liver injuries including fatty accumulation, hepatic apoptosis and the elevation of serum ALT. In UDCA pretreatment group, mice were treated with different doses of UDCA (15, 50, 150 mg/kg) 30 min before isoniazid and rifampicin. In control groups, mice were administered with PBS or UDCA (150 mg/kg) by gavage daily for one week. The doses of UDCA used in the present study referred to others (Ishizaki et al., 2008). All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University (Agreement No: 20090815003).

2.3. Biochemical parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured using

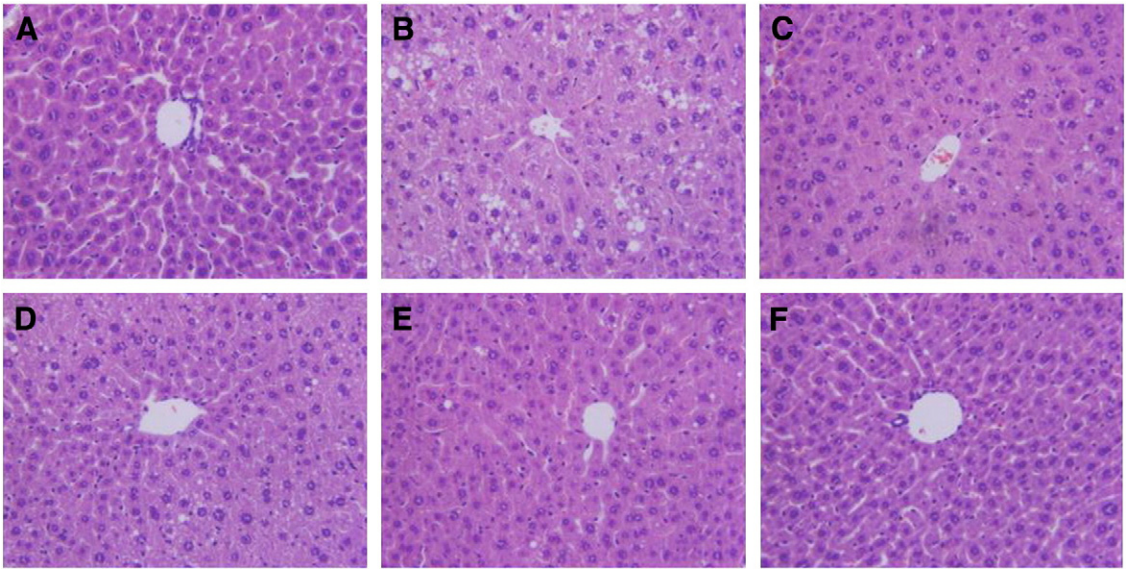

Fig. 1. Effects of UDCA on histopathology of liver treated with rifampicin plus isoniazid. (A) Liver section from mice treated with saline. (B) Liver section from mice treated with rifampicin plus isoniazid. (C) Liver section from mice treated with UDCA. (D) Liver section from mice pretreated with UDCA (15 mg/kg) before rifampicin plus isoniazid treatment. (E) Liver section from mice pretreated with UDCA (50 mg/kg) before rifampicin plus isoniazid treatment. (F) Liver section from mice pretreated with UDCA (150 mg/kg) before rifampicin plus isoniazid treatment. Liver sections were stained with hematoxylin and eosin (original magnification ×400).

Table 2
Effect of UDCA on GSH and MDA content in isoniazid and rifampicin treated mice.

Groups	GSH (μmol/g liver)	MDA (nmol/g liver)
Control	6.67 ± 0.65	117.22 ± 7.71
Isoniazid + rifampicin	5.47 ± 0.98[a]	134.25 ± 7.74[a]
UDCA (150 mg/kg)	7.20 ± 1.28	108.89 ± 9.05
Isoniazid + rifampicin + UDCA (15 mg/kg)	6.42 ± 1.02[b]	110.37 ± 10.05[c]
Isoniazid + rifampicin + UDCA (50 mg/kg)	7.00 ± 1.18[c]	112.22 ± 1.29[c]
Isoniazid + rifampicin + UDCA (150 mg/kg)	7.33 ± 1.39[c]	93.21 ± 10.55[c]

[a] $P<0.01$vs control group.
[b] $P<0.05$ vs isoniazid and rifampicin group.
[c] $P<0.01$ vs isoniazid and rifampicin group.

standard clinical methods by Central Laboratory of the First Affiliated Hospital at Anhui Medical University.

2.4. Histology

Liver specimen was fixed in 4% formaldehyde phosphate buffer. Liver sections were stained with hematoxylin and eosin and evaluated by two pathologists who were not aware of sample assignment to experimental groups.

2.5. Measurement of GSH

GSH was determined by the method of Griffith (1980). Briefly, proteins of 0.4 ml liver homogenates were precipitated by the addition of 0.4 ml of a metaphosphoric acid solution. After 40 min, the protein precipitate was separated from the remaining solution by centrifugation at 5000 rpm at 4 °C for 5 min. 400 μl of the supernatant was combined with 0.4 ml of 300 mM Na_2HPO_4, and the absorbance at 412 nm was read against a blank consisting of 0.4 ml supernatant plus 0.4 ml H_2O. 100 μl 5,5′-dithiobis-2-nitrobenzoate (DTNB) (0.02%, w/v; 20 mg DTNB in 100 ml of 1% sodium citrate) was then added to the blank and sample, and absorbance of the sample was read against the blank at 412 nm. The GSH content was determined using a calibration curve prepared with an authentic sample. GSH values were expressed as nmol/g liver.

2.6. Lipid peroxidation assay

Lipid peroxidation was quantified by measuring MDA as described previously (Ohkawa et al., 1979). Tissue was homogenized in 9 volumes of 50 mmol/l Tris–HCl buffer (pH 7.4) containing 180 mmol/l KCl, 10 mmol/l EDTA, and 0.02% butylated hydroxytoluene. To 0.2 ml of the tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, 1.5 ml of 0.9% thiobarbituric acid, and 0.6 ml of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95 °C for 40 min. After cooling on ice, 1.0 ml of distilled water and 5.0 ml of butanol/pyridine mixture (15:1, v/v) were added and vortexed. After the reaction mixture was centrifuged at 10,000 ×*g* for 10 min, absorbance of the supernatants was determined at 532 nm. MDA values were expressed as nmol/g liver.

2.7. Immunohistochemistry

Sections were mounted onto coated slides, dewaxed, and rehydrated. Antigen retrieval was performed by pressure cooking slides for 5 min in 0.01 M citrate buffer (pH 6.0). Slides were incubated for 30 min in 3% (vol/vol) hydrogen peroxide in methanol to block endogenous peroxidase activity and then washed in Tris-buffered saline [TBS; 0.05 M Tris, 0.85% (wt/vol) NaCl (pH 7.4)]. Nonspecific binding sites were blocked with an appropriate normal serum diluted 1:5 in TBS containing 5% (wt/vol) BSA before the addition of rabbit polyclonal antibody against mouse 3-NT antibody or caspase-3 antibody and overnight incubation at 4 °C. After washing in TBS, slides were incubated for 30 min with the appropriate secondary antibody conjugated to biotin goat anti-rabbit (Santa Cruz, USA). This was followed by a 30 min incubation with horseradish peroxidase-labeled avidin-biotin complex (Dako). Immunostaining was developed by application of diaminobenzidine (liquid DAB^+; Dako), and slides were counterstained with hematoxylin, dehydrated, and mounted using Pertex mounting medium (Cell Path, Hemel Hempstead, UK).

2.8. Immunoblot

Liver tissue was homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 1 mM

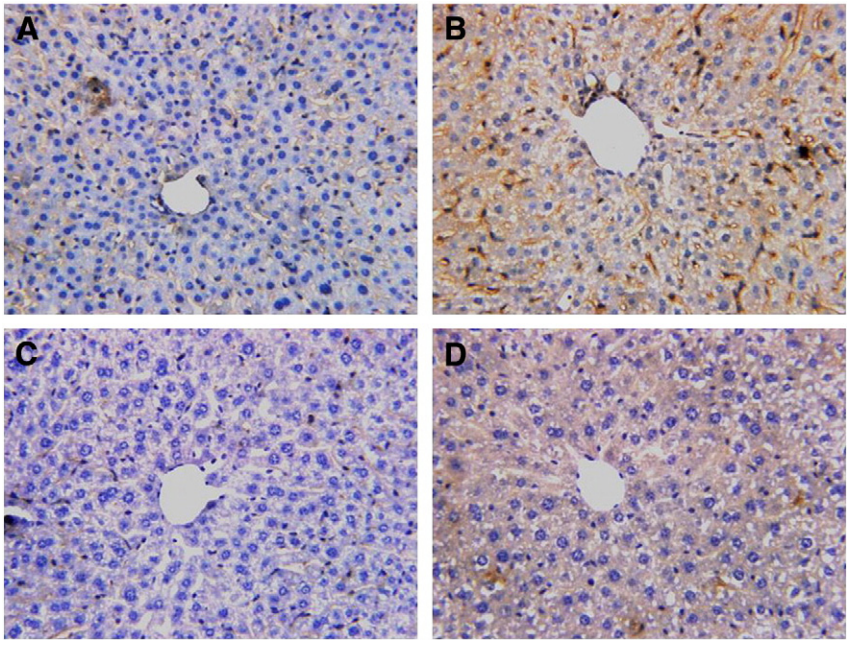

Fig. 2. Effects of UDCA on 3-NT residues in mouse liver treated with rifampicin plus isoniazid. 3-NT immunoreactivity was detected in liver section from mice treated with saline (A), rifampicin plus isoniazid (B), UDCA (C), or pretreated with UDCA before rifampicin plus isoniazid (D). Magnification: 400×.

phenylmethylsulfonyl fluoride (PMSF). Samples were then centrifuged at 15,000 ×g for 15 min. Supernatants from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 5 min. Proteins (50 μg/sample) in loading buffer were subjected to electrophoresis in 10% SDS-polyacrylamide gel for 3 h. Protein in the gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Millipore Corp., Bedford, Massachusetts, USA) and blocked in 5% nonfat powdered milk in Dulbecco's PBS (DPBS) overnight at 4 °C. The membranes were then incubated for 2 h with rabbit polyclonal antibody against mouse Bax, Bcl-2 or β-actin at room temperature. After washing in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an ECL detection kit.

2.9. Terminal dUTP nick-end labeling (TUNEL) staining

For the detection of apoptosis, paraffin-embedded sections were stained with the TUNEL technique using a TUNEL detection kit according to the manufacturer's protocols. The number of TUNEL$^+$ cells was counted in twelve randomly selected fields from each slide at a magnification of ×400.

2.10. Statistical analysis

For immunoblot, Bax and Bcl-2 were normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. All quantified data were expressed as means ± S.E.M. at each point. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups.

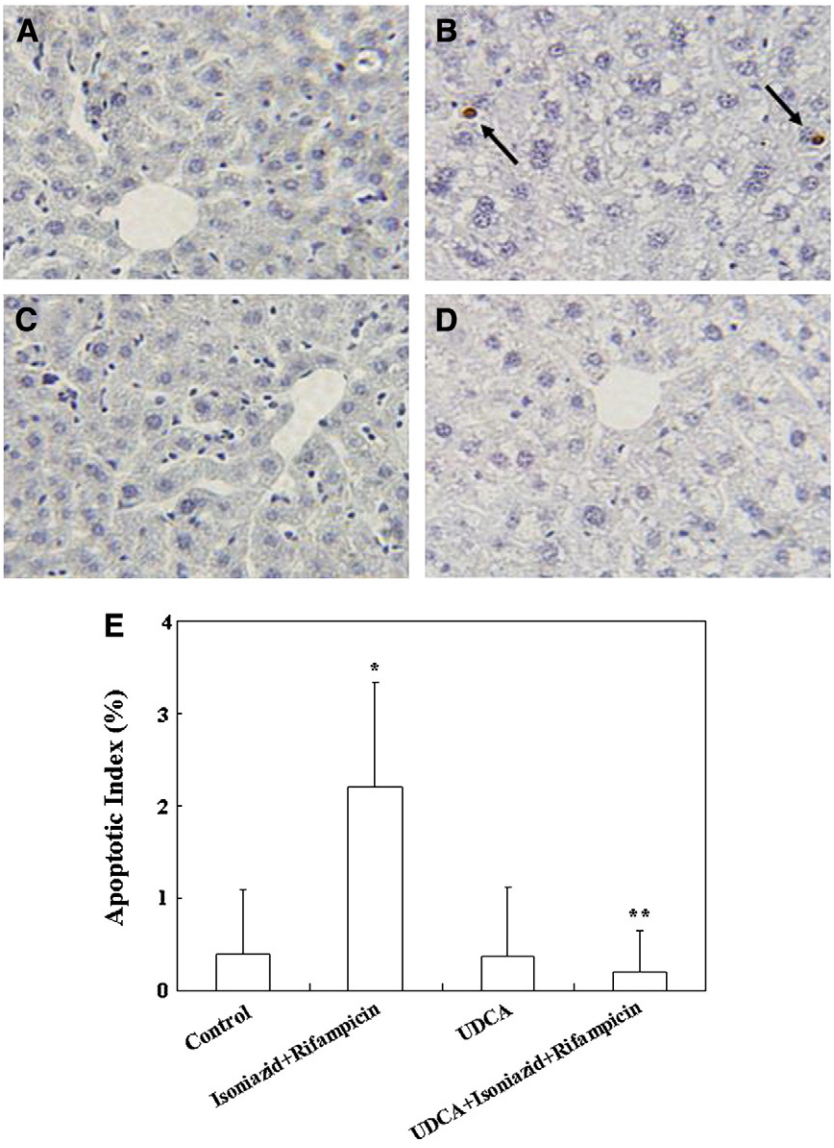

Fig. 3. Effects of UDCA on rifampicin plus isoniazid induced apoptosis in hepatocytes. Apoptosis was analyzed with TUNEL staining of liver sections from mice treated with saline (A), rifampicin plus isoniazid (B), UDCA (C), or pretreated with UDCA before rifampicin plus isoniazid (D). The number of TUNEL$^+$ cells (arrow) in liver was counted and analyzed in 12 randomly selected fields from each slide at 400× magnification (E). All data were expressed as means ± S.E.M. of six samples. *, $P<0.01$ significantly different as compared with control group; **, $P<0.01$ significantly different as compared with rifampicin plus isoniazid group.

3. Results

3.1. Effects of UDCA on isoniazid and rifampicin induced liver injury

As shown in Table 1, the level of serum ALT and ALP was significantly increased in mice administered with isoniazid and rifampicin for one week. UDCA pre-treatment significantly attenuated isoniazid plus rifampicin induced elevation of serum ALT and ALP. The effects of UDCA on isoniazid plus rifampicin induced liver injury were analyzed. As expected, the relative liver weight was significantly increased in mice administered with isoniazid and rifampicin (Table 1). An obvious fatty accumulation, accompanied by mild necrosis and inflammation, was observed in liver of mice treated with isoniazid plus rifampicin (Fig. 1B). UDCA pre-treatment significantly alleviated isoniazid plus rifampicin induced increase in the relative liver weight in a dose-dependent manner (Table 1). In addition, UDCA pre-treatment alleviated isoniazid plus rifampicin induced pathological damage (Fig. 1D, E and F).

3.2. UDCA protects against isoniazid and rifampicin induced oxidative stress in liver

As shown in Table 2, hepatic GSH content was significantly decreased in mice administered with isoniazid and rifampicin. Conversely, the level of MDA, an index of lipid peroxidation, was significantly increased in liver of mice treated with isoniazid and rifampicin (Table 2). UDCA pre-treatment significantly alleviated isoniazid plus rifampicin induced hepatic GSH depletion. In addition, UDCA pre-treatment completely prevented isoniazid plus rifampicin induced lipid peroxidation. The effects of UDCA on the intensity of 3-NT residues in liver are presented in Fig. 2. As expected, the intensity of hepatic 3-NT staining was strengthened in mice treated with

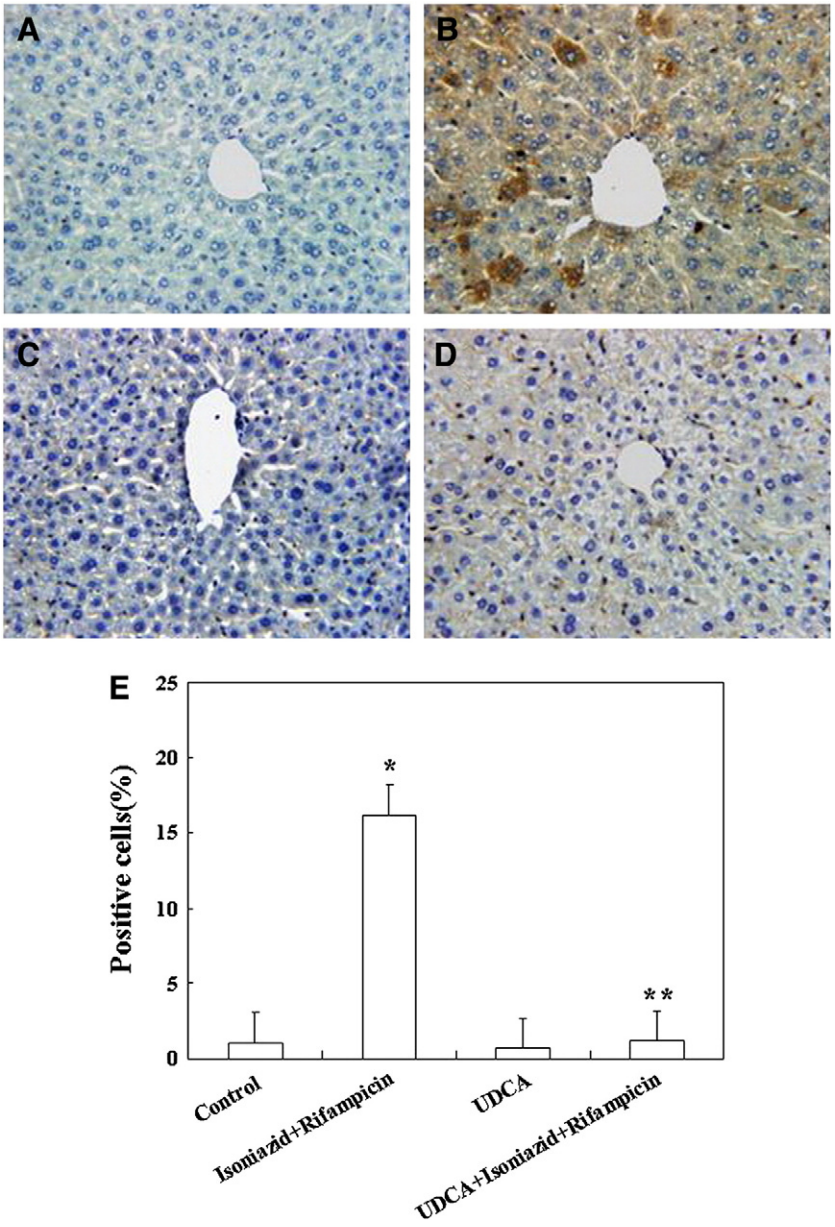

Fig. 4. Effect of UDCA on rifampicin plus isoniazid induced caspase-3 activation. Active caspase-3 was measured with immunohistochemistry. Liver sections from mice treated with saline (A), rifampicin plus isoniazid (B), UDCA (C), or pretreated with UDCA before rifampicin plus isoniazid (D). The number of active caspase-3-positive cells (arrow) in liver was counted and analyzed in 12 randomly selected fields from each slide at 400× magnification (E). All data were expressed as means ± S.E.M. of six samples per group. *, $P<0.01$ significantly different as compared with control group; *, $P<0.01$ significantly different as compared with rifampicin plus isoniazid group.

isoniazid and rifampicin (Fig. 2B). UDCA pre-treatment significantly attenuated hepatic 3-NT staining (Fig. 2D).

3.3. UDCA protects against isoniazid and rifampicin induced apoptosis in liver

Hepatic apoptosis was detected by TUNEL. As shown in Fig. 3, the number of TUNEL$^+$ cells was significantly increased in liver of mice treated with isoniazid and rifampicin. UDCA pre-treatment significantly attenuated isoniazid and rifampicin induced apoptosis in liver. The effects of UDCA pretreatment on isoniazid plus rifampicin induced caspase-3 activation are presented in Fig. 4. In accordance with the results from TUNEL, the number of hepatocytes with active caspase-3 was significantly increased in mice administered with isoniazid and rifampicin (Fig. 4B and E). UDCA pre-treatment protected against isoniazid and rifampicin induced caspase-3 activation (Fig. 4D and E). The effects of UDCA on the expression of Bax and Bcl-2 are presented in Fig. 5. As expected, the level of Bax was significantly increased in liver of mice treated with isoniazid and rifampicin (Fig. 5A and B). By contrast, the level of Bcl-2 was significantly decreased in liver of mice treated with isoniazid and rifampicin (Fig. 5A and C). UDCA pretreatment obviously protected against the alteration of hepatic Bax and Bcl-2 expression (Fig. 5A, B, and C).

4. Discussion

UDCA has been suggested as an efficient therapy in various liver diseases. First, UDCA is the currently accepted method for treating cholestasis. According to combined analysis of the three largest randomized clinical trials, UDCA improved clinical and biochemical indices and prolonged survival free of liver transplantation (Poupon et al., 1997). An earlier study in rats found that UDCA prevented from secondary biliary cirrhosis through counteracting mitochondrial oxidative stress (Serviddio et al., 2004). Second, it has been demonstrated that UDCA protects against drug-induced hepatotoxicity. A recent study showed that UDCA protected rats from liver injury induced by methotrexate, an immunosuppressive drug (Uraz et al., 2008). In addition, UDCA acts as effective hepatoprotective agent against liver dysfunction caused by the broad spectrum antibiotic combination amoxicillin-clavulanic acid (El-Sherbiny et al., 2009). In this study, we found that UDCA pretreatment significantly attenuated the elevation of serum ALT and ALP levels in mice treated with isoniazid and rifampicin. In addition, UDCA pretreatment alleviated isoniazid plus rifampicin induced pathological damage. These results provide new evidence that UDCA could protect against drug-induced liver injury.

Numerous studies have demonstrated that oxidative damage is an important mechanism of anti-tuberculosis drug-induced hepatotoxicity (Sodhi et al., 1996, 1997; Bhadauria et al., 2007; Chowdhury et al., 2006; Attri et al., 2000). In the present study, we found that the level of hepatic GSH was significantly decreased in mice treated with isoniazid plus rifampicin. Conversely, the level of hepatic MDA, a marker of lipid peroxidation, was obviously increased in mice administered with isoniazid plus rifampicin. 3-NT is a specific marker for protein nitration. A recent study demonstrated that 3-NT could cause protein denaturation and DNA damage, leading to cell death in liver (Oberley et al., 2008). The present study showed that the

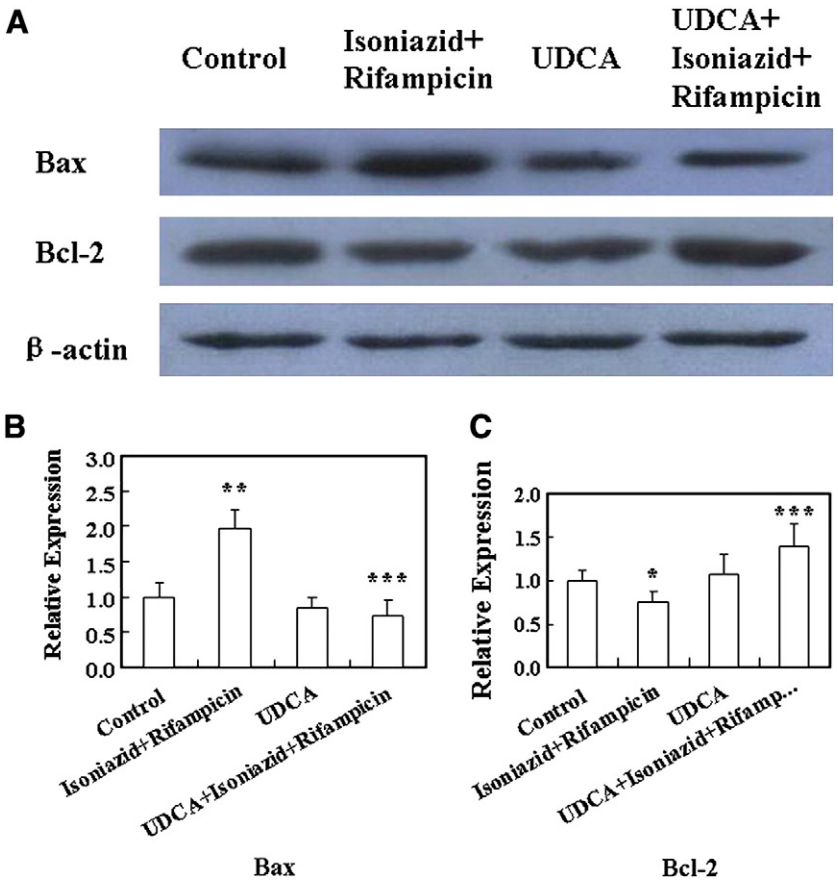

Fig. 5. Effect of UDCA on rifampicin plus isoniazid induced expression of hepatic Bax and Bcl-2 in mice. The protein expression of Bax and Bcl-2 was measured using immunoblotting. Quantitative analysis of scanning densitometry on six samples per group was performed. Bax and Bcl-2 were normalized to β-actin level in the same samples. The densitometry units of the control were assigned as 1. All data were expressed as means ± S.E.M. of six samples per group.*, $P<0.05$ significantly different as compared with control group; **, $P<0.01$ significantly different as compared with control group; ***, $P<0.01$ significantly different as compared with rifampicin plus isoniazid group.

intensity of hepatic 3-NT staining was strengthened in mice administered with isoniazid and rifampicin. These results suggest that oxidative stress might contribute, at least partially, to liver injury in mice co-administered with isoniazid and rifampicin.

A recent study showed that N-acetylcysteine, a well-known antioxidant, protected against isoniazid plus rifampicin induced hepatotoxicity through its antioxidant effect (Attri et al., 2000). UDCA is an antioxidant. An earlier study showed that UDCA alleviated hepatic lipid peroxidation in experimental cholestatic liver disease [Ljubuncic et al., 2000]. In addition, UDCA significantly increased the levels of GSH and thiol-containing proteins, thereby protecting hepatocytes against oxidative injury (Mitsuyoshi et al., 1999). A recent study demonstrated that UDCA induced the expression of detoxifying enzymes and antioxidative stress genes via the Nrf2 transcriptional pathway (Okada et al., 2008). In the present study, we found that UDCA pretreatment significantly attenuated hepatic lipid peroxidation and GSH depletion in mice administered with isoniazid plus rifampicin. In addition, UDCA pretreatment alleviated hepatic 3-NT residues in mice treated with isoniazid and rifampicin. These results indicate that UDCA-mediated protection against isoniazid plus rifampicin induced liver damage might be associated with its strong antioxidant effect.

In the present study, we found that the number of $TUNEL^+$ cells was significantly increased in liver of mice co-treated with isoniazid and rifampicin. In addition, the number of hepatocytes with active caspase-3 was significantly increased in liver of mice administered with isoniazid plus rifampicin, suggesting that apoptosis might partially contribute to isoniazid plus rifampicin induced liver injury. To investigate the molecular mechanism of isoniazid plus rifampicin induced apoptosis, the expression of hepatic Bcl-2 and Bax was analyzed. As expected, the expression of hepatic Bax was significantly increased in mice administered with isoniazid and rifampicin. By contrast, co-administration with isoniazid and rifampicin significantly reduced the level of hepatic Bcl-2. UDCA has an antiapoptotic effect. An in vitro study showed that UDCA protected against Fas-ligand-induced apoptosis in mouse hepatocytes (Azzaroli et al., 2002). In the present study, we investigated the effects of UDCA on isoniazid plus rifampicin induced apoptosis in liver. We found that UDCA pretreatment significantly reduced the number of $TUNEL^+$ cells in liver of mice administered with isoniazid and rifampicin. In addition, UDCA pretreatment obviously alleviated isoniazid and rifampicin induced hepatic caspase-3 activation. These results indicate that UDCA-mediated protection against isoniazid plus rifampicin induced liver injury might be associated with its strong antiapoptotic effect.

The mechanism of UDCA-mediated antiapoptotic effect remained unclear. Indeed, UDCA stabilizes the mitochondrial membranes of hepatocytes and protects hepatocytes from various outer injuries (Botla et al., 1995). According to several earlier studies, UDCA prevented cytochrome c release and inhibited hepatocyte apoptosis through modulating mitochondrial membrane perturbation (Rodrigues et al., 1998, 1999). Another study found that UDCA protected hepatocytes from bile acid induced apoptosis via activation of survival pathways (Schoemaker et al., 2004). A recent study demonstrated that p53 is a key molecular target of UDCA in regulating apoptosis (Amaral et al., 2007). In the present study, we found that isoniazid plus rifampicin induced up-regulation of Bax was repressed in liver of mice pretreated with UDCA. Conversely, UDCA pretreatment upregulated the expression of hepatic Bcl-2 in mice treated with isoniazid and rifampicin. These results suggest that the UDCA pretreatment might protect against isoniazid plus rifampicin induced hepatic apoptosis through regulating the expression of Bcl-2 family.

In summary, the results of the present study indicate that UDCA pretreatment protects against isoniazid plus rifampicin induced liver injury. UDCA-mediated protection against isoniazid plus rifampicin induced liver injury is associated not only with its antioxidant effect but also its antiapoptotic ability.

Acknowledgments

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