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Short communication

The protective effect of glycyrrhizin on anti-Fas antibody-induced hepatitis in mice

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Abstract

Fas ligand, which is a type II membrane protein, is a major inducer of apoptosis. The effect of glycyrrhizin on anti-Fas antibody-induced hepatitis in mice was studied. Pretreatment of mice with glycyrrhizin (100, 200 and 400 mg/kg, i.p.) inhibited the anti-Fas antibody (150 μg/kg, i.v.)-induced elevation of plasma aminotransferase activity in a dose-dependent manner. CPP32 is a cystein protease and CPP32-like activity induced by anti-Fas antibody injection was inhibited by glycyrrhizin (200 mg/kg). However, the addition of glycyrrhizin (up to 10^{-4} M) to a liver cytosol fraction isolated from mice treated with anti-Fas antibodies (150 μg/kg, i.v.) did not inhibit the CPP32-like activity in vitro. The present results clearly show that glycyrrhizin inhibited anti-Fas antibody-induced hepatitis by acting upstream of CPP32-like protease activation.

Keywords: Fas; Glycyrrhizin; Hepatitis

1. Introduction

The chronic hepatitis caused by hepatitis C virus infection has become a worldwide problem. Although interferon therapy is widely used, more than half of the patients with hepatitis C virus infection are resistant to this treatment (Lindsay, 1997). The administration of Stronger Neo-Minophargen C to patients with hepatitis C virus infection has a therapeutic effect and lowers plasma transaminase activity even in the patient who is resistant to interferon therapy (Fujisawa et al., 1980; Suzuki et al., 1983). Although Stronger Neo-Minophargen C treatment has a therapeutic effect, the mechanism by which plasma transaminase activity is lowered remains unknown. Fas ligand, which is a type II membrane protein, is a major inducer of apoptosis. In the livers of patients with chronic hepatitis caused by hepatitis C virus infection, the Fas antigen and Fas ligand are expressed, and their expression suggests a role of the Fas system in the development of hepatitis (Hiramatsu et al., 1994; Mita et al., 1994). Glycyrrhizin is the main gradient of licorice (Glycyrrhiza glabra) roots and is regarded as an effective component of Stronger Neo-Minophargen C (Fujisawa et al., 1980). Thus, it is possible that glycyrrhizin is involved in the Stronger Neo-Minophargen C inhibition of Fas-induced hepatitis in patients with hepatitis C virus infection.

In the present study, we examined the effect of glycyrrhizin on hepatitis caused by anti-Fas antibodies. Furthermore, the effect of glycyrrhizin on tumor necrosis factor-α (TNF-α), which is a mediator of hepatitis (Tiegs et al., 1989; Gantner et al., 1995), was studied.

2. Materials and methods

Female BALB/c mice obtained from Charles River Japan (Atsugi) were used at 7–10 weeks of age. The animals were kept in an air-conditioned room and given water ad libitum. Anti-Fas antibody Jo2 was purchased from Pharmingen (San Diego, CA, USA). The anti-Fas antibody (150 μg/kg) was administered to the mice via a tail vein (in a volume of 100 μl). Mice were anesthetized with ether before they were killed. Glycyrrhizin and dexamethasone were obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and Wako (Osaka, Japan), respectively. Glycyrrhizin and dexamethasone were intraperitoneally injected. The measurement of plasma alanine aminotransferase activity was performed according to a previously described method (Okamoto et al., 1999). CPP32-like activity was measured using Ac-Asp-Glu-Val-Asp-4-

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Methyl-Coumaryl-7-Amide (Ac-DEVD-MCA) as described previously (Okamoto et al., 1999). RNA isolation and reverse-transcription polymerase chain reaction (RT-PCR) analysis were performed as previously described (Okamoto et al., 1996). PCR amplification was performed within the range of the linear phase of amplification for each primer. cDNA was amplified by 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1.5 min. The PCR primers used for detection of the TNF-α and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were TNF-α (Genbank Acc#M11731) (sense) 5′-AGCCCACGTCGTAGCAAAC-CACCAA-3′ (antisense) 5′-ACACCCATTCCCTTCACA-GAGCAAT-3′. GAPDH (Genbank Acc#X02231) (sense) 5′-ATGGTGAAGGTCGGTGTGAACG-3′ (antisense)5′-GTTGTCATGGATGACCTTGGCC-3′.

The results were statistically analyzed according to one-way analysis of variance and the Dunnett multiple comparison test.

3. Results

3.1. Effect of glycyrrhizin on anti-Fas antibody-induced elevation of plasma alanine aminotransferase

Mice were treated with anti-Fas antibodies (150 μg/kg), and at 8 h after the treatment plasma alanine aminotransferase was elevated (Fig. 1A). Mice were pretreated with glycyrrhizin (100, 200 and 400 mg/kg, i.p.) at 1 h before anti-Fas antibody injection. The glycyrrhizin treatment dose dependently inhibited the anti-Fas antibody-induced elevation of plasma alanine aminotransferase (Fig. 1A).

3.2. Effect of glycyrrhizin on anti-Fas antibody-induced elevation of CPP32-like activity

Mice were treated with anti-Fas antibodies (150 μg/kg), and at 3.5 h after the treatment plasma and the liver were sampled for measurement of alanine aminotransferase and CPP32-like activities, respectively. Treatment of mice with anti-Fas antibodies induced an elevation of the CPP32-like activity ($n = 6$) (Fig. 1B). Mice were pretreated with glycyrrhizin (200 mg/kg, i.p.) at 1 h before anti-Fas antibody injection. The glycyrrhizin treatment inhibited both the anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity (alanine aminotransferase activity (U/l): anti-Fas antibody 711 ± 159, anti-Fas antibody + glycyrrhizin 247 ± 59, $n = 6$, $P < 0.01$) and CPP32-like activity (Fig. 1B).

3.3. Effect of glycyrrhizin on CPP32-like activity in vitro

Mice were treated with anti-Fas antibodies (150 μg/kg), and at 3.5 h after the treatment their livers were removed and protein was isolated for the measurement of CPP32-like activity. The anti-Fas antibody treatment caused an elevation of the CPP32-like activity in the liver cytosol to approximately 6-fold that in the normal mice liver cytosol (Fig. 1C). The addition of glycyrrhizin at the final concentration of 10^{-6}, 10^{-5} and 10^{-4} M did not inhibit CPP32-like activity (Fig. 1C), whereas, CPP32 inhibitor Ac-DEVD-CHO, as expected, abolished the activity (Fig. 1C).

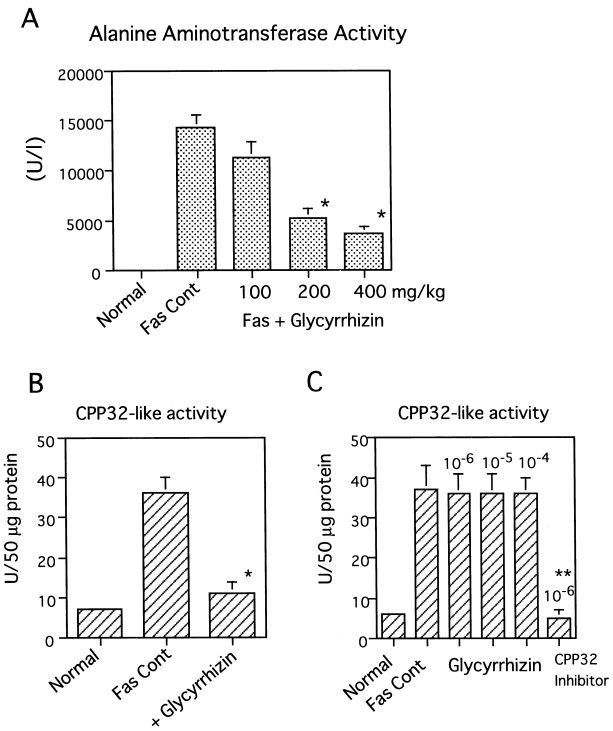

Fig. 1. (A) Effect of glycyrrhizin treatment on anti-Fas antibody-induced elevation of plasma alanine aminotransferase. Anti-Fas antibodies were injected and plasma was obtained from each mouse 8 h later. Pretreatment with glycyrrhizin was performed 1 h before anti-Fas antibody treatment. The data represent the means ± S.E. of the alanine aminotransferase levels in plasma obtained for the respective treatments. The alanine aminotransferase level (U/l) in normal mice was 37 ($n = 2$). Normal: Non-treated ($n = 2$). Fas Cont: Anti-Fas antibody (150 μg/kg, i.v.)-treated ($n = 7$). Fas + Glycyrrhizin: Anti-Fas antibody (150 μg/kg, i.v.) + glycyrrhizin (100 mg/kg, i.p., $n = 7$; 200 mg/kg, i.p., $n = 7$; 400 mg/kg, i.p., $n = 6$). $P < 0.05$ vs. Fas Cont. (B) Effect of glycyrrhizin treatment on anti-Fas antibody-induced elevation of CPP32-like activity in vivo. Glycyrrhizin treatment was given 1h before anti-Fas antibody administration and 3.5 h after injection livers were removed for preparation of protein extracts. CPP32-like activity was measured with the fluorescent substrate, Ac-DEVD-MCA (25 μM), and liver cytosol extracts. CPP32-like activity is expressed as U/50 μg protein. One unit corresponds to the activity that cleaves 1 pmol of the respective fluorescent substrate at 25°C in 30 min. Normal: Non-treated ($n = 2$). Fas Cont: Anti-Fas antibody (150 μg/kg, i.v.)-treated ($n = 5$). + Glycyrrhizin: Anti-Fas antibody (150 μg/kg, i.v.) + glycyrrhizin (200 mg/kg, i.p.)-treated ($n = 5$). $P < 0.05$ vs. liver cytosol extracts from mice treated with anti-Fas antibodies alone. (C) Effects of glycyrrhizin and a CPP32 inhibitor on CPP32-like activity in anti-Fas antibody-treated mouse liver cytosol extracts. Mice were treated with anti-Fas antibodies (150 μg/kg, i.v.), and 3.5 h after injection their livers were removed for preparation of protein extracts. CPP32-like activity was measured using the fluorescent substrate, Ac-DEVD-MCA (25 μM), with the addition of glycyrrhizin (10^{-6}, 10^{-5} and 10^{-4} M) or a CPP32 inhibitor (Ac-DEVD-CHO 10^{-6} M). $^{**}P < 0.01$ vs. Fas Cont.

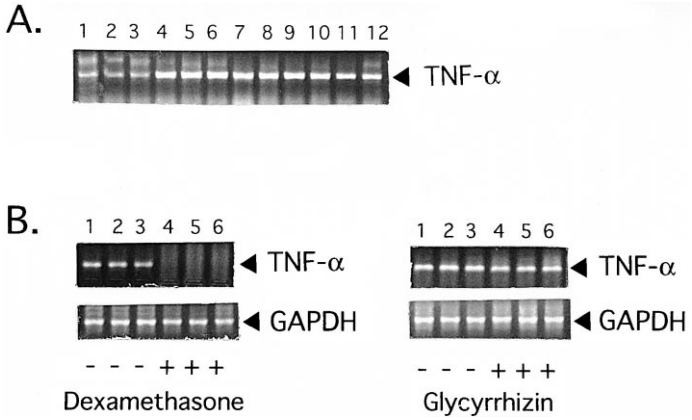

Fig. 2. RT-PCR analysis of the effect of glycyrrhizin on anti-Fas antibody-induced TNF-α mRNA expression. (A) Anti-Fas antibodies (150 μg/kg, i.v.) were administered, and livers were removed at 2, 5 and 8 h after treatment. RNA was isolated and subjected to PCR amplification with a TNF-α gene-specific primer. The contents of the gel lanes were as follows: lanes 1–3, normal liver samples; lanes 4–6, Lanes 7–9, lanes 10–12, were liver samples removed at 2, 5 and 8 h after anti-Fas antibody treatment, respectively. (B) Anti-Fas antibodies (150 μg/kg, i.v.) were administered, and livers were removed 2 h after treatment. RNA was isolated and subjected to PCR amplification with a TNF-α and GAPDH gene-specific primer. Pretreatment with dexamethasone (2.5 mg/kg, i.p.) or glycyrrhizin (200 mg/kg, i.p.) was performed 1 h before anti-Fas antibody injection. The contents of the gel lanes were as follows: Left: lanes 1–3, anti-Fas antibody-treated; lanes 4–6, anti-Fas antibody + dexamethasone (2.5 mg/kg, i.p.)-treated. Right: lanes 1–3, anti-Fas antibody-treated; lanes 4–6, anti-Fas antibody + glycyrrhizin (200 mg/kg, i.p.)-treated.

3.4. Effect of glycyrrhizin on anti-Fas antibody-induced TNF-α mRNA expression

The injection of mice with anti-Fas antibodies (150 μg/kg) induced TNF-α mRNA expression in the liver 2 h after treatment (Fig. 2A). Mice were pretreated with dexamethasone (2.5 mg/kg, i.p.) or glycyrrhizin (200 mg/kg, i.p.) at 1 h before anti-Fas antibody treatment. Their livers were removed at 2 h after anti-Fas antibody injection and RNA was isolated for PCR analysis. Treatment of mice with dexamethasone inhibited anti-Fas antibody-induced TNF-α mRNA expression in the liver (Fig. 2B). However, treatment of mice with glycyrrhizin did not inhibit the anti-Fas antibody-induced TNF-α mRNA expression in the liver (Fig. 2B). The GAPDH-gene was evenly expressed in normal and dexamethasone or glycyrrhizin-treated samples, indicating that there was no tube-to-tube variation.

4. Discussion

Hepatocytes are extremely sensitive to Fas and the injection of anti-Fas antibodies into mice causes hepatitis (Ogasawara et al., 1993). Furthermore, the Fas system is thought to play a role in the development of hepatitis in patients with hepatitis C virus infection. In the present study, anti-Fas antibody-induced hepatitis was indicated by the elevation of plasma alanine aminotransferase. Treatment with glycyrrhizin was clearly shown to inhibit anti-Fas antibody-induced hepatitis. Then we examined the effect of glycyrrhizin on CPP32-like protease activity. CPP32 is a cysteine protease that is involved in apoptosis (Nagata, 1997), and the activation of CPP32-like protease is critically involved in the development of hepatitis induced by anti-Fas antibodies (Rodriguez et al., 1996). In the present study, pretreatment of mice with glycyrrhizin inhibited the anti-Fas antibody-induced activation of CPP32-like protease in vivo. Thus, glycyrrhizin seems to inhibit anti-Fas antibody-induced hepatitis by acting upstream to the activation of CPP32-like protease. We examined the direct effect of glycyrrhizin on CPP32-like activity in vitro. The addition of glycyrrhizin at the final concentration of 10^{-4} M to the liver cytosol fraction isolated from anti-Fas antibody-treated mice did not inhibit the CPP32-like activity, whereas, the CPP32-like protease inhibitor Ac-DEVD-CHO, as expected, abolished the activity. Thus, glycyrrhizin may not directly affect CPP32-like protease.

The ability of Fas to activate other genes is not known. In the present study, treatment of mice with anti-Fas antibodies induced TNF-α mRNA expression in the liver. In several animal hepatitis models, TNF-α has been reported to play a role in the development of hepatitis (Tiegs et al., 1989; Gantner et al., 1995). Although we have not elucidated the role of anti-Fas antibody-induced TNF-α expression in the liver, dexamethasone inhibited anti-Fas antibody-induced TNF-α mRNA expression. Since glycyrrhizin is reported to have a steroidal effect (Tamaya et al., 1986), we examined the effect of glycyrrhizin on anti-Fas antibody-induced TNF-α mRNA expression in the liver. However, glycyrrhizin failed to inhibit anti-Fas antibody-induced TNF-α mRNA expression. In the liver of patients with hepatitis C virus infection, the expression of TNF-α mRNA has been reported (Larrea et al., 1996), and this cytokine was thought to contribute to the resistance to interferon therapy. The present results, however, suggest that glycyrrhizin does not inhibit such TNF-α

expression in the livers of patients with hepatitis C virus-infection.

The efficacy of glycyrrhizin in clinics to prevent liver carcinogenesis in chronic hepatitis C patients has been reported (Arase et al., 1997). In the present study, glycyrrhizin inhibited anti-Fas antibody-induced hepatitis by acting upstream to the activation of CPP32-like protease. From this, it is suggested that the decrease in the elevated level of plasma alanine aminotransferase seen in patients with hepatitis C virus infection following treatment with Stronger Neo-Minophargen C may be due to the inhibition of Fas-mediated hepatic injury by the glycyrrhizin present in Stronger-Neo-Minophargen C.

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