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Short communication

Inhibition of anti-Fas antibody-induced hepatitis by aminoguanidine in mice

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Abstract

Aminoguanidine is an inhibitor of inducible nitric oxide synthase (iNOS) and is of potential clinical usefulness. Treatment of mice with anti-Fas antibodies (150 μg/kg, i.v.) induced elevation of plasma alanine aminotransferase activity at 4 h and this elevation was inhibited by pretreatment of mice with aminoguanidine (3, 10 and 30 mg/kg, i.p.). The anti-Fas antibody-induced elevation of *caspase-3* activity was inhibited by aminoguanidine (30 mg/kg, i.p.), but the addition of aminoguanidine to the cytosol up to 10^{-4} M did not inhibit the *caspase-3* activity in vitro. Thus, aminoguanidine prevents anti-Fas antibody-induced hepatitis by affecting the apoptotic pathway upstream of *caspase-3* activation.

Keywords: Anti-Fas antibody; Aminoguanidine; Hepatitis

1. Introduction

Aminoguanidine is a selective inhibitor of the inducible isoform of nitric oxide synthase (iNOS) and has received much attention due to its low toxicity (Misko et al., 1993). Aminoguanidine is beneficial in different types of animal disease models. In particular, in diabetes, the effectiveness of aminoguanidine in animal models has been indicated by many reports (Corbett et al., 1992; Teixeira et al., 1999; Osicka et al., 2000). Furthermore, clinical trials of this drug on diabetes have been conducted (Nathan, 1995; Freedman et al., 1999). In the liver, aminoguanidine exerts a cytoprotective effect in acetaminophen-induced rat hepatitis and endotoxemia (Gardner et al., 1998; Laskin et al., 1995), but it exerts a cytotoxic effect on carbon tetrachloride-induced rat hepatitis (Tanaka et al., 1999). Hepatitis C virus-infection is one of the major causes of chronic hepatitis and the Fas-system is involved in the development of hepatitis in hepatitis C virus-infection (Hiramatsu et al., 1994; Mita et al., 1994). Furthermore, the Fas-system plays a critical role in the development of viral-induced hepatitis (Hayashi and Mita, 1997). Treatment of mice with anti-Fas antibodies induces hepatitis (Ogasawara et al., 1993), and this hepatitis model, at least in part, reflects the hepatitis in hepatitis C virus-infection. Although aminoguanidine exhibits low toxicity and is of potential clinical usefulness, its beneficial effect on hepatitis is not fully understood.

In the present study, we examined the effect of aminoguanidine on anti-Fas antibody-induced mice hepatitis.

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 chow and water ad libitum. Jo2, which is an anti-Fas antibody known to induce hepatitis through activation of receptor Fas (Ogasawara et al., 1993), was purchased from Pharmingen (San Diego, CA). The anti-Fas antibodies (150 μg/kg) were administered to the mice via a tail vein (in a volume of 100 μl). Aminoguanidine was obtained from Research Biochemical International (Natick, MA, USA) and injected intraperitoneally. Animal experiments were performed according to the experimental protocols approved by the Institutional Ethics Committee. The mice

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were anesthetized with ether before killing. The plasma alanine aminotransferase activity was measured as described previously (Okamoto et al., 1999). The measurement of *caspase-3* activity was performed using Ac–Asp–Glu–Val–Asp–4-Methyl–Coumaryl–7-Amide (Ac–DEVD–MCA) as previously described (Okamoto et al., 1999). RNA isolation and reverse-transcription polymerase chain reaction (RT-PCR) analysis were conducted according to the previously described methods (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 tumor necrosis factor-α (TNF-α), iNOS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were: iNOS (Genbank Acc#L09129) (sense); 5′-TGGGAATGGAGACTGTCCCAG-3′; (antisense) 5′-GGGATCTGAATGTGATGTTTG-3′; TNF-α (Genbank Acc#M11731); (sense) 5′-AGCCCACGTCGTAGCAAACCACCAA-3′; (antisense) 5′-ACACCCATTCCCTTCACAGAGCAAT-3′; GAPDH (Genbank Acc#X02231); (sense) 5′-ATGGTGAAGGTCGGTGTGAACG-3′; (antisense) 5′-GTTGTCATGGATGACCTTGGCC-3′.

The results were statistically analyzed by means of the Dunnett multiple comparison test.

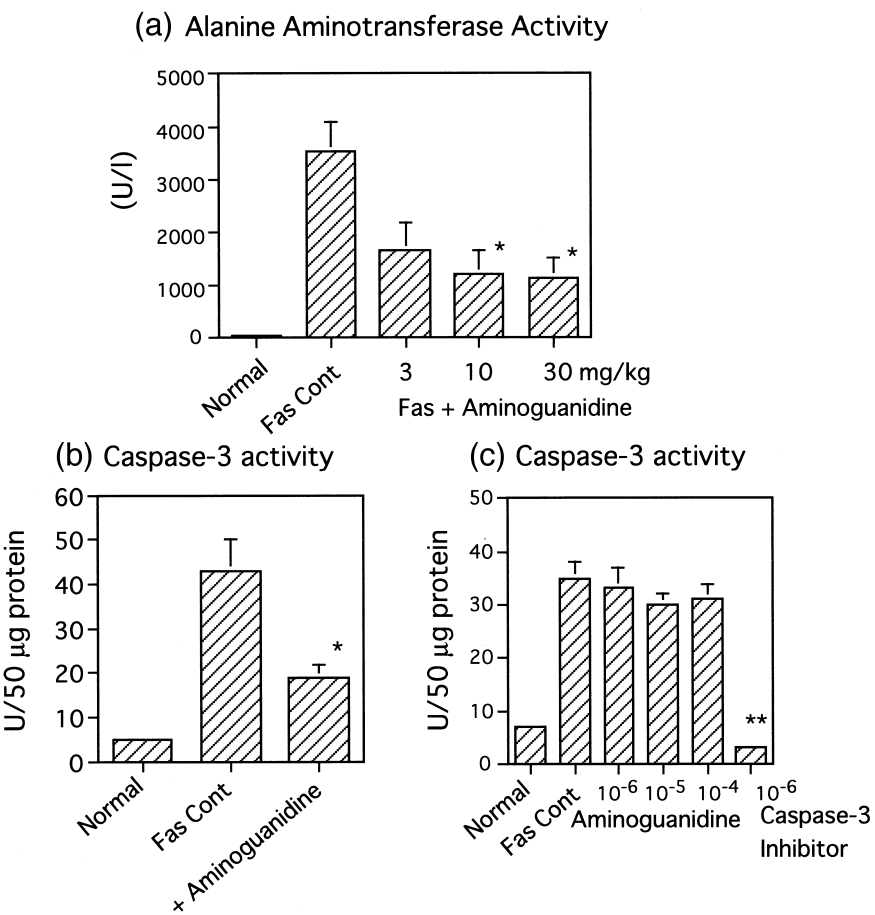

Fig. 1. (a) Effect of aminoguanidine treatment on anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity. Anti-Fas antibodies were injected. At 4 h after administration, plasma was sampled from each mouse. Pretreatment with aminoguanidine was performed at 1 h before anti-Fas antibody treatment. Data represent the means ± SE of alanine aminotransferase activity in plasma obtained for each treatment. The alanine aminotransferase activity level (U/l) in normal mice was 36 ($n = 2$). Normal: non-treated ($n = 2$). Fas Cont: anti-Fas antibody (150 μg/kg, i.v.)-treated ($n = 5$). Fas + amioguanidine: anti-Fas antibody (150 μg/kg, i.v.) + aminoguanidine (3, 10 and 30 mg/kg, i.p.)-treated ($n = 5$). $^{*}P < 0.05$ vs. Fas Cont. (b) Effect of aminoguanidine on anti-Fas antibody-induced elevation of *caspase-3* activity in vivo. Anti-Fas antibodies were injected into mice and their livers were removed at 4 h. Pretreatment with aminoguanidine (30 mg/kg, i.p.) was performed at 1 h before anti-Fas antibody treatment. Protein was extracted from the liver samples and *caspase-3* activity was measured with fluorescent substrate Ac–DEVD–MCA (25 μM) and liver cytosol extracts. *Caspase-3* activity is presented as U/50 μg protein. One unit corresponds to the activity that cleaved 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 ($n = 5$). Fas + aminoguanidine: anti-Fas antibody (150 μg/kg, i.v.) + aminoguanidine (30 mg/kg, i.p.)-treated ($n = 5$). $^{*}P < 0.05$ vs. Fas Cont. (c) Effect of aminoguanidine on the *caspase-3* activity in anti-Fas antibody-treated mouse liver cytosol extracts. Mice were treated with anti-Fas antibodies (150 μg/kg, i.v.), and then at 4 h after treatment their livers were removed for protein extraction. *Caspase-3* activity was measured using fluorescent substrate Ac–DEVD–MCA (25 μM), with the addition of aminoguanidine (10^{-6}, 10^{-5} and 10^{-4} M) or a *caspase-3* inhibitor (Ac–DEVD–CHO 10^{-6} M) ($n = 4$ for each treatment). $^{**}P < 0.01$ vs. Fas Cont.

3. Results

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

Mice were treated with anti-Fas antibodies (150 μg/kg), and then at 4 h plasma was sampled for alanine aminotransferase activity measurement. Anti-Fas antibody treatment elevated plasma alanine aminotransferase activity (Fig. 1a). Mice were pretreated with aminoguanidine (3, 10 and 30 mg/kg, i.p.) at 1 h before anti-Fas antibody treatment. The aminoguanidine pretreatment inhibited the anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity (Fig. 1a).

3.2. Effect of aminoguanidine on anti-Fas antibody-induced elevation of caspase-3 activity

Mice were treated with anti-Fas antibodies (150 μg/kg), and then at 4 h after treatment plasma and liver were sampled for measurement of alanine aminotransferase activity and the *caspase-3* activity. Anti-Fas antibody treatment elevated *caspase-3* activity ($n = 5$) (Fig. 1b). Mice were pretreated with aminoguanidine (30 mg/kg, i.p.) at 1 h before anti-Fas antibody treatment. The aminoguanidine treatment inhibited both the anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity (alanine aminotransferase activity (U/l): anti-Fas antibodies 3338 ± 396; anti-Fas antibodies + aminoguanidine, 1258 ± 335; $n = 5$, $P < 0.05$) and *caspase-3* activity (Fig. 1b).

3.3. Effect of aminoguanidine on caspase-3 activity in vitro

Mice were treated with anti-Fas antibodies (150 μg/kg), and then at 4 h after the treatment their livers were removed and protein was isolated for measurement of *caspase-3* activity. The anti-Fas antibody treatment elevated the *caspase-3* activity (Fig. 1c). The addition of aminoguanidine to final concentrations of 10^{-6}, 10^{-5} and 10^{-4} M did not inhibit the *caspase-3* activity (Fig. 1c). Whereas the activity was abolished by *caspase-3* inhibitor Ac–DEVD–CHO.

4. RT-PCR analysis of iNOS mRNA expression

Expression of iNOS mRNA in the liver was examined. Mice were treated with anti-Fas antibodies (150 μg/kg, i.v.), and then at 4 h after treatment, livers were removed and RNA was isolated for RT-PCR analysis. For normal liver, faint bands of iNOS mRNA expression were detected and anti-Fas antibody treatment slightly elevated iNOS mRNA expression (Fig. 2). Anti-Fas antibody treatment also induced TNF-α mRNA expression (Fig. 2). The expression of the GAPDH-gene in the normal samples indicated the presence of RNA.

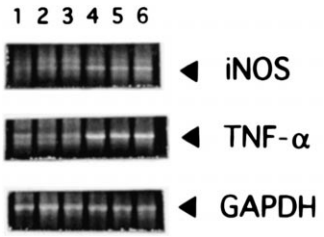

Fig. 2. RT-PCR analysis of iNOS and TNF-α mRNA expression. Anti-Fas antibodies (150 μg/kg, i.v.) were administered, and then livers were removed at 4 h after treatment. RNA was isolated and subjected to PCR amplification with iNOS, TNF-α and GAPDH gene-specific primers. Lanes 1–3: non-treated; lanes 4–6: anti-Fas antibody-treated.

5. Discussion

The Fas-system is a major inducer of apoptosis (Nagata, 1997). In the livers of patients with hepatitis C virus-infection, Fas antigen expression and Fas ligand mRNA expression are observed (Hiramatsu et al., 1994; Mita et al., 1994). Although many factors including cytokines (Andus et al., 1991) and prostanoids (Tiegs and Wendel, 1988; Nanji et al., 1997) mediate the development of chronic hepatitis, the Fas-system is thought to be one of the factors involved in the development of hepatitis in hepatitis C virus-infection. In the present study, aminoguanidine was shown to inhibit anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity, indicating its potential ability to prevent Fas-induced hepatocyte apoptosis.

Caspase-3 is a key enzyme involved in the development of Fas-mediated hepatitis (Rodriguez et al., 1996). Anti-Fas antibody treatment activates *caspase-3* and triggers apoptotic cascades in hepatocytes (Rodriguez et al., 1996). In the present study, aminoguanidine treatment inhibited anti-Fas antibody-induced elevation of *caspase-3* activity. Furthermore, an in vitro study indicated that aminoguanidine did not directly inhibit *caspase-3* activity. Thus, aminoguanidine might inhibit the anti-Fas antibody-induced elevation of plasma alanine aminotransferase activity by affecting upstream of *caspase-3* activation.

Since aminoguanidine is a selective inhibitor of iNOS, we examined the involvement of iNOS in anti-Fas antibody-induced hepatitis. The treatment of mice with anti-Fas antibodies was shown to induce slight expression of iNOS mRNA in the liver. Although the induction mechanism and its role are not known, anti-Fas antibody treatment induced TNF-α mRNA expression in the liver. Since iNOS is induced by cytokines and TNF-α reportedly induces iNOS in vitro (Binder et al., 1999), anti-Fas antibody-induced

TNF-α might trigger iNOS gene expression. However, neither elevation of the plasma nitrite/nitrate level nor immunohistochemical staining of iNOS protein in the liver was induced by anti-Fas antibody treatment (not shown). Only slight induction of iNOS mRNA expression in the liver does not necessarily indicate the involvement of iNOS in the development of anti-Fas antibody-induced hepatitis. Thus, aminoguanidine might prevent anti-Fas antibody-induced hepatitis through a mechanism other than iNOS inhibition. Furthermore, another NOS inhibitor N^G-nitro-L-arginine methyl ester failed to show any inhibitory effect on anti-Fas antibody-induced hepatitis (not shown). However, it is also possible that there might be a local production of NO and aminoguanidine inhibits anti-Fas antibody-induced hepatitis by inhibiting the locally produced NO. Further studies are required to exclude the contribution of these autocrine loops in the anti-Fas antibody-induced hepatitis.

Taken together all the results, it was suggested that there is another mode of action for aminoguanidine to inhibit hepatitis and also raised a possible therapeutic application of this drug for viral hepatitis.

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