Angiotensin II Type 1 Receptor Antagonist Improves the Prognosis in Rats Displaying Liver Cirrhosis Induced by a Choline-Deficient Diet

Kaoru Iwata, Tetsuro Sohda, Makoto Irie, Yasuaki Takeyama, Akira Anan, Satoshi Shakado, Shotaro Sakisaka

Department of Gastroenterology and Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

Abstract

Background. Angiotensin II type 1 receptor (AT1) antagonists are known to suppress TGFβ and lipid peroxidation. An experimental rat model made by feeding rats a choline-deficient diet (CDD) showed severe steatosis, fibrosis and infiltration of inflammatory cells in the liver resembling nonalcoholic steatohepatitis (NASH). NASH causes fibrosis by lipid peroxidation. In this study, we assess whether AT1 antagonists and angiotensin II type 2 receptor (AT2) antagonists can suppress the hepatic fibrosis and lipid peroxidation in CDD rats that lead to the development of NASH. Methods: Both study groups received subcutaneously aqueous solutions of AT2 antagonist (PD123319 – 1 mg/kg/day) and AT1 antagonist (L158809 – 1 mg/kg/day), respectively, 6 times per week. On day 90, some rats (5 /group) were sacrificed by excision of the liver under anesthesia, in order to assess the hepatic hydroxyproline (HP), malondialdehyde (MDA), total glutathione, superoxide dismutase (SOD) activity and TGFβ-1. The remaining rats were maintained to observe the survival rate. Results: All CDD rats developed liver cirrhosis. However, the tissue TGF and HP decreased in AT1 antagonist group in comparison with the other two groups. All groups of CDD rats showed strong adipose hyperoxidation. The AT1 antagonist group demonstrated a markedly improved survival rate in comparison to the other two groups. Conclusion: Hepatic fibrosis progression in the AT1 antagonist group was slower than that in the other groups. This observation suggests that AT1 antagonists delayed the progression of liver failure, which thus led to an improved survival rate.

Keywords

Angiotensin II receptor – choline-deficient diet – nonalcoholic steatohepatitis – lipid peroxidation - hepatic fibrosis

Introduction

Angiotensin II type 1 receptor (AT1) antagonists have been widely used in the treatment of hypertension. The unique characteristics of this biomolecule have attracted interest from a variety of fields. Two types of angiotensin II receptors have been so far been described, type 1 receptor and type 2 receptor [1-8], which trigger opposing effects. AT1 antagonists have been shown to protect the heart and kidney both experimentally and clinically [9-15]. The suppression of TGFβ by angiotensin conversion enzyme (ACE) inhibitors and AT1 antagonists, which inhibit the development of fibrosis in the heart, kidney and blood vessels, has been elucidated [16-19].

In liver diseases, the development of fibrosis can lead to liver cirrhosis. Therefore, an effective preventive treatment for fibrosis is of utmost importance. ACE inhibitors or AT1 antagonists have been reported to suppress mRNA of TGFβ-1 and fibrosis markers in rats with biliary duct ligation (animal model for liver cirrhosis) [20-22]. In addition, a hepatic fibrosis animal model by administration of a swine serum demonstrated that ACE inhibitors and AT1 antagonists suppressed liver fibrosis despite the presence of inflammation due to immune reaction [23]. In most studies, however, a dose 5 to 10 times higher than the clinical dose of AT1 antagonist was used for experiments.

Rats that are fed a choline-deficient diet (CDD) develop hepatic fibrosis as a result of fat deposits and lipid peroxidation as well as inflammation of the liver. The lesions observed in this animal model are very similar to those of nonalcoholic steatohepatitis (NASH) [24-27]. The aim of this study was to determine whether a clinical dose of AT1 or AT2 antagonist can suppress the development of hepatic fibrosis and lipid peroxidation in CDD rats.
Material and methods

Animal model

Sixty Fischer 344 male rats (Charles River Japan Inc., Yokohama, Japan) weighing 150-180 grams were randomly divided into four groups (15 rats each group). In the normal diet group (Normal group), the rats were fed with ad libitum access to commercial standard rat chow (K.B.T Oriental Co., Ltd., Tosu, Japan) and water. In the remaining three groups the rats were fed with ad libitum access to a CDD (K.B.T Oriental Co., Ltd., Tosu, Japan) and water. Subsequently, the CDD rats were assigned to either the control group (CDDC; n=15), the AT2 antagonist group (CDDAT2A; n=15) or the AT1 antagonist group (CDDAT1A; n=15). Normal group and the CDDC rats received subcutaneously physiological saline 6 times per week. The CDDAT2A and CDDAT1A rats received subcutaneously aqueous solutions of PD123319 (1 mg/kg/day) (PD123319: SIGMA, St Louis, Missouri, USA) and L158809 (1 mg/kg/day) (L158809: MERCK, Rahway, New Jersey, USA), respectively, 6 times per week.

At day 90, 20 rats (5 of each group) were sacrificed by excision of the liver under anesthesia, which was achieved via the intraperitoneal administration of pentobarbital (6 mg/100 g body weight, Dainihonnseiyaku Ltd., Osaka, Japan). The remaining 40 rats were maintained under feeding conditions in order to observe the survival rate. Liver tissue specimens were fixed in 4% paraformaldehyde for haematoxylin-eosin (H-E) staining, sirius red staining and immunohistochemical examination. A part of the liver was frozen in liquid nitrogen and stored at -80°C in order to assess the superoxide dismutase (SOD) activity as well as the levels of hydroxyproline (HP), malondialdehyde (MDA), total glutathione and TGFβ-1 (Fig. 1).

Histopathologic assessment of hepatic fibrosis

To assess hepatic fibrosis, four micrometer-thick liver sections were stained with sirius red in picric acid. To histologically quantify sirius red staining, image software (Image Gauge, Fujifilm) was used to calculate the percent area staining positively in 15 random low-power views.

Hepatic hydroxyproline, TGFβ-1 content and lipid peroxidation

The collagen content was determined by measurement of liver hydroxyproline (HP), TGFβ-1 was measured in liver tissue specimens as described previously. Lipid peroxidation was assessed by measurement of SOD activity and MDA and total glutathione levels in liver.

Statistical analysis

The data are shown as the mean ± SD. Statistical comparisons were performed using the ANOVA test. The

Fig.1 Protocol: 60 male rats were randomly divided into four groups (15 rats each group) (see text). CDDAT2A and CDDAT1A rats received the subcutaneous administration of aqueous solutions of PD123319 (1 mg/kg/day) and L158809 (1 mg/kg/day), respectively, 6 times per week. At day 90, 20 rats (5 of each group) were sacrificed by excision of the liver under anesthesia and the HP, TGF-β1, SOD activity, MDA and total GSH were all measured in liver tissue specimens. The remaining 40 rats were maintained under normal feeding conditions in order to observe the survival rate.

Fig.2. Sirius red staining of liver section (x40). All CDD rats exhibited liver cirrhosis (A). The sirius red positive area was expressed as the percentage of the total area of the specimen (B).
Angiotensin II type 1 receptor antagonists

survival curve and survival rate, which were calculated utilizing the Kaplan-Meier method, were compared with the Log-rank test. A p-value of less than 0.05 was considered statistically significant.

Results

Histologically (H-E staining and sirius red staining), all CDD rats exhibited inflammatory cell infiltration as well as strong fibrosis (Fig. 2). Fibrosis was colored red due to the sirius red staining. All CDD rats developed liver cirrhosis. A semi-quantitative analysis of fibrotic areas evidenced no significant differences among the three CDD groups (Fig. 2).

The CDDAT1A group also displayed markedly lower liver TGFβ-1 values relative to the other two groups (Fig. 3A). In addition, CDDAT1A rats demonstrated lower liver hydroxyproline content in comparison to control rats (Fig. 3B). In terms of lipid peroxidation, all three CDD groups exhibited decreased levels of total glutathione and SOD activity as well as elevated MDA in liver tissue relative to the Normal group. No significant differences were observed among the three CDD groups (Table I).

A survival analysis of the CDD groups revealed an increase in the number of deaths from the 100th day in CDD and CDDAT2A rats. The CDDAT1A group displayed a significantly extended survival time in comparison to the other two groups (p < 0.001, log rank test).

Discussion

The current investigation used angiotensin II receptor antagonists in order to suppress hepatic fibrosis in CDD

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<th>Table I. Lipid peroxidation in the rat liver tissue</th>
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NOTE: Results are expressed as mean ± SD. SOD, superoxid dismutase; MDA, Malondialdehyde; GSH, glutathion. *p < 0.01 vs Normal
rats. The administration of AT1 antagonist was found to significantly reduce the levels of TGFβ-1 in the liver tissue in CDDAT1A group in comparison to the two other groups as well as the HP levels relative to the control group. AT1 antagonists were found to suppress fibrosis in diabetic nephropathy via TGFβ-1 [28, 29]. Furthermore, AT1 antagonist also led to the suppression of TGFβ-1 mRNA expression in liver tissue in rats with bile duct ligation [20, 21].

This study revealed that the administration of AT1 antagonists suppressed hepatic fibrosis mediated by TGFβ-1 in CDD rats. AT1 antagonists are known to possess an antioxidative effect [30-33]. Consequently, we also examined the degree of lipid peroxidation. We found that MDA, an indicator of the lipid peroxidation in the liver tissue, increased following the administration of AT1 antagonist in a manner consistent with that observed in the other two groups. In addition, the SOD activity and total glutathione level in the liver tissue significantly decreased in all three CDD groups. These data suggested that the suppression effect of AT1 antagonist on lipid peroxidation attributable to the administration of a CDD was rather weak. Furthermore, even rats from the CDDAT1A group displayed strong evidence of fibrosis (almost to the point of liver cirrhosis). Therefore, in order to prevent fibrosis in CDD rats, the usage of a more robust antioxidative agent is considered to be necessary instead of obtaining an antioxidative effect via AT1.

The CDDAT1A group revealed a markedly improved survival rate in comparison with the other two groups. All of the rats that died eventually progressed to liver cirrhosis with a few cases of ascites; as a result, the most likely cause of death was liver failure. The progression of hepatic fibrosis in the CDDAT1A group was slower than that in the other groups. Consequently, this observation suggested that AT1 antagonists delayed the progression of liver failure, thus leading to an improved survival rate. It is also possible that the protective effect exerted on the heart and kidney by the AT1 antagonists was also associated with improvement of the survival rate [16, 17, 34, 35].

At first, we expected that the AT2 antagonist would enhance hepatic fibrosis and increase the lipid peroxidation. However, our data showed that AT2 antagonist did not act on hepatic fibrosis in the used dose. We speculate that AT2 antagonists are not related to hepatic fibrosis at all or the dose was too small to act.

In conclusion, we demonstrated that a clinical dose of AT1 antagonist delayed hepatic fibrosis. The effect of AT1 antagonists in terms of other types of organ failure, such as heart and kidney in CDD rats, is therefore considered to be a suitable topic for future investigation.

Conflicts of interests
None to declare.

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