A Novel Soluble Beta-Glucan Salecan Protects against Acute Alcohol-Induced Hepatotoxicity in Mice

Peng Chen,1,* Zhongqiu Wang,3,* Liyuan Zeng,2 Xiao Yang,1 Shiming Wang,1 Wei Dong,1 Aiqun Jia,1 Chun Cai,1,2 and Jianfa Zhang1,*

1 Center for Molecular Metabolism, Nanjing University of Science and Technology, Nanjing, 210094, China
2 School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, 210094, China
3 Nanjing Jinling Hospital, Nanjing, 210002, China

Received May 26, 2011; Accepted July 20, 2011; Online Publication, October 7, 2011

This investigation was designed to determine the effect of a novel soluble beta-glucan salecan on acute alcohol-induced hepatic injury in mice. Mice were given salecan (15 or 30 mg/kg) or PBS for 4 d. Ethanol (6 g/kg) was administered orally 1 h after the last injection. The animals were sacrificed at 10 h after alcohol administration. Pretreatment with salecan significantly ameliorated the hepatic damage induced by ethanol, as evidenced by markedly reduced serum aminotransferase activities and hepatocyte steatosis. Salecan administration remarkably alleviated the formation of thiobarbituric acid-reactive substances and counteracted glutathione depletion. The mRNA level of peroxisome proliferator activated receptor alpha, a major gene responsible for fatty acid oxidation, was significantly increased after salecan pretreatment. The expression of diacylglycerol acyltransferase 1, an important gene responsible for triacylglycerol synthesis, was markedly decreased after salecan was administrated. These findings suggest that salecan might represent a novel protective strategy against alcoholic liver injury.

Key words: beta-glucan; salecan; alcoholic liver injury; mice

Beta-glucans are naturally occurring polysaccharides with poly-branched beta-1,3-(D)-glucans or beta-1,6-(D)-glucose side chains, originally found in the cell walls of fungi and cereal plants.1 Due to a lack of toxic and adverse effects, beta-glucan has attracted the attention of many researchers studying its biological activities.2,3 Recently, it was found to play a beneficial role in regulating the immune system.4,5 Additionally, evidence also indicates that it has anti-oxidative stress, anti-virus, and anti-tumor effects.6,7 A novel water-soluble extracellular beta-glucan, salecan, produced by a novel strain of Agrobacterium sp. ZX09, has been found to consist of the following repeating unit: \(-\beta-D-\text{Glc}p-(1 \rightarrow 3)-\beta-D-\text{Glc}p-(1 \rightarrow 3)-\beta-D-\text{Glc}p-(1 \rightarrow 3)-\beta-D-\text{Glc}p-(1 \rightarrow 3)-\alpha-D-\text{Glc}p-(1 \rightarrow)

This special structure of salecan is related to its water-soluble property and has potential biological activities owing to several beta-1,3 glucans.

Alcoholic liver disease (ALD) is an extremely common disease with high mortality around the world.8,9 Steatosis and oxidative stress are the main well-documented pathological factors in the development of ALD.9,10 It has long been known that ethanol is metabolized predominantly by a well-characterized pathway, wherein it is first metabolized to acetaldehyde and further oxidized to acetate. Both steps are coupled with the reduction of NAD to NADH. The metabolism of lipids is then disturbed by an increasing NADH/NAD ratio.11 Steatosis occurs due to impaired fatty acid catabolism and increased lipogenesis in the liver.12 Peroxisome proliferator activated receptor \(\alpha\) (PPAR\(\alpha\)) is the main fatty acid catabolism regulator.13 It has been reported that PPAR\(\alpha\)-/- mice exhibited much more alcoholic fatty liver development after ethanol administration.14 Ethanol also generated reactive free radicals, which can lead to oxidative stress. In addition, alcohol exposure also impaired the system that protects cells against reactive oxygen species (ROS), including lowered levels of glutathione (GSH).15 Enhanced ROS production and compromised antioxidant activity resulted in protein and DNA function impairment and hepatocyte necrosis/apoptosis eventually. Although the pathogenesis of ALD has become increasingly clear, there is no satisfactory therapy for ALD at present. Here, we found that pretreatment with Salecan, a novel soluble beta-glucan, significantly ameliorated the hepatic damage induced by ethanol ingestion. This might provide a novel protective strategy against ALD.

Materials and Methods

Preparation of salecan. The preparation of salecan was described previously.6 Strain ZX09, used in this study, was isolated from a soil sample from the ocean coast of Shandong province, China. Cultures were maintained on Hmun agar consisting of NaH\(\text{PO}_4\) 1 g, \(\text{KNO}_3\) 3 g, \(\text{CaCl}_2\) 0.07 g, \(\text{MgCl}_2\) 0.2 g, \(\text{FeSO}_4 \cdot 7\text{H}_2\text{O}\) 0.0125 g, \(\text{MnSO}_4\) 0.003 g, \(\text{ZnCl}_2\) 0.0075 g, sucrose 20 g, agar 9 g, and H\(\text{O}_2\) 1.000 mL, pH 7.2. A colony of the strain was inoculated into a 250-mL flask containing 50 mL of medium consisting of sucrose and a mineral salt solution. The inoculated preparation was incubated at 28°C on a rotary shaker at 220 rpm for 24 h. A 0.5-mL portion was transferred to a 250-mL flask.
containing 50 mL of fermentation medium. Fermentation was done on a rotary shaker at 220 rpm for 48 h. The culture broth was diluted more than 3 times with de-ionized water and centrifuged at 12,000 × g for 30 min to separate the cells from the supernatant. The supernatant was added to 2 volumes of 95% ethanol. The productivity of salecan was expressed in terms of weight after ethanol precipitation collected by centrifugation at 6,000 × g for 15 min and dried under a reduced press. The salecan was further purified according to previously described methods. Gel filtration chromatography was conducted with a Sepharose CL-4B (Pharmacia, Shanghai, China) column (1.5 by 60 cm), and the polysaccharides were eluted with 50 mMol/L phosphate buffer, pH 7.2, at a rate of 1 mL/min. Fractions containing polysaccharides were collected, and the total sugar contents of the fraction were determined by the phenol–sulfuric acid method. The average molecular weight of the purified salecan was estimated from a calibration curve of standard dextrans obtained by gel filtration on Sepharose CL-4B to be about 2 × 10^6. The purity of the purified salecan was more than 99%.

Animals and treatment. Male 7–8 week old C57BL/6 mice were used. The animals were maintained under a 12/12 h light/dark cycle with lights on at 7:00 am and off at 7:00 pm, and with free access to regular chow food and water. All animal care and use procedures were in accordance with the Guidelines of the Institutional Animal Care and Use Committee at Nanjing University of Science and Technology. The salecan was further purified according to previously described methods. Gel filtration chromatography was conducted with a Sepharose CL-4B (Pharmacia, Shanghai, China) column (1.5 by 60 cm), and the polysaccharides were eluted with 50 mMol/L phosphate buffer, pH 7.2, at a rate of 1 mL/min. Fractions containing polysaccharides were collected, and the total sugar contents of the fraction were determined by the phenol–sulfuric acid method. The average molecular weight of the purified salecan was estimated from a calibration curve of standard dextrans obtained by gel filtration on Sepharose CL-4B to be about 2 × 10^6. The purity of the purified salecan was more than 99%.

Serum biochemical and hepatic TC and TG assay. Serum alanine transaminase (ALT), aspartate aminotransferase (AST) activity, total triglycerides (TG), and total cholesterol (TC) were measured with a commercial kit (Jiancheng, Nanjing, China). The animals were sacrificed 10 h after ethanol dosing.

Histopathological analysis. Liver specimens were fixed in 10% v/v buffered formalin solution over 24 h and processed routinely by embedding them in paraffin. Tissue serial sections (5 μm) were stained with hematoxylin and eosin (H and E, Jiancheng, Nanjing, China) following the manufacturer’s protocol. The real-time PCR reaction was carried out on ABI 7300 real-time PCR system with a cDNA sample and was amplified in a 20 μL reaction volume containing 1 × SYBR Green PCR master mix (Applied Biosystems, Foster City, CA). The primers used were PPARα, forward: GGGTAGCCACTACGAGGTCACG, reverse: CAGACAGGACCTTGTGAACL, DGAT1, forward: GTGGACAAATGTGGTCATCG, reverse: CAGTTGGGATCTGAGCCATCTC; and Gapdh, forward: CATCCACTGGTGCTGCAAGGCGT, reverse: ACAACCTGTCTCTACATGCTG-TAGCCCA. Relative expression in comparison with Gapdh was calculated by the comparative CT method.

**Statistical analysis.** The results were expressed as mean ± SEM. Statistical evaluation was done using Student’s t-test. A p value of less than 0.05 was considered a statistically significant difference.

**Results and Discussion**

**Salecan administration ameliorated acute alcohol-induced hepatic injury**

Acute alcohol-induced liver injury was calibrated by elevated serum ALT/AST activity, hepatic triglyceride content, and liver pathological changes characterized by swelling and hydropic degeneration of hepatocytes around the central and interlobular veins. As shown in Fig. 2, serum ALT/AST, TC, TG, and hepatic TC and TG dramatically increased at 10 h after administration of alcohol (6 g/kg) to the mice, but pretreatment with salecan remarkably decreased the levels of all the parameters in a dose-dependent manner. Figure 2 also shows the histopathological changes in the livers after acute ethanol exposure. H and E staining revealed obvious swelling and hydropic degeneration of the hepatocytes around the central and interlobular veins in the ethanol-treated mice, as compared to the livers of the controls. Salecan pretreatment markedly ameliorated the histopathological changes. Oil Red O staining confirmed the histopathological results by demonstrating that the intense staining of neutral triglycerides in the liver samples from acute ethanol treated mice was diminished in the salecan treated mice. These data clearly indicate that salecan can markedly ameliorate acute alcohol-induced hepatic injury.

**Salecan treatment decreased lipid peroxidation and reverse GSH depletion induced by alcohol**

Alcohol-induced liver injury is associated with increased lipid peroxidation and weakened hepatic antioxidant defenses. Hepatic lipid peroxidation was assessed by measuring the thiobarbituric acid-reactive substance contents. Acute ethanol exposure induced a significant increase in the thiobarbituric acid-reactive substance content, which was dramatically attenuated by salecan pretreatment in a dose-dependent manner (Fig. 3A). Compared to the control group, acute ethanol administration led to a significant decrease in the GSH level in the liver, but salecan pretreatment restored the GSH concentration in a dose-dependent manner (Fig. 3B). These results indicate a protective effect of salecan against acute alcohol-induced liver injury, partially through alleviation of oxidative stress.
Effects of salecan on PPARα and DGAT1 expression

Fat accumulation is an important pathological feature in alcoholic liver disease. PPARα and DGAT1 are enzymes responsible for fatty acid oxidation and triacylglycerol synthesis respectively. Both are involved in alcohol-induced steatosis.12) As shown in Fig. 4A and B, the mRNA level of PPARα fell after ethanol treatment, while salecan increased the expression of PPARα to certain extent. The DGAT1 mRNA level increased in response to ethanol, the expression of which fell in the salecan pretreatment group.

In the current study, we identified a novel water-soluble β-glucan, salecan, produced by Agrobacterium sp. ZX09 as a new potential protective agent against...
acute alcohol-induced hepatic injury. After we isolated this glucan, the toxic and adverse effects of salecan were studied by our group. Acute and subchronic experiments demonstrated the safety properties of this glucan.20) Also, salecan alone did not affect normal hepatic activity.21) Hence salecan can be used as hepatoprotective agent. Our work indicates that salecan can attenuate hepatic oxidative stress and decrease intra-cellular lipid accumulation by modulating PPARα and DGAT1 expression to alleviate the liver injury induced by ethanol ingestion. Ethanol administration can induce oxidative stress, which includes reactive oxygen species (ROS) over-production and antioxidants such as GSH depletion. It is believed that oxidative stress plays a major role in the mechanisms by which ethanol produces liver injury.10,15) Previous work indicates the antioxidative effect of certain β-glucans.22) In the present study, salecan restored GSH depletion and decreased lipid peroxidation, and this anti-oxidative effect played an important role in protecting against ethanol-induced hepatotoxicity. A 30 mg/kg dose of salecan lowered hepatic TBARS content to even lower than the control group. ROS also occurred in the normal cells, which means that in the control group the TBARS content could have been further lowered by an anti-oxidative agent. A high dose of salecan (30 mg/kg) decreased the TBARS content to even less than the control group, which strongly indicates that salecan is an excellent anti-oxidative agent. Another key pathological change after alcohol exposure is the development of hepatic steatosis. It is generally believed that the accumulation of triglyceride in the hepatocytes is first hit, which leads to inflammation and acts as lipotoxicity in alcohol-induced liver injury.23) Our findings indicate that salecan alleviates ethanol-induced hepatic fatty accumulation, as evidenced by H and E and Oil Red O staining. Furthermore, an important component responsible for triacylglycerol synthesis, DGAT1, was downregulated by salecan after ethanol ingestion. Another interesting finding of the current work is that salecan enhanced PPARα expression to more than control group. It has been reported that β-glucan lowered lipid contents in vivo,23,24) but the mechanism remains elusive. We noticed that PPARα, a key gene responsible for fatty acid catabolism, was an important inhibitor of lipid accumulation.13) Hence we speculate that salecan can enhance PPARα expression to reduce lipid deposition. Here, our data confirm this. Salecan markedly enhanced the gene expression of PPARα over than controls. Therefore, inhibition of hepatic steatosis might also account for the protective effect of salecan against alcoholic liver injury.

Collectively, our results indicate that salecan alleviates acute alcoholic liver injury via inhibiting hepatic steatosis and oxidative stress. This might represent a novel, effective therapeutic agent for ethanol-induced liver injury.

Acknowledgment

This work was supported by the National Science Foundation of China (30730030) and the Nanjing University of Science and Technology fund (2010ZDJH14).

References