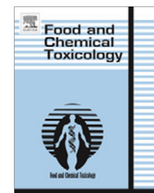
Food and Chemical Toxicology 50 (2012) 2149–2154

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Corn oligopeptides protect against early alcoholic liver injury in rats

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ARTICLE INFO

Article history:
Received 15 February 2012
Accepted 31 March 2012
Available online 13 April 2012

Keywords:
Corn oligopeptides
Alcoholic liver injury
Antioxidation
Lipid metabolism

ABSTRACT

Background: The present study aimed to investigate the effects of corn oligopeptides (COPs) on early alcoholic liver injury in rats. A total of 70 Wistar rats were randomly assigned to 7 groups, including a normal control group, 3 alcohol control groups (2.0, 4.0 and 6.0 g/kg/BW ethanol), and 3 COP intervention groups (2.0, 4.0 and 6.0 g/kg/BW ethanol, with 900 mg/kg/BW COPs). The study duration lasted for 4 weeks. Serum markers were assayed, and a histopathological examination was conducted.
Results: We found that the COP treatment prevented the elevation of serum aminotransferase and alleviated the hepatic histological damage that was induced by alcohol. In addition, the COPs counteracted the changes in the SOD activity and the MDA content in serum. Furthermore, the COPs ameliorated the abnormal lipid metabolism.
Conclusion: These findings suggest that COPs have a significant protective effect on early alcoholic liver injury in rats.

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1. Introduction

Alcoholism remains a serious health problem worldwide, and it is reported that there are approximately 2 billion alcohol consumers worldwide and 76.3 million with diagnosable alcohol use disorders (WHO, 2004). The excessive and chronic intake of alcohol can lead to alcoholic liver disease (ALD) of which alcoholic fatty liver, alcoholic hepatitis and alcoholic cirrhosis are the most widely diagnosed forms (O'Shea et al., 2010). There are more than 2 million ALD patients estimated in the United States (McClain et al., 2004); in addition, it is reported that patients with alcoholic hepatitis and cirrhosis had greater than 60% mortality over a 4-year period in the Veterans Administration Cooperative Studies (Arteel et al., 2003). Several mechanisms have been identified to contribute to ALD, such as oxidative stress, mitochondrial damage and inflammatory factors (Albano, 2008; Tilg and Diehl, 2000), and increasing evidence now supports the notion that oxidative stress plays a pivotal role in the progression of liver damage (Arteel, 2003; Yuan et al., 2007).

ALD has been well recognized a major cause of morbidity and mortality in the world; yet, other than abstinence, few satisfactory treatments for ALD have been described. Although incomprehensive, it has been reported that several bioactive peptides, including Ganoderma lucidum peptides (Shi et al., 2008) and shark hepatic stimulator substance (Lu et al., 2004), have protective effects against the liver injury induced by D-galactosamine or acetaminophen. Indeed, these hepatoprotective agents found both in plants and animals are currently attracting much attention.

Corn gluten meal (CGM) is a byproduct of the starch industry and contains approximately 60% (w/w) protein (Lin et al., 2011); corn oligopeptides (COPs) are low molecular weight peptides derived from CGM by enzymatic hydrolysis. The various multifunctional properties of COPs have been described previously, such as the inhibition of angiotensin I-converting enzyme (Lin et al., 2011), the alleviation of fatigue (Chang, 2004), resistance to lipid peroxidation (Xu et al., 2002) and the facilitation of alcohol metabolism (Yamaguchi et al., 1997). However, the effect of COPs on alcoholic liver injury has not yet been elucidated.

Therefore, in the present study, the effect of COPs on early alcoholic liver injury was investigated in rats. Liver injury was evaluated by both biochemical parameters and histopathological changes. In addition, as indicators of hepatic lipid peroxidation, we evaluated the levels of MDA and SOD to determine the possible mechanisms.

2. Materials and methods

2.1. Materials

Commercial kits used for the determination of T-SOD and MDA in serum were purchased from the Jiancheng Institute of Biotechnology (Nanjing, China). Ethanol and the other chemicals used were all of analytical grade and purchased from Beijing Chemical Co. (Beijing, China).

2.2. Preparation and identification of COPs

The COPs, which were prepared from CGM, were donated by CF Haishi Biotechnology Ltd. Co. (Beijing, China). Briefly, after being ground through a 60-mesh sieve, the CGM was suspended in distilled water (1:10, w/w) and then hydrolyzed at pH

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http://dx.doi.org/10.1016/j.fct.2012.03.083

11.0 and 90 °C for 1 h. The suspension was neutralized and centrifuged to recover the insoluble protein precipitate, which was then resuspended and subjected to the same procedure above to produce the wet corn protein isolate (CPI). The wet CPI was resuspended to a concentration of 6% (w/w) and subjected to a two-step enzymatic hydrolysis. The first step, with crude alkaline proteinases, was performed at pH 8.5 and 55 °C for 3 h. The second step, with crude neutral proteinases, was performed at pH 7.0 and 45 °C for 2 h. The resulting hydrolysates were centrifuged to remove the impurities, and the supernatant was filtered successively through 10 and 1 kDa MWCO ceramic membranes. A procedure of nanofiltration was then performed to remove the mineral salts, and the purified liquid was condensed by cryoconcentration under a vacuum at 70 °C with an evaporation rate of 500 kg/h. When the concentration was almost 30 Baume degrees, it was decolored with 12% active carbon at 75 °C for 1 h, and then the carbon was removed by filtration. Most of the water was removed by spray drying with a pressure of 20 MPa. This scheme produced the COP powder that was used in the following procedures.

The COP sample contained approximately 91.61% hydrolyzed protein, 5.56% ash, 0.82% carbohydrate, 1.49% water and 0.52% fat. The molecular weight distributions of the COPs were determined using an HPLC system (LC-20AD, Shimadzu, Kyoto, Japan) and a TSK-gel 2000 SWXL column (7.8 × 300 mm, Tosoh, Tokyo, Japan), according to the method of Kong et al. (2008). The HPLC was performed with the mobile phase (45% acetonitrile with 0.1% TFA, v/v) at a flow rate of 0.5 ml/min. A molecular weight calibration curve was prepared from the average retention times of the following standards (Sigma, St. Louis, MO, USA): cytochrome C (12.5 kDa), aprotinin (6.5 kDa), bacitracin (1450 Da), tetrapeptide GGYR (451 Da) and tripeptide GGG (189 Da). In addition, a total amino acid analysis was conducted using an amino acid analyser (835–50, Hitachi, Tokyo, Japan), according to the method of Yang et al. (2007). The results indicated that 96.77% of the peptides in the COPs were distributed below 1000 Da and that the average molecular weight in the COP mixture was 363 Da. The average molecular weight of the amino acids was 137 Da, and the mean peptide length was approximately 2.7 residues. The composition of amino acids is shown in Table 1.

2.3. Animals

A total of 70 male Wistar rats, weighing 180–200 g, were obtained from the Animal Service of Health Science Center, Peking University and adapted to the vivarium for 1 week before the treatment began. The conditions consisted of a filter-protected air-conditioned room, with a controlled temperature (25 ± 28 °C), relative humidity (60 ± 5%) and 12-h light/dark cycles (light on between 07:30 and 19:30 h). The animal treatment and maintenance were conducted in complete accordance with the Principle of Laboratory Animal Care (NIH Publication No. 85–23, revised 1985) and the guidelines of the Peking University Animal Research Committee.

2.4. Experimental procedure

All of the rats were fed with a model AIN-93M rodent diet (Vital River Limited Company, Beijing, China), with casein as the main protein source. The animals were randomly assigned to 7 groups, including a normal control group (defined as NC group), 3 alcohol control groups (2.0, 4.0 and 6.0 g/kg/BW ethanol, designated as the LA, MA and HA groups), and 3 COP intervention groups (2.0, 4.0 and 6.0 g/kg/BW ethanol, with 900 mg/kg/BW COP, designated as the CLA, CMA and CHA groups). COP intervention started on the same day when the rats received alcohol. The rats were intragastrically administered alcohol, except for the rats in the NC group, which received an equivalent of distilled water. After 1 h, the rats of the three intervention groups were intragastrically administered COPs, whereas the others received an equal volume of vehicle as a control. The study lasted for 4 weeks. The animal body weights were obtained weekly to determine the effects of the COPs on the body weight and to adjust the injection volumes. At 12 h after the last alcohol treatment, the rats were anesthetized by CO_2 inhalation and then sacrificed. The blood was obtained, and the serum was separated by centrifugation at 3000 rpm for 10 min; portions of the liver for histopathological examination were also obtained.

Table 1
Amino acid composition of corn oligopeptides.

Amino acid	No. of residues/100 residues
Alanine	9.68
Aspartic acid	5.28
Arginine	1.79
Cysteine	0.36
Glutamic acid	24.21
Glycine	1.61
Histidine	1.36
Isoleucine	3.49
Leucine	18.27
Lysine	0.25
Methionine	2.51
Phenylalanine	5.82
Proline	8.29
Serine	4.83
Threonine	2.84
Tryptophan	0.26
Tyrosine	5.51
Valine	3.63

2.5. Histological study

Portions of tissues from the same lobe of the liver in each rat were immediately prepared for frozen sections (10 μm) and Oil Red-O staining and were then observed at 400× magnification under a light microscope (Nikon Y-FL light microscope, Tokyo, Japan).

2.6. Biochemical assays

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) in the serum were detected using an automatic biochemistry analyser (Hitachi 7020, Tokyo, Japan). The SOD activity and the MDA contents in the serum were determined using detection kits, according to the manufacturer's respective protocols.

2.7. Statistical analysis

All of the values were expressed as the means ± SD. Statistical analyses were performed using SPSS for Windows, version 17.0 and the analysis of variance (ANOVA) and post hoc LSD tests. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Histopathological observation

The hepatoprotective effect of COP was confirmed by histopathological examination (Fig. 1). Morphological changes in the liver were observed using the Oil Red-O staining method. There were no pathological changes in the livers in the NC group (Fig. 1a). Alcohol intoxication (Fig. 1b–d) induced the assembly of lipid droplets in the hepatocytes, especially in the HA group (Fig. 1d), and the COP treatment effectively reduced these changes (Fig. 1e–g).

3.2. Effects of COPs on body weight

During the first week, there was no significant difference observed among the different groups. After the second week, the body weights of the CMA and CHA groups had declined significantly ($p < 0.05$), compared to the NC group. From the third week, the body weights of the HA group had reduced markedly ($p < 0.05$), and the COP treatment did not change the body weight notably in comparison to the rats administered alcohol alone (Table 2).

3.3. Serum ALT and AST levels

The serum levels of ALT and AST were used to evaluate the early liver injury induced by the alcohol. The rats intoxicated with alcohol (the MA and HA groups) developed hepatocellular injuries, with a significant change ($p < 0.05$) in the serum ALT activities, which increased by 19.6% and 21.4%, respectively, compared to the NC group. The COP treatment demonstrated protection against alcohol-induced injury by lowering the elevation of the ALT level to the normal level. The ALT level decreased by 15.8% in the CMA group compared to the MA group ($p < 0.05$), whereas a 13.6% reduction was found in the CHA group compared to the HA group ($p < 0.05$), which was similar to the NC group. In contrast, neither alcohol administration nor the COP treatment resulted in significant changes in the serum AST activities compared to the NC group (Table 3).

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