Hepatoprotective effects of dual-coated and uncoated mixture of probiotics in rats

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Probiotics have been used for the treatment of various disorders or as alternative therapies. The stability of dual-coated probiotics is increased in the gastrointestinal environment. The aim of the present study was to evaluate the hepatoprotective effects of dual-coated and uncoated probiotic supplements, following liver injury. Albino Wistar rats were orally treated with probiotics daily and carbon tetrachloride (CCl\textsubscript{4}) was administered on the seventh and eighth days to induce acute liver damage. Hepatoprotective effects were determined by assessment of serum glutamic--oxaloacetic transaminase (SGOT) and serum glutamic--pyruvic transaminase (SGPT) activities, as well as by histopathological examination. The CCl\textsubscript{4}-treated control group showed increased SGOT and SGPT activities as compared with the normal control group. However, treatment with probiotics reduced SGOT and SGPT activities, following CCl\textsubscript{4} administration. Animals treated with probiotics showed reduced liver weight than that in the standard CCl\textsubscript{4} group which did not receive probiotics. Histopathological analysis showed that administration of probiotics minimized liver damage by reducing the level of morphological changes and necrosis. Therefore, probiotics may be effective hepatoprotective agents and should be considered useful for the treatment and prevention of hepatic disorders.

Keywords: carbon tetrachloride; glutamic--oxaloacetic transaminase; glutamic--pyruvic transaminase; probiotics

Introduction

The liver is a vitally important organ that protects the body from various harmful substances and toxic metabolic byproducts.[1,2] Exposure of the liver to xenobiotics and other therapeutic agents has been reported to cause hepatic damage,[3] and the incidence of liver disease continues to increase worldwide.[4]

Carbon tetrachloride (CCl\textsubscript{4}) is widely used to induce acute toxic liver injury in animal models.[5] The toxic effects of CCl\textsubscript{4} are caused by oxidative stress.[4,6,7] Despite the advances in modern medicine, few reliable drugs for liver disorders are available.[8] Therefore, there is a need to find new effective and safe drugs, without notable side effects.

The use of probiotics is considered an effective and safe alternative treatment for hepatotoxicity.[9,10] Probiotics confer general health benefits on the host. Moreover, they contribute to the reduction in the risk of diseases.[11] Probiotics play important roles in the body, such as inhibition of harmful bacteria by lowering the pH of the intestinal environment, improvement of diarrhoea, synthesis of vitamins and lowering of blood cholesterol levels. [12] Several studies have found that probiotics have beneficial effects against intestinal diseases and liver diseases.[12,13]

A recent study reported that dual-coated probiotics show improved viability when exposed to gastrointestinal conditions as compared with non-coated probiotics. The dual-coating offers better protection from heat, pH conditions and moisture.[14,15] Thus, the stability of dual-coated probiotics is increased in the gastrointestinal environment. The purpose of the present study was to examine the hepatoprotective effects of dual-coated probiotics and uncoated probiotics on CCl\textsubscript{4}-induced acute hepatic injury in rats.

Materials and methods

Dual-coated and uncoated probiotics

Dual-coated and uncoated probiotic strains were manufactured and supplied by Cell Biotech Co. Ltd, Korea. The dual-coated and uncoated probiotics contained a combination of three types of live probiotic bacteria, \textit{Lactobacillus acidophilus} (LA), \textit{L. plantarum} (LP) and \textit{Streptococcus thermophilus} (ST), mixed in a 1:1:1 ratio. These probiotics were suspended in phosphate-buffered saline (PBS) and were used for animal experiments.
**Animal experiments**

Thirty male albino Wistar rats (aged 4 weeks) were purchased from Raon Bio (Raon Bio, Korea) and housed in a temperature-controlled animal facility (22 ± 2 °C/humidity 55% ± 5%) under a 12 h light/dark cycle. Food and water were provided ad libitum from the day of the rats’ arrival until the completion of the experiment. The rats were randomly assigned to five groups (six rats per group); Group I served as normal controls and received only PBS; Group II served as CCl₄ controls and received PBS and 1 mL/kg body weight (BW) of CCl₄ per os (p.o.); Group III served as the standard group and received silymarin (100 mg/kg BW p.o.) and CCl₄ (1 mL/kg BW p.o.); Group IV served as a test group and received dual-coated probiotic supplement (10⁹ CFU/kg BW p.o.) and CCl₄ (1 mL/kg BW p.o.); Group V served as another test group and received uncoated probiotic supplement (10⁹ CFU/kg BW p.o.) and CCl₄ (1 mL/kg BW p.o.). We carried out the experiment after a stabilization period of one day. All treatments were administered by oral gavage continuously for nine days. Animals in Groups II–V were administered with CCl₄ on the 7th and 8th day. At the end of the experimental period, on the 10th day, they were sacrificed under mild ether anaesthesia and blood was collected from the animals by cardiac puncture. The serum was separated for determination of biochemical parameters. The liver, spleen, and kidneys from each animal were carefully excised and washed in ice-cold normal saline solution and weighed. Liver tissues were then kept in 10% formalin solution for histopathological studies. Animal studies were performed according to the guidelines for the care and use of laboratory animals issued by Sahmyook University (SYUIACUC 2014-032).

**Biochemical analysis**

The blood samples were allowed to clot for 45 min at room temperature. The serum was separated by centrifugation at 2500 × g for 15 min and utilized for the quantification of serum glutamic–oxaloacetic transaminase (SGOT, E.C. 2.6.1.1) and serum glutamic–pyruvic transaminase (SGPT, E.C. 2.6.1.2), using commercially available assay kits (BioVision Inc., USA), according to the manufacturer’s protocol. Briefly, 100 μL of reaction mixture was added to the serum sample present in each well of a 96-well microplate (Nunc, Denmark), and the mixture was incubated at 37 °C for 60 min. SGOT and SGPT were then quantified at 450 nm and 570 nm, respectively, by using an ELISA plate reader (Molecular Devices, USA). All measurements were calculated using a standard curve.

**Histopathological studies**

Processing of liver tissue for histopathological analysis was performed by following the modified method of Luna,[16] and hematoxylin and eosin (H&E) staining was carried out using the standard protocol.

**Statistical analysis**

The results from the study were expressed as means with standard deviations (±SD). Data were analysed with one-way analysis of variance (ANOVA), followed by Dunnett’s t-test for multiple comparisons. These statistical tests were performed with SPSS for Windows software V.15.0.2 (SPSS, Chicago, IL, USA). Values with p < 0.05 were considered statistically significant.

**Results and discussion**

**Body weight and weight of the liver, spleen and kidneys**

The body weight of Group II was significantly decreased from 161.1 g on day 7 to 149.8 g on day 8, but the body weights in Groups IV and V were almost comparable to those in Group III (Figure 1). The drop in the body weight in Group IV was minimal and the final body weight in this group was higher than that in Group V. Table 1 shows that in Groups IV and V the gain of body weight in rats was not significantly affected by CCl₄ treatment. In particular, Groups IV and III showed very similar gain in body weight (Table 1).

In addition, the assessment of liver and kidney weights showed that the increase in weight in Group II was greater than that in all other groups. Moreover, there was no difference in body, liver and kidney weight gains between Groups III, IV and V. However, the spleen weight gain was less in Groups II–V compared to Group I, and probiotic treatment had no effects against CCl₄ treatment (Table 1).

Thus, the present study showed that CCl₄ caused body weight loss but organ weight gain in the rat model. On the other hand, probiotics (dual-coated probiotics and uncoated probiotics) maintained the body weight and organ weights at almost normal values. These results are similar with other reports on the effect of other probiotics against CCl₄-induced hepatotoxicity.[3,15,17,18]

**Biochemical analysis**

SGOT and SGPT are important hepatic metabolic enzymes that indicate liver damage by xenobiotics or other causes; these enzymes are released from the liver into the blood serum when liver damage occurs. Therefore, the activities of SGOT and SGPT are considered to reflect the degree of liver damage.[19]

The experiments showed statistically significant differences in the levels of SGOT in all groups; however, the levels of SGPT in Groups II–V were not statistically different (Figure 2). The levels of SGOT and SGPT in
Group IV were 151 UI/L and 55 UI/L, respectively. The levels of SGOT and SGPT in Group V were 151.8 UI/L and 51.5 UI/L, respectively, and those in Group II were 206.5 UI/L and 67 UI/L, respectively. Whereas, Group II showed an increase in the activities of SGOT and SGPT as compared to those observed in Group I, Groups IV and V showed a significant reduction in SGOT and SGPT activities compared to those observed in Group II. These results demonstrate that the marker enzymes were released in much lesser quantities in the probiotic groups than in the group treated with CCl4 alone.

**Light microscopy findings**

CCl4 is a hepatotoxin that induces liver injury in rats. The metabolisms of CCl4 by cytochrome P450 can generate trichloromethyl radicals (CCl3 and/or CCl3OO), which can lead to membrane lipid peroxidation and cell necrosis.

The histopathological analysis showed that the livers of rats from Group I had no noticeable histological changes (Figure 3). In contrast, in the livers of all rats from the CCl4-treated groups, there were consistently observed liver morphological changes such as necrosis and cytoplasmic vacuolization. However, in Groups IV and V, there was a marked reduction in these types of liver morphological changes. That is, the histopathological status of the liver was improved in all animals from the probiotic Groups IV and V. Therefore, it could be suggested that the studied probiotics could have an antioxidant and anti-necrotic effect. In Figure 3, the dual-coated probiotic and the uncoated probiotic demonstrated equally effective hepatoprotective potential. However, according to a study on dual-coated probiotics, double coating increases the survival of probiotic bacteria in the intestine. Therefore, dual-coated probiotics may be expected to be more health beneficial and economically viable than uncoated probiotics.

**Final remarks**

A recent in vitro study showed that a dual-coated probiotic supplement was highly resistant to acidic...
environment in the stomach and was more heat stable compared to the non-coated probiotic supplement.\[14\] Another recent in vivo and in vitro study showed that dual-coated LAB (lactic-acid bacteria) have stronger probiotic effects compared to uncoated LAB.\[21\] In in vivo experiments, double-coated live L. plantarum KCTC3928 has demonstrated hypocholesterolaemic effects in mice.\[22\] In the present study, we showed that dual-coated and uncoated probiotic supplements have hepatoprotective effects.

Although the hepatoprotective mechanisms of probiotics were not addressed in this study, potential mechanisms were identified. Probiotics and their products have antioxidant activities and free-radical scavenging properties in vitro and in vivo.\[23,24\] For example, LcZ (L. casei Zhang) may be related to down-regulation of toll-like receptor 4 (TLR4), which may cause inhibition of oxidative stress and tumour necrosis factor alpha (TNF-α). In addition, probiotic products are important mediators of cell growth and differentiation and prevent lipid peroxidation in liver microsomes.\[25,26\] Our findings suggest that dual-coated and uncoated probiotic supplements may exhibit hepatoprotective effects by affecting the oxidative stress levels and TNF-α.

Conclusions

Our findings in Wistar rats as a model demonstrated the possible use of dual-coated and uncoated probiotic supplements (containing probiotic bacteria: L. acidophilus, L. plantarum and S. thermophilus) for the prevention of liver injury. However, the mechanism of probiotic action in relation to hepatoprotective effects needs to be investigated further under various preclinical conditions.

Figure 2. Effects of dual-coated and uncoated probiotics on SGOT (A) and SGPT (B) levels against CCl₄-induced hepatotoxicity in rats. Group I: normal control; Group II: CCl₄ control, Group III: silymarin standard and CCl₄; Group IV: dual-coated probiotics and CCl₄; Group V: uncoated probiotics and CCl₄. Note: Values represent means from six determinations ± SD. a–c Means with different superscripts differ (p < 0.05) by ANOVA.

Figure 3. Histopathological changes in the liver tissue of normal and CCl₄-treated rats. (A) Group I: normal control rats; centrally located and round nuclei and homogeneous cytoplasm (arrows). (B) Group II: CCl₄-treated rats, cell necrosis (arrows). (C) Group III: rats treated with silymarin plus CCl₄. (D) Group IV: rats treated with dual-coated probiotics plus CCl₄. (E) Group V: rats treated with uncoated probiotics plus CCl₄. Note: H&E staining; magnification ×400; bar = 200 μm.
Disclosure statement

No potential conflict of interest was reported by the authors.

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