Antifibrotic Activity of LCC, a Cerebroside of *Lycium chinense* Fruit, in Bile Duct-Ligated Rats

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Abstract — We previously reported that a novel cerebroside, LCC, isolated from the fruits of *Lycium chinense* (Solanaceae), significantly exerted hepatoprotective activity against both the carbon tetrachloride-induced and galactosamine-induced toxicities in primary cultures of rat hepatocytes. In the present study, we further attempted to determine the effect of LCC on hepatic fibrosis in animal model. Hepatic fibrosis was induced in rats by bile duct ligation/scission (BDL) for a period of 5 weeks. Treatment of BDL rats with LCC significantly reduced collagen deposition and the activities of serum alkaline phosphatase and \( \gamma \)-glutamyl transpeptidase. In addition, the LCC treatment of BDL rats significantly preserved the decreased hepatic glutathione as well as the activities of glutathione reductase and catalase in BDL rats. From the results, it can be speculated that LCC might exert antifibrotic activity in rats with BDL, in part, through the preservation of antioxidant enzymes and hepatic glutathione.

Keywords — LCC, Bile duct ligation/scission, *Lycium chinense*, Hepatic fibrosis, Antifibrotic activity, Antioxidative activity

Introduction

Hepatic fibrosis is one of the most frequent lesions in chronic liver diseases and has been characterized by an increased accumulation of extracellular matrix (ECM) protein including collagen (Hui and Friedman, 2003; Friedman, 2004). It is well recognized that hepatic fibrosis has been associated with oxidative stress. That is, lipid peroxidation has been observed in hepatic fibrosis caused by iron overload (Gualdi et al., 1994), ethanol (Kamimura et al., 1992; Niemela et al., 1995), carbon tetrachloride (CCL\(_4\)) (Tsukamoto et al., 1990) and bile duct ligation/scission (Fox et al., 1997; Pastor et al., 1997). Therefore, enhancement of antioxidative defense mechanism has been proposed as a mean to protect against some clinical manifestations of fibrosis (Hernandez-Munoz et al., 1997; Muriel and Moreno, 2004; Loguercio et al., 2007).

In our previous study, we reported that a novel cerebroside isolated from the fruits of *Lycium chinense* (Solanaceae), 1-\( \text{O-} \beta\text{-D-glucopyranosyl-(2S,3R,4E,8Z)-2-N-palmitinoldeca-4,8-diene} \) (LCC) (Fig. 1), significantly exerted hepatoprotective activity against both the CCL\(_4\)-induced and galactosamine-induced toxicities in primary cultures of rat hepatocytes through maintaining the hepatic glutathione (GSH) redox system (Kim et al., 1997, 1999 and 2000). As a continuation of the study, we attempted to determine the antifibrotic activity of LCC in an animal model.

Experimental

Animal model for hepatic fibrosis — Female Wistar rats weighing around 200 g were obtained from the Laboratory Animal Center, Seoul National University. They were kept on standard rat chow with free access to tap water, in temperature- and humidity-controlled animal quarters under a 12-h light-dark cycle. All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of

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Secondary biliary fibrosis was induced by BDL as described previously (Kountouras et al., 1984; Kim et al., 1993). The sham-operated rats and BDL rats served as control groups. LCC was daily given at 10 a.m. per os at a dose of 6.25 and 12.5 mg/kg body weight (in 5% Tween 80) to the BDL rats throughout the experimental period. After 5 weeks of biliary obstruction, sham-operated rats, BDL rats and BDL rats treated with LCC were sacrificed under urethane and blood was collected by heart puncture. A lobe of the liver was rapidly removed for the determination of the activity of antioxidant enzymes and the contents of GSH and 4-hydroxyproline. Liver sections fixed by 10% formalin and frozen were used to determine hepatic collagen content. Serum was obtained for the determination of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT).

**Determination of hepatic collagen content**—The collagen content in liver section was determined by the method of Lopez de Leon and Rojkind (1985), as validated by Jimenez et al. (1985).

**Determination of 4-hydroxyproline content**—The frozen middle lobe of liver (30-50 mg) was lyophilized overnight. This lyophilized tissue was ground into powder and subsequently extracted five times with 2 ml of disopropylether to remove vitamin A. It was then hydrolyzed with 6 N HCl for 20 h at 100°C. The hydrolysate was filtered through a 0.22-μm Millipore filter. The content of 4-hydroxyproline in the liver hydrolysate was determined as described by the method of Jamall et al. (1981).

**Biochemical analysis of blood**—The activity of ALT was measured according to the method of Reitman and Frankel (1957) using an assay kit obtained locally (Youngdong Pharmaceuticals, Seoul, Korea). ALT activity was measured according to the method of Tietz et al. (1983), and GGT activity was determined by the method of Glossman and Neville (1972) using γ-glutamyl-p-nitroaniline as a substrate.

**Determination of GSH content and antioxidant enzyme activity**—The content of GSH was determined by the method of Hissin and Hilf (1976). GSH-transferase (GST), GSH peroxidase (GPx) and GSH disulfide reductase (GR) activities were determined as described by Carlberg and Mannervik (1975); Flohe and Gunzler (1984); Habig et al. (1974), respectively. Catalase activity was measured by the method of Beers and Sizer (1952). Protein concentration was determined by the Lowry method (Lowry et al., 1951).

**Statistical analysis**—All data are expressed as the mean ± S.D. Group means are evaluated for statistically significant differences using analysis of variance (one way ANOVA). Values of p < 0.05 were considered statistically significant differences.

**Results and Discussion**

The BDL rat model has been recognized as a representative experimental model for studying cholestasis and cirrhosis (Kountouras et al., 1984; Mullen and McCullough, 1989; Kim et al., 1993). Dueland et al. (1991) reported that after the BDL for 4 to 5 weeks, liver cirrhosis in rats occurred by the newly formed duct-like structure associated with collagen deposition, mainly type I and III, in liver tissue. It was also reported that cirrhotic liver contained more than 15 mg of collagen per gram tissue than normal tissue and the content of other ECM material was also increased (Rojkind et al. 1983). In this experiment, we found similar morphological changes in BDL rat after 5 weeks of BDL. In addition, BDL results in the increase in spleen weight, an indicator of portal hypertension, and liver weight because of pathological defense mechanism and collagen deposition (Kountouras et al., 1984). We also found similar histological changes in BDL rats after 5 weeks of BDL (Table 1). Weights of liver and spleen were significantly increased in BDL rats compared to the sham-operated control rats (p < 0.001). However, the treatment of BDL rats with LCC at a dose of 6.25 and 12.5 mg/kg body weight/day, respectively, significantly lowered the increased weights of liver and spleen (p < 0.05) in BDL rats. There were no significant differences in body weight between BDL rats and BDL rats treated with LCC.

Excessive production and deposition of ECM such as collagen are important characteristics of hepatic fibrosis (Hui and Friedman, 2003). Thus, we determined collagen deposition by the selective dye-binding method of collagen quantitative assay (Lopez de Leon and Rojkind, 1985). After 5 weeks of BDL, hepatic collagen content in BDL rats increased to 1.2-fold compared to that in sham-operated controls (Table 2). The treatment of BDL rats...