J. Dairy Sci. 97:3281–3291
http://dx.doi.org/10.3168/jds.2013-7505

Lactoferrin protects against chemical-induced rat liver fibrosis by inhibiting stellate cell activation

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ABSTRACT

Liver diseases, which can be caused by alcohol abuse, chemical intoxication, viral hepatitis infection, and autoimmune disorders, are a significant health issue because they can develop into liver fibrosis and cirrhosis. Lactoferrin (LF), a siderophilic protein with 2 iron-binding sites, has been demonstrated to possess a multitude of biological functions, including antiinflammation, anticancer, and antimicrobial effects, as well as immunomodulatory-enhancing functions. In the current study, we induced hepatotoxicity in rats with dimethylnitrosamine (DMN) to establish a situation that would enable us to evaluate the hepatoprotective effects of LF against hepatic injury. Our results showed that DMN-induced hepatic pathological damage significantly decreased the body weight and liver index, increased the mRNA and protein levels of collagen α-1(I) (ColIα-1) and α-smooth muscle actin, and increased the hydroxyproline content. However, treatment with LF significantly increased body weight and liver index, decreased the mRNA and protein levels of ColIα-1 and α-smooth muscle actin, and suppressed the hydroxyproline content when compared with the DMN-treated group. Liver histopathology also showed that low-dose LF (100 mg/kg of body weight) or high-dose LF (300 mg/kg of body weight) could significantly reduce the incidences of liver lesions induced by DMN. These results suggest that the LF exhibits potent hepatoprotection against DMN-induced liver damage in rats and that the hepatoprotective effects of LF may be due to the inhibition of collagen production and to stellate cell activation.

Key words: lactoferrin, liver fibrosis, dimethylnitrosamine, hepatic stellate cells

INTRODUCTION

According to statistics tabulated by the Department of Health in Taiwan, chronic liver disease and cirrhosis constituted the ninth leading cause of death in 2010. Recent studies show that alcohol abuse, chemical intoxication, viral hepatitis infection, and autoimmune disorders contribute to chronic liver fibrosis, which often advances to liver cirrhosis (Friedman, 2003; Kisseleva and Brenner, 2006). Liver cirrhosis is generally irreversible, and treatment usually focuses on preventing progression and complications. In advanced stages of cirrhosis, the only option is a liver transplant.

Dimethylnitrosamine (**DMN**), a family of N-nitrosamine compounds, is a potent hepatotoxin, carcinogen, and mutagen. Dimethylnitrosamine causes liver necrosis, fibrosis, and cirrhosis through the metabolic activation of cytochrome P450 2E1 (Guengerich et al., 1991). Activation of liver cytochrome P450 2E1 stimulates Kupffer cells to generate reactive oxygen species, thus leading to liver cell damage (Teufelhofer et al., 2005). The injured liver cells release several cytokines that cause further liver damage. Therefore, DMN-induced liver fibrosis presents a valuable animal model for studying the mechanisms of hepatic fibrosis that may facilitate the rapid screening of antifibrotic agents.

The DMN-induced liver fibrosis model closely resembles the development of liver damage in humans, which includes ascites, nodular regeneration, overproduction of the extracellular matrix (including collagen), histopathological manifestations, and biochemical alterations (George and Chandrakasan, 2000; Bataller and Brenner, 2005). Lee et al. (2013) also noted that DMN intoxication inhibited the growth of rats; damaged liver function; activated transforming growth factor beta 1

Received September 20, 2013.
Accepted December 28, 2013.
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or Smad signaling; increased the expression of α-smooth muscle actin (**α-SMA**), matrix metalloproteinase-2, and collagen; and relieved hepatic fibrogenesis. Thus, the rat model of DMN-induced liver injury, which displays similar characteristics to human patients based on clinical observations, biochemical alterations, and histopathological determinations, has been studied to gain insight into the underlying mechanisms and to discover potential therapeutic interventions.

Lactoferrin (**LF**), an siderophilic protein with 2 iron-binding sites, is mainly found in exocrine secretions, such as breast milk, tears, saliva, and biliary tracts, with the highest concentration (5–7 mg/mL) being found in colostrum (Levay and Viljoen, 1995). Lactoferrin has been reported to have a wide range of biological activities, including antiinflammatory, anticancer, antimicrobial, antioxidant, and immunomodulatory-enhancing effects (Yamauchi et al., 1998; Hayashida et al., 2004; Ishikado et al., 2005). Lactoferrin has been demonstrated to have protective effects against cutaneous inflammation, colitis, and rheumatoid arthritis by inhibiting the levels of the proinflammatory cytokines tumor necrosis factor-α and IL-1b and stimulating the expression of the antiinflammatory cytokine IL-10 (Cumberbatch et al., 2000; Togawa et al., 2002). Lactoferrin has also been demonstrated to possess anti-inflammatory activity, as it can directly modulate cytokine production by immune cells, such as macrophages and lymphocytes, through receptor-mediated signaling pathways (Van Snick and Masson, 1976; Mazurier et al., 1989). Lactoferrin can also downregulate inflammatory responses by preventing iron-catalyzed free radical damage at inflammation sites (Chodaczek et al., 2007). In the current study, we induced hepatotoxicity in rats with DMN to establish a situation that would enable us to evaluate the hepatoprotective effects of LF against hepatic injury induced by DMN in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (aged 8 wk and weighing 271 ± 12 g) were purchased from the Livestock Research Institute (Taipei, Taiwan), given a standard laboratory diet (Altromin, Lage, Germary) and distilled water ad libitum, and kept on a 12 h light-dark cycle at 21 to 27°C (Liu et al., 2013). This animal study was approved by the Institutional Animal Care and Utilization Committee of National Chung Hsing University (IACUC Approval No: 100–104). In the current study, we induced hepatotoxicity in rats with DMN (Sigma, St. Louis, MO) to evaluate the hepatoprotective effects of LF from bovine milk, the purity approximately 90% (Sigma; L9507). For the examination of both aspartate aminotransferase and alanine aminotransferase activities in the serum, 24 rats were randomly assigned to 4 groups (n = 6). The normal control group received 0.90% wt/vol NaCl by gastric gavage plus a 0.90% wt/vol NaCl injection. The DMN group received 0.90% wt/vol NaCl by gastric gavage plus a DMN injection. The low-dose LF (**LF-L**) group received oral administration of LF at 100 mg/kg of BW by gastric gavage (concentration = 100 mg of LF/1 mL of 0.90% wt/vol NaCl; volume = 1 mL of LF/1 kg of BW) plus a DMN injection. Finally, the high-dose LF (**LF-H**) group received oral administration of LF at 300 mg/kg of BW by gastric gavage (concentration = 300 mg of LF/1 mL of 0.90% wt/vol NaCl; volume = 1 mL of LF/1 kg of BW) plus a DMN injection. The DMN-induced hepatotoxic rats were injected i.p. with 10 mg/kg of DMN (3 consecutive days each week) for 4 wk, whereas rats in the normal control group were injected with 0.90% wt/vol NaCl only. Ochoa et al. (2008) observed no adverse effects of children that received 1 g of LF for 6 d/wk for 9 mo. Thus, in the current study, we simulate the diet of LF to treat the mice (300 mg/kg of BW of mice = 33.294 mg/kg of human = 33.294 × 30 mg/30 kg of children = 998.82 mg/30 kg of children). At the end of the experiment, BW, liver index (liver-to-BW ratio), and spleen index (spleen-to-BW ratio) were recorded. Accordingly, each rat was anesthetized, and the liver was immediately perfused with ice-cold 0.90% wt/vol NaCl, then carefully removed, rinsed in ice-cold 0.90% wt/vol NaCl, blotted dry, and weighed. All samples were stored at −80°C for further assays.

High-Frequency Ultrasound Examination

The animals fasted for 3 h before high-frequency ultrasound (**HFU**) scanning. During the surgical procedures, animals were lightly anesthetized with gas consisting of 0.5 to 1.0 L/min of oxygen-enriched air mixed with 2.0 to 2.5% isoflurane vapor. The animals were placed in supine positions and were able to breathe freely. After being anesthetized, each rat abdomen was shaved and further cleaned with a chemical hair remover to minimize ultrasound attenuation. For ultrasound imaging, the Vevo 770 micro-imaging system (VisualSonics Inc., Toronto, Canada) with a single element probe at a center frequency of 40 MHz was used for small animal examinations. The Vevo 770 ultrasound probe has a 40-MHz center frequency with a 6-mm focal depth, providing an axial resolution of 40 μm with a 14.6-mm field of view. Ultrasound gel was placed on the skin as a coupling fluid before using the transducer (Chen et al., 2012b).