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Ciliary Neurotrophic Factor Analogue Aggravates CCl₄-induced Acute Hepatic Injury in Rats

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Abstract

Ciliary neurotrophic factor (CNTF) and CNTF analogs were reported to have hepatoprotective effect and ameliorate hepatic steatosis in *db/db* or high fat diet-fed mice. Since hepatic steatosis and injury is also commonly induced by hepatotoxin, the aim of the present study is to clarify whether CNTF could alleviate hepatic steatosis and injury which induced by carbon tetrachloride (CCl₄). Unexpectedly, when combined with CCl₄, CNTF aggravated hepatic steatosis and liver injury. The mechanism is associated with effects of CNTF which inhibited lipoproteins secretion and drastically impaired lipoproteins ability to act as transport vehicles for lipids from the liver to the circulation. While injected after CCl₄-cessation, CNTF could improve liver function. These data suggest that CNTF could be a potential hepatoprotective agent against CCl₄-induced hepatic injury after the cessation of CCl₄ exposure. But it is forbidden to combine rhmCNTF treatment with CCl₄.

Keywords: ciliary neurotrophic factor (CNTF); carbon tetrachloride (CCl₄); fatty liver; liver injury; lipid peroxidation.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by the triglyceride (TG) accumulation within hepatocytes and ranges from simple steatosis to steatohepatitis, which often progresses into advanced fibrosis and cirrhosis (Finelli and Tarantino 2012). Liver has an important role in detoxification and is a crucial target of the toxicity of drugs and xenobiotics (Jaeschke et al. 2002). Liver damage due to facultative hepatotoxins is scarcely foreseeable. NAFLD conveys a nearly fourfold increase of drug-induced liver injury risk in obese middle-aged patients (Tarantino et al. 2007).

Previous reports have suggested that ciliary neurotrophic factor (CNTF) or CNTF analogs ameliorated the metabolic abnormalities (Liu et al. 2007; Rezende et al. 2012) including fatty liver in obese mice (Cui et al. 2010; Sleeman et al. 2003). The therapeutic effect is accompanied by improved liver function and antioxidant ability. In order to further clarify the underlying mechanisms of CNTF against obesity-related hepatic steatosis and whether CNTF could relieve the liver injury which induced by hepatotoxin, the present study induced acute hepatic injury by carbon tetrachloride (CCl₄) in rats and investigated the effects of a recombinant mutant of human CNTF (rhmCNTF).

CNTF activates leptin-like pathways and reduces body fat (Lambert et al. 2001). But excessively high doses of CNTF can signal through receptors for related cytokines, such as IL-6 (Schuster et al. 2003) which can induce stress responses and cachexia (Lambert et al. 2001; Schooltink et al. 1992) and is strongly associated with fatty liver and steatohepatitis

(Tarantino et al. 2009). Taking into account the immune adverse effects of CNTF, a safe and effective dose of rhmCNTF, 0.1 mg/kg/d, was used in the present study.

Materials and Methods

Animals and drug. Twenty-five male SPF Wistar rats (9-week-old) were obtained from Slac Inc. (Shanghai, China), housed in standard environmental conditions, and had free access to tap water and standard rodent chow. All animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals (CCAC 1993). RhmCNTF in the form of a freeze-dried powder injection was a gift from Lanzhou Institute of Biological Products (Lanzhou, China). The purity of this protein is higher than 95%.

Experimental design. As show in Figure 1. Hepatic injury model rats were induced by subcutaneous (s.c.) injection of 40% (v/v) of CCl₄ in corn oil three times (3, 2, and 2 ml/kg, respectively) in one week and randomly divided into 4 groups of 5 rats each. Rats were s.c. injected with vehicle or rhmCNTF 0.1 mg/kg/d for one week after or along with CCl₄-treatment. At the end of the experiment, all rats were fasted overnight and anaesthetized. Blood was collected from the abdominal aorta and the serum samples were taken to determine lipids and biochemical markers of liver function with an automatic biochemistry analyzer. Serum total antioxidant capacity (T-AOC) was detected according to the manufacturer's protocol. All kits were products of Jiancheng Bioengineering Institute (Nanjing, China). One part of the liver was kept at -20°C for determination content of triglyceride (TG. Kit was product of Sichuan Maker Biotechnology Co., Ltd. Sichuan, China). Another part of the liver

was fixed in 10% formalin and ready for hematoxylin and eosin (H&E) staining.

Statistical analysis. The data are expressed as mean $\pm$ S.D. Statistical comparisons between groups were analyzed by a two-tailed Student's *t*-test. $P < 0.05$ was considered significant.

Results and Discussion

CCl₄-induced liver injury is a commonly used experimental model for hepatoprotective drug studying (Tlili et al. 2016; Wu et al. 2016). In the present study, CCl₄ s.c. injection three times a week induces marked hepatic steatosis and injury (Figure 2). The significant decrease of serum T-AOC (Figure 3) demonstrated that CCl₄ inhibited the antioxidant capacity in rats. CCl₄ damaged hepatocyte and inhibited lipoproteins secretion which drastically impaired lipoproteins ability to act as transport vehicles for lipids from the liver to the circulation (Boll et al. 2001). In this study, hepatic total protein (TP) content is positively correlated with serum TG level and negatively correlated with hepatic TG level. Serum lipids and hepatic TP content were both dramatically reduced by CCl₄, which may be part of the causes of CCl₄-induced hepatic lipid accumulation in rats.

CNTF analogs have been reported to have hepatoprotective and therapeutic effects on fatty liver in obese mice (Cui et al. 2010; Sleeman et al. 2003). In present study, rhmCNTF treatment combined with CCl₄, unexpectedly, aggravated hepatic steatosis and injury which induced by CCl₄. The rats showed obviously increased hepatic TG content, upward of serum biochemical markers of liver function, and downward trend of liver TP and serum lipids level.

One week after CCl₄-cessation, the degree of hepatic steatosis was attenuated obviously. Increased serum liver enzymes were also recovered but still much higher than that of control rats. These results showed that liver function which was damaged by CCl₄ was partly improved after CCl₄-cessation one week later. The recovery of liver TP production led to a large amount of lipids, especially cholesterol, transferred from liver to peripheral blood. As a result, serum lipids level was increased while liver lipids accumulation was attenuated. Serum TC, HDL-C, and LDL-C levels even rebounded and were much higher than that of control group. Different from combined with CCl₄, rhmCNTF injected for a week after cessation of CCl₄ did not result in aggravation of hepatic steatosis and could further improve liver function to near normal condition.

Antioxidative action plays an important role in protection against CCl₄-induced liver injury (Choudhury et al. 2016). Recently, it's suggested the antioxidative, anti-inflammatory properties of Maresin 1 in CCl₄-induced liver injury. The possible mechanism is partly associated with its abilities to inhibit reactive oxygen species (ROS) production and activation of NF- κ B and MAPK pathway (Li et al. 2016). Besides, adhering to the hypothesis that the excess free fatty acids lead to the increase in ROS generation and mitochondrial damage is balanced by the presence of antioxidant substances, circulating levels of cytochrome c, triglycerides and unconjugated bilirubin (Tarantino et al. 2011). In this study, regardless of injection for one week combined with CCl₄ or after CCl₄-cessation, rhmCNTF could significantly increase serum T-AOC which was obviously inhibited by

CCl₄. These findings indicate that rhmCNTF enhances systemic antioxidant capacity and protects rats against CCl₄-induced oxidative stress. However, the effect of rhmCNTF on hepatic antioxidant activity needs further study.

In conclusion, the present study suggests that rhmCNTF has protective effect against CCl₄-induced hepatic injury after CCl₄-cessation. But hepatic steatosis and injury could be aggravated when combine rhmCNTF treatment with CCl₄.

Conflict of interest

The authors have no conflict of interest to disclose.

Acknowledgments

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Figure legends

Figure 1. Diagram of the experimental protocol. ▲#: subcutaneous (s.c.) injection with 40% CCl₄ 3 ml/kg. ▲: s.c. injection with 40% CCl₄ 2 ml/kg. ●: s.c. injection with rhmCNTF 0.1 mg/kg/d.

Figure 2. Effect of rhmCNTF on hepatic steatosis and abnormal liver function in CCl₄-intoxication rats. (A) H&E staining (×100), vacuoles are lipid droplets. (B) The degree of hepatic steatosis was evaluated by light microscopy and graded as follows: absent (no hepatocyte steatosis), mild (present in < 30% hepatocytes), moderate (30–60% hepatocytes), or severe (> 60% hepatocytes). (C and D) Hepatic content of triglyceride (TG) and total proteins. (E) Serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). (F) Serum lipids level. *n* = 5 in each group. **P* < 0.05, ***P* < 0.01. Group 1 to 5 were normal control, rats were injected vehicle or rhmCNTF for a week after CCl₄-cessation, and vehicle or rhmCNTF combined with CCl₄ for a week, respectively.

Figure 3. Effect of rhmCNTF on serum total antioxidant capacity (T-AOC) in CCl₄-intoxication rats. *n* = 5 in each group. **P* < 0.05, ***P* < 0.01. Group 1 to 5 were normal control, rats were injected vehicle or rhmCNTF for a week after CCl₄-cessation, and vehicle or rhmCNTF combined with CCl₄ for a week, respectively.

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119x68mm (300 x 300 DPI)

Figure 2. Effect of rhmCNTF on hepatic steatosis and abnormal liver function in CCl₄-intoxication rats. (A) H&E staining ($\times 100$), vacuoles are lipid droplets. (B) The degree of hepatic steatosis was evaluated by light microscopy and graded as follows: absent (no hepatocyte steatosis), mild (present in $< 30\%$ hepatocytes), moderate ($30\text{--}60\%$ hepatocytes), or severe ($> 60\%$ hepatocytes). (C and D) Hepatic content of triglyceride (TG) and total proteins. (E) Serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). (F) Serum lipids level. $n = 5$ in each group. * $P < 0.05$, ** $P < 0.01$. Group 1 to 5 were normal control, rats were injected vehicle or rhmCNTF for a week after CCl₄-cessation, and vehicle or rhmCNTF combined with CCl₄ for a week, respectively.

157x137mm (300 x 300 DPI)

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70x71mm (300 x 300 DPI)