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Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats

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ABSTRACT

Background & Aims: Recent studies have revealed that ghrelin may be an antioxidant and antiinflammatory agent. Oxidative stress are considered to play a prominent causative role in the development of various hepatic disorders. We investigated whether ghrelin plays a protective role against carbon tetrachloride (CCl_4)-induced acute liver injury in rats.
Methods: Forty adult male Sprague–Dawley rats were randomly divided into four equal groups as; control, ghrelin, CCl_4 and ghrelin plus CCl_4. Evaluations were made for lipid peroxidation, enzyme activities and biochemical parameters. Pathological histology was also performed.
Results: CCl_4 treatment increased plasma and liver tissue malondialdehyde (MDA) content and plasma nitric oxide (NO) level, and decreased erythrocyte and liver tissue superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities when compared to control group. At the same time, CCl_4 treatment increased the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alcaline phosphatase (ALP) activities. By contrast, ghrelin pretreatment reduced plasma and liver MDA content and plasma NO level, and increased erythrocyte and liver tissue SOD, CAT and GPx activities when compared with CCl_4-treated group. Moreover, both ghrelin alone and ghrelin plus CCl_4 treatment elevated serum glucose level. The CCl_4-induced histopathological changes were also reduced by the ghrelin pretreatment.
Conclusion: Our results show that ghrelin can be proposed to protect the liver against CCl_4-induced oxidative damage in rats, and the hepatoprotective effect may be correlated with its antioxidant and free radical scavenger effects.

1. Introduction

Ghrelin, a 28 amino acid peptide, mainly synthesized by rat and human stomach, and recognized as the main endogenous ligand for the growth hormone secretagogue receptor (GHSR) [1]. Ghrelin is secreted primarily from the stomach, but ghrelin transcripts have been founded in many other organs, including the liver, kidney, lung, pancreas, hypothalamus, heart, suggesting an endocrine as well as extra-endocrine action of ghrelin [1,2].

Previous reports have indicated that ghrelin has beneficial effects on gastrointestinal, cardiovascular, reproductive, immune and coagulation systems [3–8]. Recent studies have revealed that ghrelin may be an antioxidant and anti-inflammatory agent. In this context, Kheradmand et al. [9] reported that ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in the rat ovary. Obay et al. [10] have shown that ghrelin prevents lipid peroxidation and reduction of antioxidant enzyme activities and glutathione level against pentylenetetrazole-induced oxidative stress in the erythrocytes, liver and brain of rats. Similarly, Xu et al. [11] demonstrated that ghrelin significantly reduced the concentration of malondialdehyde and increased the activity of antioxidant enzymes such as superoxide dismutase and catalase in primary cultured cardiomyocytes. In addition, Moreno et al. [12] showed that ghrelin may attenuate fibrosis by exerting a hepatoprotective effect. Several hormones such as growth hormone, melatonin and estrogen have been reported to reduce CCl_4-induced hepatotoxicity [13–15]. However, whether ghrelin reduces the lipid peroxidation or increases the activity of antioxidant enzymes in CCl_4-induced acute liver injury of rats is unknown. Therefore, the aim of this study was to investigate the possible protective effect of ghrelin using measurement of the activities of antioxidant enzymes (SOD, CAT and GPx) and oxidative stress markers (MDA and NO), and detecting the alteration of hepatic microscopy in CCl_4-induced acute liver injury of rats.

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2. Materials and methods

2.1. Animals

Forty male Sprague–Dawley rats weighing 280–300 g were used in this study. They were obtained from the Experimental and Clinical Research Center of Erciyes University, Kayseri, Turkey. Rats were housed in a continuously ventilated room at a mean temperature of 22 ± 2 °C with a lighting period of 12 h dark and 12 light. Throughout the study, the animals had free access to standard pellet rat chow and drinking water. The experiments were performed in accordance with Guide for the care and use of Laboratory Animals (National Research Council, 1996). The protocol of this study was approved by Ethic Committee, University of Erciyes.

2.2. Experimental design

Ghrelin (Sigma Chemical, St. Louis, MO) was dissolved in 0.9% physiological saline and injected subcutaneously at a dose of 10 ng/kg body weight according to previous report [16]. Acute liver injury was induced in rats through a single intraperitoneal injection of CCl_4 (Merck; diluted 1:1 with corn oil) at a dose of 1.6 g/kg body weight as described previously [17]. The rats were randomly divided into four groups containing ten rats each and were treated as follows. The control group was given 1 ml of physiological saline, subcutaneously, daily for 5 days. The CCl_4 group was treated with a single intraperitoneal injection of CCl_4. The Ghrelin group was injected subcutaneously with ghrelin daily for 5 days. The Ghrelin plus CCl_4 group was pretreated with ghrelin daily for 5 days prior to treatment with a single dose of CCl_4.

2.3. Collection and processing of blood and tissue samples

Animals were anesthetized with ketamine/xylazine mixture 24 h after the last injection and blood was collected by cardiac puncture. The blood samples collected into heparinized and nonheparinized tubes, and then heparinized tubes were centrifuged at 5000 rpm for 10 min for the separation of plasma and sera. The lower erythrocyte layer in the heparinised tubes was washed three times with phosphate buffered saline (pH:7.4) and diluted with an equal volume of the indicated solution. Next the erythrocytes were hemolysed with ice-cold distilled water (1:5) [18]. Immediately after blood collection, the animals were sacrificed by an overdose of pentobarbitone. The liver of the animals was promptly excised and used to determine the level of lipid peroxidation and antioxidant enzyme activities, and for further histopathological study. The excised tissues were washed with deionized water for the removal of blood. Homogenization was performed in a phosphate buffer solution with a pH value adjusted to 7.4, and later the supernatant was separated by means of centrifugation at 20,000 rpm for 1 h. The supernatant and hemolysate obtained were used for the analyses of SOD, GPx and CAT. Furthermore, erythrocyte haemoglobin, plasma MDA and NO levels were measured. The serum obtained was used for the analyses of certain biochemical parameters (AST, ALP and ALT activities, and glucose, TP and TG levels).

2.4. Determination of tissue protein and erythrocyte haemoglobin levels

Tissue protein content was determined by the method of Lowry et al. [19] and modified by Miller [20]. Erythrocyte haemoglobin level was assessed according to the method of Fairbanks and Klee [21]. Measurements were performed spectrophotometrically. (Shimadzu UV-1700).

2.5. Determination of oxidative stress markers and serum biochemical parameters

Plasma MDA level was estimated by the method of Yoshioka et al. [22]. Tissue MDA level was assessed according to the method of Ohkawa et al. [23]. The measurements were performed using a spectrophotometer.

Plasma NO level was determined in accordance with the Griess method [24]. Tissue and erythrocyte superoxide dismutase activity was measured as described by Sun et al. [25], catalase activity was determined as described by Luck [26] and glutathione peroxidase activity was measured according to Paglia and Valentine [27] with minor modifications. Serum glucose, total protein (TP) and triglyceride (TG) level, and AST, ALT and ALP activities were measured with a spectrophotometer (Shimatzu UV-1700) using commercial assay kits (Chema, Italy) according to the manufacturer's direction.

2.6. Histological evaluation

Histopathological evaluation was made in liver tissues. For light microscopic examinations, liver samples were fixed in 10% neutral buffered formalin solution. The tissues were embedded in paraffin. The paraffin blocks were cut in 5 μm thick. The sections were stained with Hematoxylin-Eosin (H&E).

2.7. Statistical analysis

Statistical analyses were carried out using SPSS 12.0 for Windows statistical package (SPSS Inc., Chicago, IL). Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $p<0.05$ was considered statistically significant. All values were expressed as mean values ± standard deviation (SD).

3. Results

3.1. Effect of ghrelin on oxidative stress markers in plasma and liver tissue

The plasma MDA and NO content and tissue MDA level were significantly increased ($p<0.05$) in the liver tissue of animals receiving a single injection of CCl_4 compared to controls. Ghrelin pretreatment prior to CCl_4 injection significantly prevented these elevations (Table 1,2).

Table 1
Plasma MDA and NO levels and erythrocyte SOD, CAT and GSH-Px activities in the control and experimental groups.

Groups	MDA (nmol/ml)	NO (nmol/ml)	SOD (U/mgHb)	CAT (k/gHb)	GPx (μmol NADPH+H^+/min/gHb)
Control	10.51 ± 0.61	215.23 ± 36.07	1.19 ± 0.15	70.31 ± 13.08	32.18 ± 5.68
Ghrelin	10.99 ± 0.81	207.01 ± 26.59	1.22 ± 0.12	79.50 ± 11.13	31.20 ± 3.14
CCl_4	17.70 ± 1.85[a]	297.17 ± 35.36[a]	0.73 ± 0.15[a]	22.72 ± 6.71[a]	18.16 ± 2.53[a]
Ghrelin + CCl_4	11.63 ± 1.88[b]	221,80 ± 37.19[b]	1.26 ± 0.10[b]	67.28 ± 7.80[b]	35.90 ± 6.89[b]

[a] Significantly different from the control group ($p<0.05$).
[b] Significantly different from the CCl_4 group ($p<0.05$).