Life Sciences 190 (2017) 91–96

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Protective effect of chlorogenic acid on the inflammatory damage of pancreas and lung in mice with L-arginine-induced pancreatitis

Tatsuya Ohkawara[a,*], Hiroshi Takeda[a], Jun Nishihira[b]

[a] Pathophysiology and Therapeutics, Hokkaido University, Faculty of Pharmaceutical Sciences, Kita-12, Nishi-6, Kita-ku, Sapporo 060-8638, Japan
[b] Department of Medical Management and Informatics, Faculty of Business Administration and Information Science, Hokkaido Information University, Nishinopporo, 59-2, Ebetsu 069-8585, Japan

ARTICLE INFO

Keywords:
Chlorogenic acid
Cytokines
Macrophage migration inhibitory factor
Pancreatitis

ABSTRACT

Aims: Pancreatitis is characterized by inflammatory disease with severe tissue injury in pancreas, and the incidence of pancreatitis has been recently increasing. Although several treatments of acute pancreatitis have been developed, some patients have been resistant to current therapy. Chlorogenic acid (CGA) is one of the polyphenols, and is known to have an anti-inflammatory effect. In this study, we investigated the effects of CGA on experimental pancreatitis in mice.

Materials and methods: Pancreatitis was induced by twice injection of L-arginine (5 g/kg body weight). Mice were intraperitoneally injected with CGA (20 mg/kg or 40 mg/kg) 1 h before administration of L-arginine.

Key findings: Administration of 40 mg/kg of CGA decreased the histological severity of pancreatitis and pancreatitis-associated lung injury. Moreover, administration of CGA inhibited the levels of pancreatic enzyme activity. Interestingly, CGA reduced the serum and pancreatic levels of macrophage migration inhibitory factor (MIF) in mice with L-arginine-induced pancreatitis.

Significance: Our results suggest that CGA has an anti-inflammatory effect on L-arginine-induced pancreatitis and pancreatitis-associated lung injury.

1. Introduction

Pancreatitis is characterized by the inflammatory infiltration and tissue injury of pancreas, and the incidence of pancreatitis has been recently increasing [1]. Furthermore, severe acute pancreatitis often leads to necrotizing pancreatitis and lethal multiple organ failures [1]. In fact, about 30% of patients with acute severe necrotizing pancreatitis and its complication die [2]. Although various treatments for acute pancreatitis such as proteinase inhibitors have been used, some patients are resistant to current therapies. Thus, the fatality rate of severe pancreatitis has been still high [3]. The mechanism underlying the development of pancreatitis has been well investigated. Several cytokines such as IL-8 contribute to development of pancreatitis and pancreatitis-associated complication [4–6]. Recently, in vivo study has indicated that blockade of chemokine bioactivity ameliorates the severity of pancreatic injury in experimental pancreatitis [7].

Chlorogenic acid (CGA) is one of the polyphenols, and it is abundant in a variety of plant foods such as coffee bean and apple [8]. Interestingly, CGA has anti-inflammatory, anti-cancer and anti-oxidant properties [9–11]. CGA suppresses the production of proinflammatory cytokines in vitro [12–14]. In vivo study, CGA has protected against inflammation and tissue injury in rheumatoid arthritis, hepatitis and colitis [15–17]. Although the benefit of CGA is well-investigated in many kinds of inflammatory diseases, the effects of CGA on pancreatitis have not been examined. In this study, we tested the effect of CGA on acute pancreatitis using a mouse model of L-arginine-induced pancreatitis. A mouse model of L-arginine-induced pancreatitis is well known as a mimic of clinical pancreatitis, and is commonly used to evaluate the effects of products [18,19].

2. Materials and methods

2.1. Animals and grouping

Eight week-old male C57B6 mice (20-24 g) were obtained from CLEA Japan Inc. (Tokyo, Japan). Mice were bred and housed in standard cages in a climate-controlled room with comfortable temperature (23 ± 2 °C) and a 12 hour light-dark cycle. All animal procedures were conducted according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee under an approved

* Corresponding author.
E-mail address: tokawara@pharm.hokudai.ac.jp (T. Ohkawara).

http://dx.doi.org/10.1016/j.lfs.2017.09.015
Received 19 July 2017; Received in revised form 30 August 2017; Accepted 12 September 2017
Available online 15 September 2017
0024-3205/ © 2017 Elsevier Inc. All rights reserved.

protocol.

Mice were randomly divided into 3 groups: non-treated normal group, phosphate buffered saline (PBS)-pretreated and L-arginine-treated group, CGA-pretreated and L-arginine-treated group. In histological evaluation of pancreas tissue, doses of 20 and 40 mg/kg of CGA were used. In the other experiments, 40 mg/kg of CGA was used for evaluation and analyses.

2.2. Administration of chlorogenic acid

Mice were treated by intraperitoneal injection of 20 or 40 mg/kg of CGA (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 mL of PBS solution (Sigma-Aldrich, St. Louis, MO, USA) 1 h before initial administration of L-arginine (Sigma-Aldrich, St. Louis, MO, USA). Mice injected with only PBS (0.1 mL) were served as control.

2.3. Induction of L-arginine-induced pancreatitis

Acute pancreatitis was induced by L-arginine as described previously with minor modification [19]. Mice were intraperitoneally injected with L-arginine (5 g/kg body weight) hourly for 2 h. Seventy two hours after injection of L-arginine, mice were sacrificed and pancreatic and lung tissues were removed, and blood samples were collected. Blood samples were coagulated at 4 °C overnight, and serum samples were separated from blood samples after centrifugation at 3000*g* for 15 min. Pancreas tissues were weighed and homogenized in PBS solution with protein cocktail inhibitor (Roche Diagnostics, Indianapolis, IN, USA). The homogenates were centrifuged at 22,000*g* for 10 min at 4 °C, and then the supernatants were collected. The protein concentrations in the supernatants were measured with a Micro BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) in accordance with protocols. Separated supernatants and serum samples were stored at − 20 °C in the freezer until analyses.

2.4. Histological evaluation of pancreatitis and lung injury

Samples of pancreas and lung tissues were fixed in 10% neutral buffered formalin solution, and embedded in paraffin blocks. Thin slice samples of pancreas and lung were stained with hematoxylin and eosin (H & E). Histological examination was performed at a light microscope. Histological severity of pancreatic damage were scored in interstitial edema, inflammatory infiltrate and acinar cell necrosis using scoring system on a scale of 0–3 (0: none, 1: mild, 2: moderate, 3: severe) as described previously [19]. Histological evaluation of pancreatitis-associated lung injury was also performed using scoring system as described previously [19]. Microscopic findings of lung sections were scored in alveolar thickness and inflammation on a scale of 0–3 (0: none, 1: mild, 2: moderate, 3: severe).

2.5. Measurement of serum lipase and amylase activities

Lipase and amylase activities in serum were determined with the QuantiChrom lipase/amylase activity kits (BioAssay Systems, Hayward, CA, USA) according to protocol.

2.6. Measurement of macrophage migration inhibitory factor, interleukin-6 and macrophage inflammatory protein-2

In the supernatants isolated from pancreatic tissues and serum samples, the levels of interleukin-6 (IL-6) and macrophage inflammatory protein-2 (MIP-2) were measured with Milliplex MapMouse Cytokine/Chemikine Magnetic Bead Panel kit according to manufacturer's instructions. Furthermore, the level of macrophage migration inhibitory factor (MIF) in these samples was measured with MIF-ELISA kit (USCN Life science, Wuhan, China) according to protocol.

2.7. Myeloperoxidase staining in lung in L-arginine-induced pancreatitis

A slice of tissue cells on a slide glass was deparaffinized and incubated with an antigen activator (Nichirei Science, Tokyo, Japan) at 95 °C for 20 min. After washing with Tris buffered saline (TBS) solution, the sample was incubated with 0.3% H_2O_2 at room temperature (RT) for 10 min. Then, the sample was incubated with an anti-myeloperoxidase (MPO) antibody (dilution 1:300) at 4 °C overnight following twice TBS washing. After twice washing, sample was incubated with Histfine simplestain MAX-PO® (Nichirei Bioscience, Tokyo) at RT for 30 min. The sample was reacted with 3,3′-Diaminobenzidine Tetrahydrochloride at RT for 5 min, and was stained with hematoxyline. Numbers of MPO-positive staining cells were counted in high power field (HPF) at a microscope.

2.8. Statistical analysis

All results were shown as average ± standard error. In results from histological scores, Mann-Whitney *U* test was performed. In the other results, one-way analysis of variance (ANOVA) was performed followed by Bonferroni's post hoc test. Values were considered as significant at $p < 0.05$.

3. Results

3.1. Effect of CGA on histological findings in L-arginine-induced pancreatitis

At 72 h after initial injection of L-arginine, none of mice died in all groups. Histological findings were evaluated in the pancreas tissue of mice with L-arginine-induced pancreatitis (n = 10 in each groups). Administration of L-arginine induced the moderate to severe pancreatitis in mice. Histological findings showed the acinar cell necrosis, edema and inflammatory infiltrate in mice with L-arginine-induced pancreatitis (Fig. 1A). Remarkable increases of histological scores for edema (1.9 ± 0.2), inflammatory infiltrate (1.9 ± 0.2) and acinar cell necrosis (0.7 ± 0.2) were observed in the pancreas tissues of mice with L-arginine-induced pancreatitis (Fig. 1B). On the other hand, 40 mg/kg of CGA treatment significantly reduced the histological scores for edema (0.8 ± 0.1), inflammatory infiltrate (1.1 ± 0.1) and acinar cell necrosis (0.1 ± 0.1) in the pancreas tissues of mice (Fig. 1A and B). In mice treated with 20 mg/kg of CGA, histological findings of pancreatic damage seemed to be reduced, but the histological scores were not statistically significantly decreased (edema;1.4 ± 0.2, inflammatory infiltrate; 1.3 ± 0.2, acinar cell necrosis; 0.3 ± 0.2) (Fig. 1A and B).

3.2. Effect of CGA on the levels of serum lipase and amylase activities in L-arginine-induced pancreatitis

In lipase and amylase activities in serum, administration of L-arginine increased the levels of both activities in the serum from mice compared to those in normal mice (Table 1). Administration of CGA significantly suppressed the increases of these activities in mice with L-arginine induced pancreatitis (n = 5 in each groups) (Table 1).

3.3. Effect of CGA on increase of serum and pancreatic tissue levels of IL-6, MIP-2 and MIF in L-arginine-induced pancreatitis

The levels of IL-6, MIP-2 and MIF were markedly increased in the levels of serum and pancreatic tissue of mice with acute pancreatitis induced by L-arginine (Tables 2 and 3). In contrast, increases of the serum and pancreatic levels of IL-6, MIP-2 and MIF were significantly suppressed by CGA treatment in the mice compared to PBS-treated mice 72 after injection of L-arginine (Tables 2 and 3).

Download English Version:

https://daneshyari.com/en/article/5556726

Download Persian Version:

https://daneshyari.com/article/5556726

Daneshyari.com