Original Research Article

Colchicine protects against acute pancreatitis via down-regulation of cytokine levels

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Abstract

Purpose: To investigate the effect of colchicine on acute pancreatitis (AP) in a rat model, and the molecular mechanism involved.
Methods: Acute pancreatitis (AP) was induced in rats by injection with 5 % sodium taurocholate. Changes in histology of pancreatic tissues were determined following treatment with colchicine. Serum amylase activity was measured using Automated Biochemistry Analyser.
Results: Hematoxylin and eosin (H & E) staining showed that colchicine prevented histopathological changes such as infiltration of interstitial leukocytes and erythrocytes, cell necrosis, oedema formation and vacuolization in the rat pancreas. Treatment of AP rats with colchicine significantly and dose-dependently decreased ascite volume in the abdominal cavity. Serum amylase activity was significantly suppressed in AP rats on treatment with 100 mg/kg colchicine. Furthermore, treatment of the AP rats with colchicine caused marked decrease in the expressions of interleukin 6 and tumor necrosis factor α, and upregulated expressions of IL 10 in serum. Colchicine treatment of AP rats also caused significant increase in CGRP level in the plasma.
Conclusion: Colchicine prevents pancreatic tissue damage induced by AP by down-regulating pro-inflammatory cytokines, upregulation of anti-inflammatory cytokines, and enhancing CGRP release. Therefore, colchicine may be useful for the treatment of acute pancreatitis.

Keywords: Colchicine, Acute pancreatitis, Anti-inflammation, Calcitonin, Interleukin

INTRODUCTION

Acute pancreatitis (AP) is characterised by inflammation of pancreatic tissues. The incidence of AP has increased from 13 to more than 45 patients per 100,000 people [1,2]. It is generally a mild disorder, but approximately 30 % of the patients develop severe AP which leads to tissue necrosis, impairment of the barrier between intestines, and bacterial infection which eventually cause multiple organ failure [3]. The degree of death in patients with severe AP is above 25 % [3]. Although the mechanism of AP is not fully understood, it is believed that the disease is associated with

disturbance in microcirculatory system and secretion of inflammatory mediators [4].

The main factor that contributes to AP is disturbance in microcirculatory system of the pancreas caused due to several factors [5]. Pancreatic tissue damage leads to release of various pro-inflammatory cytokines from the injured cells [6]. The higher production of cytokines leads to multiple organ failure through increased disturbance in microcirculatory system [4]. The available therapies for AP generally act by suppression of pancreatic secretions and prevention of secondary injuries. However, the use of these therapies is limited because of various side effects and patient non-compliance, thereby increasing the incidence of the disease [7]. The understanding of the molecular mechanism involved in AP, and development of effective treatment strategies for the disease pose serious challenges to researchers in medical and biomedical sciences.

It has been reported that secretion of calcitonin gene-related peptide (CGRP) after vanilloid receptor subtype 1 (VR1) activation has an important role in the treatment of AP [8]. The sensory nerves which express capsaicin receptor known as VR1 are densely distributed in several organs and play vital role in the regulation of resistance to peripheral vascular system [9]. The activated VR1 secretes higher levels of various neurotransmitters, including CGRP [10]. Studies have demonstrated that CGRP administration offers protection against pancreatic injury [11].

Colchicine is an alkaloid used for the treatment of various diseases like familial Mediterranean fever, Sweet's syndrome and liver cirrhosis [12]. Colchicine has shown very impressive results in the treatment of mucosal ulcers and arthritis [13]. It reduces skin stiffness during scleroderma and improves proteinuria in amyloidosis [14]. Colchicine treatment is very effective against the familial Mediterranean fever which induces acute inflammatory episodes [15]. In the present study, the effect of colchicine on AP, and the molecular mechanism involved were investigated in a rat model of AP.

EXPERIMENTAL

Animals

Male Sprague-Dawley rats (n = 50; mean weight = 230 ± 40 g) were provided by the Animal Laboratory of Shandong University (Jinan, China). The rats were housed under controlled conditions of temperature (25°C) and humidity (50%) with 12-h day/12-h night cycle. The animals were provided access to feed and water. The experimental protocols involving animals were performed in accordance with the guidelines issued by Care and Use of Laboratory Animals National Institute of Health standards (revised in 1996) [16]. The study received approval from the Committee for Research Ethics of Central South University, Changsha, China (CSU/17.0019).

Chemical and reagents

Sodium taurocholate (45 % solution) and dimethyl sulfoxide (DMSO). were obtained from the Sigma-Aldrich (St. Louis, MO, USA).

Establishment of rat model of AP and treatment

The rats were assigned randomly to 5-groups: normal control, untreated group, and three colchicine treatment groups (30, 50 and 100 mg/kg). Colchicine was given to the rats through sublingual vein l h before the surgery. The rats in normal control and untreated groups received a mixture of DMSO and ethyl alcohol (1:4, v:v) instead of colchicine. In order to induce AP, the rats were fasted for 12 h and subjected to anesthetization using 3 % solution of pentobarbital sodium. Disinfection was carried out using conventional procedure consisting of initial washing with 2 % iodine inunction, followed cleaning twice with ethyl alcohol. A 2-cm incision was made on the abdominal cavity of each rat along the white line. Then, 4.5% solution of sodium taurocholate was administered through the cholangiopancreatic duct using laparotomy. Rats in normal control group were given normal saline instead of sodium taurocholate. The incision was sutured using silk thread under sterilized conditions, and the rats were placed in sterilized cages. After 48 h, the rats were sacrificed, and arterial blood samples were collected, and pancreatic tissues were excised. The blood samples were subjected to centrifugation at 1,500 x g for 10 min at 4°C, and the resultant serum samples were kept at a temperature of -20°C. The pancreas were fixed in phosphate-buffered formaldehyde (4 %) and kept at 4°C for histopathological analysis.

Measurement of ascite volume

The rats were sacrificed 48 h post-surgery, and their abdominal cavities were opened. Then, 5-mL syringes were used to remove ascites from the abdomen and transfer into measuring

cylinders. The volume of ascites were recorded using graduated 100-mL measuring cylinders.

Histopathological examination

The pancreatic tissues excised from the rats 48 h post-surgery were fixed in formaldehyde and subsequently paraffin-embedded. The tissues were cut into thin 3-μm sections and heated in xylene, followed by dehydration in ethyl alcohol. Then, the tissue sections were rinsed with PBS, and subjected to staining for 5 min in hematoxylin and then treated with eosin for 2 min. The tissue sections were blindly examined for histopathological changes by pathologists under the light microscope. The pathological changes in the tissues were scored according to the established criteria.

Determination of amylase activity

The blood sample collected from aortic artery of the rats after 48 h of surgery was subjected to centrifugation at 1,500 x g for 10 min at 4°C, and the serum samples were obtained. Measurement of amylase activity in the rat serum was performed using completely-automatic Biochemical Analyser (Hitachi, Ltd., Tokyo, Japan) in accordance with the manufacturer's instructions.

Determination of cytokine levels

The serum levels of various cytokines in the rats 48 h after surgery were determined with ELISA assay. The pancreatic tissues were subjected to homogenization using ice-cold solution of homogenization buffer [HEPES (12 mM; pH 7.8), potassium chloride (12 mM), magnesium chloride (2.2 mM), EDTA (0.2 mM), dithiothreitol (1.2 mM) and phenyl methylsulphonylfloride (0.6 mM)]. The tissue homogenates were subjected to centrifugation at 4°C for 20 min at 3,000 x g, and the supernatants were kept at -78°C. Commercially available ELISA test kits were used to determine the levels of IL-6 (cat. no. KRC0061; BioSource Europe SA, Nivelles, Belgium), and IL-10 and TNF-α (Diaclone, Besançon, France) in the homogenates.

Western blot analysis

The protein concentrations in the pancreatic tissue lysates were determined using bicinchoninic acid (BCA) kit (Sigma-Aldrich). The separation of proteins was achieved on SDS-PAGE and the separated proteins were subsequently transferred onto polyvinylidene membranes. The membranes were subjected to incubation overnight at 4 °C with rabbit polyclonal primary antibodies against IL-6, IL-10 and TNF-α (dilution 1:1,000; Cell Signaling Technology, Danvers, MA, USA). After washing twice with Tris-buffered saline and Tween 20, the membranes were incubated for 1 h with horseradish peroxidase conjugated secondary antibody (dilution 1:1,000) at room temperature. The bands of proteins were visualized using enhanced chemiluminescence reagent (Amersham Pharmacia Biotech, Tokyo, Japan).

Determination of CGRP level

The level of CGRP was measured in the serum of rats collected 48 h post-surgery. The blood samples were subjected to centrifugation at 4°C for 45 min at 1,200 x g. Radioimmunoassay kit was used for measurement of CGRP level in the rat serum in accordance with the instructions of the manufacturer.

Statistical analysis

Data are presented as mean ± standard deviation, and were analysed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). Differences were determined statistically using Student's t-test and one-way analysis of variance. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

Effect of colchicine on histopathological changes in rat pancreas

The histopathological changes in pancreatic tissues of the AP rats treated with colchicine are shown in Figure 1. The pancreatic tissues of rats in AP group showed evident histopathological changes such as infiltration of interstitial leukocytes and erythrocytes, cell necrosis, oedema formation and vacuolization. In the pancreas of sham rats, no such histopathological changes were observed. Treatment of AP rats with colchicine prevented AP-induced histopathological changes in the pancreatic tissues in a dose-based manner (Figure 2). The pancreatic tissues of AP rats showed histological features similar to those of the sham group on treatment with colchicine at a dose of 100 mg/kg. Histopathological changes in the pancreas of AP rats were completely prevented on treatment with colchicine at a dose of 100 mg/kg. AP rats treated with colchicine at doses of 30 and 50 mg/kg showed significant accumulation of leukocytes as well as erythrocytes, oedema formation and vacuolization in the pancreatic tissues.
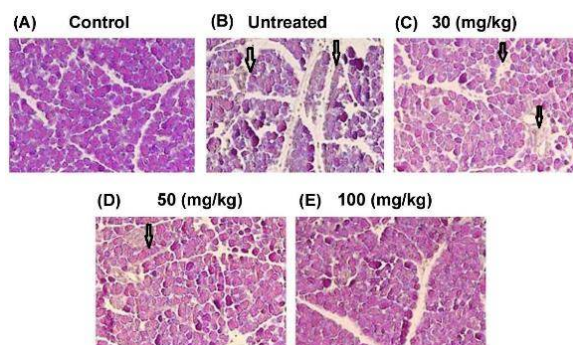
Figure 1: Effect of colchicine on AP-induced histopathological changes in rat pancreas. The AP rats were kept untreated or treated with colchicine at doses of 30, 50 and 100 mg/kg. The rats were then sacrificed and pancreatic tissues were excised for examination of histopathological changes. (A) Sham; (B) untreated group; (C) 30 mg/kg colchicine treatment group; (D) 50 mg/kg colchicine treatment group, and (E) 100 mg/kg colchicine treatment group (x 200 magnification)
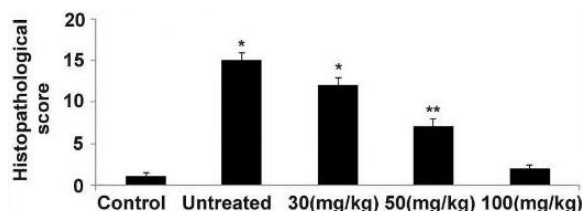

Figure 2: Effect of colchicine on AP-induced histopathological changes in rat pancreas. The AP rats were either untreated or treated with colchicine at doses of 30, 50 and 100 mg/kg. The rats were then sacrificed to excise the pancreatic tissues for examination of histopathological changes. Histological scores in different rat groups were quantified. The values are expressed as mean ± SD of three independently examined regions; *$p < 0.05$ and **$p < 0.02$ vs. untreated AP rats

Effect of colchicine on ascite volume and activity of serum amylase in AP rats

In the AP rat group, abdominal cavity showed very large ascite volume, and serum amylase level was significantly increased, when compared to the sham group (Figure 3). However, treatment of the AP rats with colchicine significantly decreased ascite volume in the abdominal cavity in a dose-dependent manner. Colchicine at a dose of 100 mg/kg markedly inhibited development of ascites in the abdominal cavity, and significantly suppressed serum amylase activity in the AP rats.

Effect of colchicine on inflammatory cytokine levels in AP rats.

Acute pancreatitis (AP) significantly increased the serum levels of interleukin-6 and tumor

necrosis factor-α in the rats, when compared to the sham group (Figure 4). Treatment of the AP rats with colchicine caused marked decreases in the expression of interleukin-6 and tumor necrosis factor-α. Colchicine treatment at the dose of 100 mg/kg reduced interleukin-6 and tumor necrosis factor-α levels comparable to those of the sham group. The level of IL-10 in the AP rats was decreased significantly on treatment with colchicine, when compared to the sham group ($p < 0.05$). Moreover, colchicine increased the expression of IL-10 in the serum. The AP rats treated with colchicine at doses of 30 and 50 mg/kg showed lower serum level of IL-10 than those in the sham group. Treatment of the AP rats with colchicine at a dose of 100 mg/kg increased the level of IL-10 close to that in the sham group. These findings suggest that colchicine treatment decreases inflammatory cytokine levels and promotes anti-inflammatory cytokine level in the serum of AP rats.
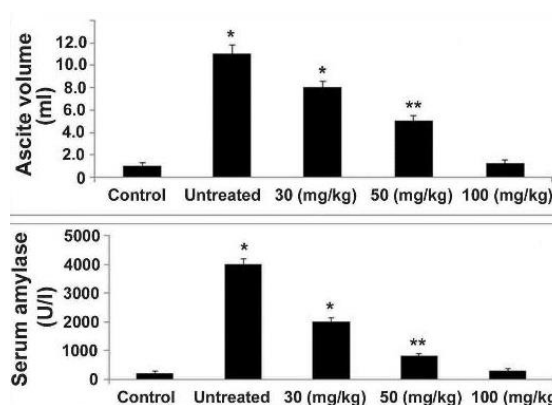

Figure 3: Effect of colchicine on ascite volume and serum amylase activity in AP rats. (A) Ascite volume, and (B) serum amylase activity in the AP rats were measured after treatment with colchicine at doses of 30, 50 and 100 mg/kg; *$p < 0.05$; **$p < 0.02$ vs. untreated AP rats
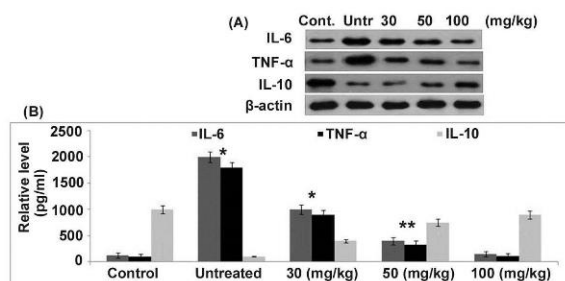

Figure 4: Effect of colchicine on serum IL-6, TNF-α and -IL-10 in the AP rats. After AP-induction, the rats were either untreated or treated with colchicine at doses of 30, 50 and 100 mg/kg. (A) IL-6, TNF-α and IL-10 levels in the serum of rats were assayed with western blotting. (B) Densitometric analysis of the results; *$p < 0.05$; **$p < 0.02$ vs. untreated AP rats

Effect of colchicine on CGRP in AP rat plasma

The level of CGRP in the plasma of AP rats was significantly ($p < 0.05$) lower than that of the sham group (Figure 5). Colchicine treatment of the AP rats caused significant increase in the CGRP level in the plasma in a dose-based manner. Treatment of the AP rats with colchicine at a dose of 100 mg/kg increased the plasma CGRP to a higher level than that due dose of 30 or 50 mg/kg.
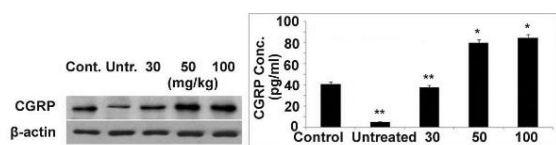

Figure 5: Effect of colchicine on CGRP in AP rat plasma. The AP rats were either untreated or treated with colchicine at doses of 30, 50 and 100 mg/kg, and then CGRP level was assayed in the plasma; *$p < 0.05$; **$p < 0.02$ vs. untreated AP rats (CGRP: calcitonin gene-related peptide)

DISCUSSION

The present study has demonstrated the effect of colchicine on a rat model of AP and investigated the molecular mechanism involved. The study revealed that colchicine prevents AP through down-regulation of interleukin-6 and tumor necrosis factor-α, up-regulation of interleukin-10, and enhancement of CGRP levels. Acute pancreatitis (AP) occurs frequently, and the morbidity of AP patients has increased markedly over the last decade. The disease is worsened by non-availability of effective treatment strategies. The high morbidity and non-availability of the treatment strategies require investigation of the mechanism of AP at the molecular level so that novel and effective treatments can be developed.

Disorders in the microcirculatory system play an important role in the pathogenesis of AP by damaging focal tissues, leading to oedema and cell necrosis [17]. The injured tissues of the pancreas release various types of pro-inflammatory cytokines which mediate the process of AP [6]. Development of AP causes induction of injuries to multiple organs through the aggravation microcirculatory disorders [17]. It has been reported that CGRP acts as a promising vasodilator and is associated with the regulation of blood flow in the regional organs [18]. Up-regulation of the CGRP level through VR1 activation has great significance in the blood flow regulation in peripheral organs [9,17].

The nerve fibres densely distributed throughout the pancreas of mammals have shown immunoreactivity for CGRP [19]. Studies have shown that the development of AP is inhibited by the stimulation of sensory nerves or CGRP [11]. The present study demonstrated that treatment of AP rats with colchicine inhibited inflammation and cell necrosis. The colchicine treatment caused reductions in ascite volume and serum amylase activity, while it increased plasma CGRP. These findings provide evidence that colchicine prevents pancreatic tissue damage in AP rats by promoting CGRP level. It is known that mediators of inflammation which cause disturbance in the microcirculatory system, are major factors in the pathogenesis of AP [6,17].

Secretion of pro-inflammatory cytokines by leukocytes causes damage to the pancreatic tissues [20]. The damage due to inflammatory cytokines is diminished by the secretion of IL-10, which is an anti-inflammatory molecule [21]. Studies have shown that CGRP exhibits its inflammation-inhibitory effect by down-regulating the expressions of IL-6, TNF-α and nuclear factor-κB [22]. Besides, CGRP enhances the level of inflammation-suppressing cytokine, IL-10 [22]. In the current study, colchicine treatment of AP rats down-regulated the levels of IL-6 and TNF-α, and increased the level of IL-10. The level of CGRP was also increased in the plasma of the AP rats on treatment with colchicine.

CONCLUSION

Colchicine prevents pancreatic tissue damage caused by AP by down-regulation of pro-inflammatory cytokines, promotion of anti-inflammatory cytokines and enhancement of CGRP release. Therefore, colchicine can potentially be used for the treatment of AP. However, more investigation is required to fully understand the mechanism of colchicine-mediated prevention of AP.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Nan Zhang,

Meng Liang contribute to this work equally. Zhanbiao Yu designed the study and wrote the paper. Nan Zhang, Meng Liang, Kuo Wang, Tao Sun, Xiaoxu Ding and Yan Zhou performed the experimental work. Nan Zhang, Meng Liang and Kuo Wang carried out the literature study and compiled the data. Tao Sun, Xiaoxu Ding and Yan Zhouper formed literature survey, analyzed and compiled the data. All the authors approved the research article for publication.

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