Pancreatology 13 (2013) 347–354

Contents lists available at SciVerse ScienceDirect

Pancreatology

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

Original article

Effects of curcumin on proinflammatory cytokines and tissue injury in the early and late phases of experimental acute pancreatitis

Ahmet Gulcubuk [a,*], Damla Haktanir [a], Aris Cakiris [b], Duran Ustek [b], Ozlem Guzel [c], Muzaffer Erturk [d], Murat Karabagli [c], Ibrahim Akyazi [e], Hayriye Cicekci [f], Kemal Altunatmaz [c], Hafize Uzun [f], Kenan Ates [b]

[a] Department of Pathology, Faculty of Veterinary Medicine, Istanbul University, 34320 Avcilar, Istanbul, Turkey
[b] Department of Genetics, The Research Institute for Experimental Medicine, Istanbul University, 34093 Istanbul, Turkey
[c] Department of Surgery, Faculty of Veterinary Medicine, Istanbul University, 34320 Avcilar, Istanbul, Turkey
[d] Deparment of Gastroenterology, Gaziantep American Hospital, 27300 Gaziantep, Turkey
[e] Department of Physiology, Faculty of Veterinary Medicine, Istanbul University, 34320 Avcilar, Istanbul, Turkey
[f] Deparment of Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, 34098 Cerrahpasa, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
15 April 2013
Accepted 8 May 2013

Keywords:
Acute pancreatitis
Curcumin
Tissue injury
Proinflammatory cytokine
Rat

ABSTRACT

Background & aims: Acute pancreatitis (AP) varies from mild to severe necrotizing changes with high mortality. The objective of the current study was to investigate the effects of curcumin on tissue injury and proinflammatory cytokines in the early and late phases of AP.
Methods: AP was induced by sodium taurocholate in rats ($n = 140$). First group was left untreated. Group II received 100 mg/kg curcumin daily starting 20 days before AP induction. The rats were allocated into 7 sub-groups (n:5) and were sacrificed at 2, 6, 12, 24, 72, 144 and 288 h following the induction of AP. Blood and pancreatic tissue samples were collected for biochemical and histopathologic evaluations and the assessment of protein and mRNA levels, as well.
Results: Curcumin decreased total histopathologic scores in comparison with those of the taurocholate group ($P < 0.05$). Curcumin increased Caspase-3 activity and decreased trypsin activity, while inhibited nuclear factor-κ (NF-κB) at all time points ($P < 0.05$) and moreover reduced activator protein-1 (AP-1). Curcumin decreased chemokine (except for 288 h), TNF-α (except for 2 and 24 h), IL-6 (except for 2, 6 and 288 h) and iNOS (except for 144 and 288 h) mRNA levels ($P < 0.05$). Curcumin serum nitric oxide (NO) (except for 144 and 288 h) levels were reduced, as well.
Conclusions: In conclusion, curcumin reduced tissue injury, trypsin activation and inhibited NF-κB and AP-1. However TNF-α, IL-6 and iNOS and NO were not inhibited at all time points. Therefore no direct correlation was detected in the subgroups between tissue injury, proinflammatory cytokines and oxidative enzymes.

1. Introduction

Acute pancreatitis is a pathological condition ranging from mild to severe necrotizing forms [1,2]. The most common form is a mild, self-limiting pancreatitis which resolves spontaneously within a few days. However, severe hemorrhagic and necrotizing pancreatitis requiring intensive treatment is seen in 25% of cases with high mortality rate between 30 and 50% [1]. In severely damaged pancreatitis, death occurs due to pancreatic autodigestion associated with intracellular trypsinogen activation of zymogen granules followed by the activation of other digestive enzymes as the result of acinar cell injury [1,3]. Such injury in pancreatic acinar cell causes local and systemic inflammatory reactions which lead to systemic inflammatory response, multiple organ dysfunction and acute respiratory distress syndromes [1]. Free oxygen radicals [4] and proinflammatory cytokines [1,5] which are released by neutrophil leukocytes and macrophages during acute pancreatitis exacerbate the inflammatory response by causing an increase in local and systemic capillary permeability and by promoting leukocyte adhesion and extravasation [2,6]. Studies have demonstrated increased interleukin-1β, IL-6, IL-8 TNF-α levels in both sera and tissues of patients with AP [2–10].

* Corresponding author. Tel.: +90 212 4737070x17084; fax: +90 212 4737241.
E-mail addresses: gulcu@istanbul.edu.tr, agulcubuk@yahoo.com (A. Gulcubuk).

1424-3903/$ – see front matter
http://dx.doi.org/10.1016/j.pan.2013.05.005

The cellular mechanism of proinflammatory cytokines is regulated by nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). NF-κB and AP-1 are two transcriptional complexes required for the early response gene expression of inflammatory molecules [8,11,12]. NF-κB and AP-1 regulate many gene expressions which participate in immune and inflammatory responses. NF-κB is found in an inactive form in the cell cytoplasm attached to inhibitor protein kappa B (IκB). When activated, IκB is phosphorylated by specific IKK kinases and rapidly degraded though proteasome-dependent pathways [8,11]. The other transcription factor, AP-1 is a homo- or heterodimer complex molecule formed from Jun, Fos or the activating transcription factor subunits [12]. AP-1 is also involved in tissue proliferation, differentiation and transformation as observed in adult tissues and plays a key role in the regulation of the inflammatory process. AP-1 can be activated by growth factors, cytokines, chemokines, hormones and multiple environmental factors [13].

Curcumin is a yellow-colored substance derived from turmeric (*curcuma longa*) and has commonly been in use as a spice in the eastern cuisine for thousands of years. Its curative/medicinal effects on diseases have been known for ages [14]. Studies conducted on curcumin for the last few decades have demonstrated antioxidant [15,16], anti-inflammatory [8,9,11,17], antifibrotic [18], apoptotic [14,19] and anticancerogenic [14,19,20] feautures of curcumin. Curcumin decreases the production of proinflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, TNF-α, IL-12, through inhibition of NF-κB, AP-1, JAK-kinase, cyclooxygenase and lipoxygenase [2,20,21].

Studies evaluating the effects of curcumin in acute pancreatitis are still considered novel. Five experimental studies [8, 9, 15, 22, and 23] and one clinical trial [24] have been conducted so far. Two of them were carried out by us [9,15]. In above studies except for ours, the pathological alterations were investigated 6 h after induction of a mild edematous pancreatitis by cerulein [22,23], cerulein and ethanol + cholecystokinin (CCK) [8]. However, their effects in the late phase of AP were not investigated. The objective of this study was to investigate the effects of curcumin on tissue injury, oxidative injury, proinflammatory cytokines and pancreatic enzymes (trypsin, amylase, lipase) in AP, at seven different time points (2 h, 6 h, 12 h, 24 h, 72 h, 144 h and 288 h).

2. Material and method

2.1. Animals

A total of 140 male Wistar-Albino rats, weighing approximately 250–350 g were used in our study. The animals were inbred by the Department of Laboratory Animals in the Institute of Experimental Medical Research, Istanbul University. They were kept at room temperature throughout the study under a 12 h light–dark cycle and fed on commercial pellet diet containing 21% protein and received tap water ad libitum. The study was approved by the Ethics Committee of Istanbul University.

2.2. Experimental groups:

Each group contained 35 animals and 7 subgroups were formed, each including five animals. AP was induced by infusion of 0.1 ml of 3% sodium taurocholate solution (Sigma, T-9034) freshly prepared under sterile conditions by being dissolved in 0.9% physiologic saline, into the biliopancreatic ducts of the animals in Group I (taurocholate group) and Group II (curcumin group). Curcumin (Sigma C-1386) was freshly prepared at a dose of 100 mg/kg containing 1 ml of active substance by being dissolved in 9% ethanol. Group II rereceived this solution once a day via an intragastric tube starting 20 days before induction of AP [15]. Daily administration of curcumin was maintained until the last day of the study (288 h after the induction of AP). Meanwhile, Group III (Alcohol Control Group) received 1 ml of 9% ethanol for the same period. Animals in Group IV (sham-operated) were subjected only to the surgical procedure and no further treatment was carried out.

2.3. Induction of pancreatitis

After a short-term diethyl ether anesthesia, the abdominal regions of the animals in Group I, Group II and Group IV were shaved, disinfected and prepared for surgery. Then the rats were subjected to inhalation anesthesia by isoflurane, which was initiated at a concentration of 5% and maintained with 2%. The surgical procedure and the induction of AP were performed according to the method described in our previous study [15]. Five animals from each subgroup were sacrificed at seven different time points (2, 6, 12, 24, 72, 144 and 288 h) following the induction of AP. The animals were sacrificed under prolonged anesthesia after intracardiac blood samples were collected and then necropsy was performed.

3. Laboratory tests

3.1. Biochemical analysis

Serum amylase and lipase levels were measured on the day of sacrifice. Spectrophotometric measurements of amylase and lipase

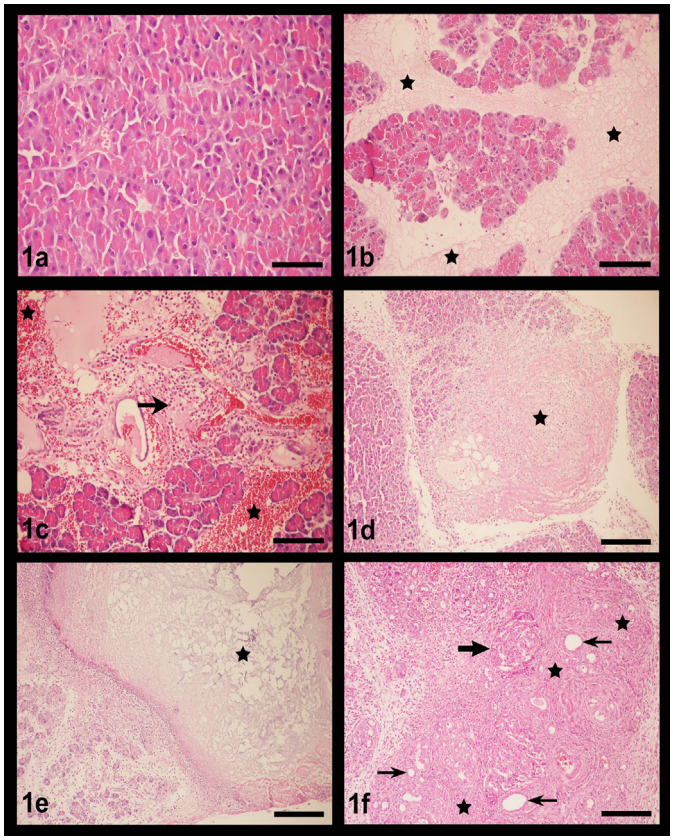

Fig. 1. a Sham operated group. HE. Bar:100 μm. b Lobules were separated from each other as a result of severe edema (stars) in pancreas. 6 h. HE. Bar:100 μm. c Prominent hemorrhage (stars) in interglandular area in pancreas and leukocyte infiltration (arrow). 12 h. HE. Bar:100 μm. d Prominent coagulation necrosis in pancreatic tissue (star). 24 h. HE. Bar:200 μm. e Severe fat necrosis starting from peripancreatic tissue extending into pancreas, the middle part is appearing slightly blue (star) and liquefactive areas developed accordingly and leukocyte infiltrations. 144 h. HE. Bar:200 μm. f Fibrosis occurred as a result of tissue destruction in pancreas (stars), duct-like acini with the loss of zymogen granules (fine arrows) and remaining normal islet cells (bold arrow). 288 h. HE. Bar:200 μm. (b–f) are the histolopathologic images of the taurocholate group.