Life Sciences 87 (2010) 64–68

Contents lists available at ScienceDirect

Life Sciences

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

Protective effect of cyclosporin A on brain injury in rats with acute necrotic pancreatitis

Nian-Song Qian [a], Xiao-Ping Xu [b], Yong Chen [a], Ke-Feng Dou [a], Ya-Yun Wang [c,*], Yan-Ling Yang [a,*]

[a] Department of Hepatobiliary Surgery, Xi-Jing Hospital, Fourth Military Medical University, Xi'an 710032, People's Republic of China
[b] Department of General Surgery, Zhu-Jiang Hospital, South Medical University, Guangzhou 510282, People's Republic of China
[c] Department of Anatomy and K.K. Leung Brain Research Centre, Fourth Military Medical University, Xi'an 710032, People's Republic of China

ARTICLE INFO

Article history:
Received 26 September 2009
Accepted 20 May 2010

Keywords:
Acute pancreatitis
Cyclosporin A
Pro-inflammatory cytokines
Anti-inflammatory cytokines
Brain injury

ABSTRACT

Aims: This study was designed to evaluate the protective effect of cyclosporin A (CsA) on brain injury in rats with acute necrotic pancreatitis (ANP) in order to provide a scientific basis for the use of the drug in treating brain injury caused by pancreatitis.
Main methods: The rats were divided into a sham group, an ANP group and an ANP + CsA group. The ANP model was induced by administering 5% sodium taurocholate through the biliopancreatic duct. Five minutes before the preparation of the ANP model, 1 ml of CsA (10 mg/kg) was injected in a clinically used pharmaceutical formulation (Sandimmun®) via the dorsal vein of the penis. Twelve hours after the model was established, samples were taken from the rats in all groups for measurement of appropriate indexes. The serum levels of pro-inflammatory tumor necrosis factor-alpha (TNF-α), interleukin 1β (IL-1β), and anti-inflammatory interleukin 10 (IL-10) were determined by ELISA. The pancreatic mRNA expressions of these cytokines were evaluated by RT-PCR. Brain water content was tested by the dry–wet method, and brain malondialdehyde (MDA) content was detected by the chemical colorimetry method.
Key findings: Both the serum levels and the pancreatic tissue mRNA expression of TNF-α and IL-1β, as well as the brain water content and brain MDA content, were significantly increased in the ANP group. CsA treatment noticeably reduced both the serum levels and the pancreatic mRNA expression of TNF-α and IL-1β and decreased brain water and MDA contents. In contrast to the pro-inflammatory cytokines, the serum levels and the pancreatic tissue mRNA expression of IL-10 were markedly increased by the injection of CsA.
Significance: CsA could alleviate acute pancreatitis-associated brain injury.

Introduction

Pancreatic encephalopathy caused by pancreatitis-associated brain injury may lead to neuropsychic disturbance syndrome, making it a major concern for clinicians. However, the etiology and pathogenesis of pancreatitis-associated brain injury remain unclear. At present, there are many hypotheses that relate this disease to trypsin, fungal infection, electrolyte disturbance, vitamin deficiency, alcohol intoxication, hypoxemia, etc. (Bourgeois and Fakhri 2007; Zhang and Tian 2007).

Recent studies have shown that overactivation of leukocytes, overexpression of pro-inflammatory cytokines and immunologic dysfunction are important factors that result in the deterioration caused by acute pancreatitis and play crucial roles in the pathogenesis of pancreatitis-associated brain injury (Bartha et al. 2006; Ohkubo et al. 2004). Immunosuppressive drugs, such as 5-fluorouracil and glucocorticoid, have a therapeutic effect on pancreatitis in clinical practice. In the present study, the immunosuppressant cyclosporin A (CsA) was used to alleviate excessive immune responses caused by pancreatitis in order to block or alleviate rat brain injury caused by acute necrotic pancreatitis.

Materials and methods

Animals

This study was approved by the Institutional Animal Use and Care Committee of Xi'jing Hospital (The Fourth Military Medical University, China), and this protocol complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication, 1996). Male Sprague–Dawley rats weighing 230–260 g were supplied for this study by the Animal Experimental Center of The Fourth Military Medical University. The animals were maintained at the Animal Research Center of The Fourth Military Medical University with a 12 h light/dark cycle and free access to standard laboratory feed and water.

* Corresponding authors. Yang is to be contacted at Tel.: +86 29 84773772; fax: +86 29 83283229. Wang Tel.: +86 29 84773074; fax: +86 29 83283229.
E-mail addresses: wangyy@fmmu.edu.cn (Y.-Y. Wang), yangyanl@fmmu.edu.cn (Y.-L. Yang).

0024-3205/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2010.05.014

Preparation of the acute necrotic pancreatitis (ANP) rat model

Twelve hours before operation, rats were fasted but had free access to water. A total of 48 rats were randomly divided into a sham-operated group ($n=16$), an ANP group ($n=16$) and an AP + CsA group ($n=16$).

The rats were anesthetized by intraperitoneal injection of 10% sodium pentobarbital (30 mg/kg), a median abdominal incision was made, and a small artery clamp was used to temporarily clamp the bile duct on the porta hepatis side of the hepatoduodenal ligament. A needle was inserted into the duodenal anterior wall. When it reached 0.5 cm within the choledoch, the biliopancreatic duct was clamped, and 5% sodium taurocholate (0.1 ml/kg, Sigma) was injected uniformly at 0.1 ml/min. The artery clamp was removed 10 min later, and the abdomen was closed layer by layer.

Serving as controls, the sham-operated group received a retrograde infusion of sterile saline. In the ANP + CsA group, 5 min before the preparation of the ANP model, 1 ml of CsA (10 mg/kg) was injected via the dorsal vein of the penis.

Twelve hours after the model was established, the rats in all groups were sacrificed by cervical dislocation. Blood samples were immediately centrifuged at 3000 *g* for 10 min at room temperature, and the serum samples were examined the same day for TNF-α, IL-1β and IL-10 levels. The pancreatic tissues were dissected out and immediately frozen in powdered dry ice. Total RNA was isolated using Trizol reagent (Gibco BRL, Grand Island, NY, USA) according to the manufacturer's protocol and then used for RT-PCR. The brains were excised following decapitation, and the brain samples were randomly divided into two parts for the determination of brain water content and determination of brain malondialdehyde (MDA) content.

Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-10 (IL-10) enzyme-linked immunosorbent assay (ELISA)

Serum TNF-α, IL-1β and IL-10 levels were determined by ELISA using kits purchased from Endogen Laboratories (Endogen, Bioreba, Basel, Switzerland). These kits use polyclonal ovine anti-TNF-α, anti-IL-1β and anti-IL-10 antibodies conjugated to horseradish peroxidase (HRP), which are bound by free TNF-α, IL-1β and IL-10 in samples or standards in competition with TNF-α-/IL-1β-/IL-6-conjugated proteins coated on the plate well surface. Samples were incubated with the antibodies, washed with PBS with 0.05% Tween 20, and then treated with TMB (3,3′,5,5′-tetramethylbenzidine), which reacts with the HRP enzyme to form a blue end product. The addition of 0.3 M sulfuric acid turned the blue to yellow, and the plate was read on a Novo-Star plate reader (BMG, USA) at 450 nm. Analyses of TNF-α, IL-1β and IL-10 concentrations in the standard curve and samples were performed using ELISA data-processing software provided by Biosense Laboratories.

Real-time quantitative RT-PCR of TNF-α, IL-1β and IL-10 assay

Real-time quantitative RT-PCR was performed to analyze the mRNA expression levels of TNF-α, IL-1β and IL-10 in the pancreatic tissue. Total RNA was extracted from the pancreatic samples using RNA Extract Kit and converted to first-strand cDNA according to the manufacturer's instructions. Quantitative real-time PCR was performed using SYBR PrimeScript RT-PCR Kit in the Light Cycler System (Roche Diagnostics, Lewes, UK). The primer sequences used for PCR were as follows: TNF-α sense 5′-CCA GGA GAA AGT CAG CCT CCT-3′ and antisense 5′-TCA TAC CAG GGC TTG AGC TCA-3′ resulting in a 87-bp product; IL-1β sense 5′-CAC CTC TCA AGC AGA GCA CAG-3′ and antisense 5′-GGG TTC CAT GGT GAA GTC AAC-3′ resulting in a 79-bp product; IL-10 sense 5′-GGC TCA GCA CTG CTA TGT TGC C-3′ and antisense 5′-AGC ATG TGG GTC TGG CTG ACT G-3′ resulting in a 116-bp product; and b-actin sense 5′-GAA CAC GGC ATT GTA ACC AA CTG G-3′ and antisense 5′-GGC CAC ACG CAG CTC ATT GTA-3′ resulting in a 77-bp product. Amplification was performed with the following cycles: 95 °C for 30 s, followed by 40 cycles of denaturing at 95 °C for 5 s and annealing at 60 °C for 20 s. All of the reactions were performed in triplicate. Data analysis was performed using the $2^{-\Delta\Delta CT}$ method described by Livak and Schmittgen (2001); β-actin was used as a reference gene.

Determination of brain water content

Brain water content was determined by desiccation, also known as the dry–wet method. After sacrificing the animal, the brain tissue was rapidly excised without exsanguination and weighed. The excised tissue was then cut into small pieces to increase the surface area for evaporation and subsequently dried at 40 °C–50 °C to constant weight. Assuming a density of 1 g/ml, the tissue water content (V_W) was calculated from the difference between the wet (Wwet) and dry (Wdry) weights of the tissue: V_W (ml/g) = (Wwet − Wdry)/Wwet × 100%. The tissue water content was corrected for the water present in the residual tissue blood considering that the percentage of water in rat blood is 81.1% (Altman and Dittmer 1961).

Determination of brain malondialdehyde (MDA) content

After rats were anesthetized, their brains were excised. Following excision, the cortex was dissected, frozen immediately using liquid nitrogen and stored at −80 °C. Due to the size of the cortex, only a portion of it was collected and analyzed in this study. Briefly, a coronal cut at the level of the optic chiasm was performed, and the cortical tissue caudal to the cut and superficial to the hippocampus was collected and analyzed. Prior to the analysis of malondialdehyde (MDA), tissue samples were placed in 50 μl of 5 mM butylated hydroxytoluene (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China), to prevent further lipid peroxidation. MDA level was assessed using the commercially available colorimetric MDA-586 assay kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) with the absorbance read on a microplate reader (ELX808, BIO-TEK Instruments, USA) at a wavelength of 586 nm. All experimental procedures for these assays were performed using the instructions provided by the manufacturer. MDA of the cortex was normalized to wet tissue weight (mg) and expressed as nmol/mg.

Statistical analysis

Data are presented as means ± SD. A one-way repeated-measures ANOVA was used for the analysis of differences between the experimental groups. $P<0.05$ was considered statistically significant.

Results

Survival rate

The mortalities of the rats in the sham-operated, ANP and ANP + CsA groups were all 0% at 12 h.

Serum TNF-α, IL-1β and IL-10 levels

Ductal infusion of sodium taurocholate resulted in significant increases in serum TNF-α and IL-1β levels at 12 h. As shown in Fig. 1, the serum TNF-α and IL-1β levels in the sham-operated group were significantly lower than those of the ANP group ($P<0.05$). Compared with the ANP group, the treatment with CsA significantly reduced the serum TNF-α and IL-1β levels ($P<0.05$). However, the serum TNF-α and IL-1β levels in the CsA-treated ANP group were still significantly higher than those of the sham-operated group ($P<0.05$).

The serum IL-10 level moderately increased after the induction of ANP, whereas it significantly increased in the CsA-treated ANP group (Fig. 1).