

Effects of TCMP-1 on the changes of platelet endothelial cell adhesion molecule-1 expression in acute edematous pancreatitis

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BACKGROUND: Traditional Chinese medicine is a potent agent in the management of clinical and experimental acute pancreatitis (AP), but the molecular mechanism of its therapeutic action is unclear. Numerous experimental and clinical studies have shown that platelet endothelial cell adhesion molecule-1 (PECAM-1) is pivotal to leukocyte recruitment, which results in microcirculatory injury during inflammation, but its role in acute pancreatitis is poorly understood. We investigated the effects of a compound of traditional Chinese medicine pancreatitis-1 (TCMP-1) on the changes of platelet endothelial cell adhesion molecule-1 (PECAM-1) expression on polymorphonuclear leukocytes (PMNs) in acute edematous pancreatitis (AEP).

METHODS: The model of acute pancreatitis was established by subcutaneous injection of caerulein, and TCMP-1 treated groups were given TCMP-1 by catheterization from mouth to stomach (20 ml/kg) immediately after first time subcutaneous injection of caerulein. The changes of expression of PECAM-1 on leukocytes from the blood of the splenic vein and inferior vena cava were determined by flow cytometry.

RESULTS: In the AEP group, expression of PECAM-1 on PMNs was not significantly different between pancreatic microcirculation and systemic circulation at AEP2h and AEP4h time point. Then from AEP4h time point to AEP8h time point, expression of PECAM-1 was up-regulated in systemic circulation while it was down-regulated in pancreatic microcirculation and was significantly different between pancreatic microcirculation and systemic circulation at AEP8h time point ($P < 0.05$). In the TCMP-1 treated group, compared with the AEP group, expression of PE-

CAM-1 on PMNs decreased in different levels between pancreatic microcirculation and systemic circulation and was of significant difference at AEP8h time point ($P < 0.05$).

CONCLUSION: Inhibition of PECAM-1 expression on PMNs may prevent PMNs from transmigration through the endothelium and may be one of the treatment mechanisms of TCMP-1 decoction on AEP.

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KEY WORDS: acute pancreatitis; platelet endothelial cell adhesion molecule-1; Chinese medicine

Introduction

Acute pancreatitis is a potentially lethal disease and its etiology remains poorly understood. Several studies have documented that platelet endothelial cell adhesion molecule-1 (PECAM-1) plays an important role in the sequestration of leukocytes, particularly polymorphonuclear leukocytes (PMNs),^[1-3] which subsequently results in injury to the pancreas. Recent studies on the efficacy of traditional Chinese medicine like TCMP-1 (traditional Chinese medicine for pancreatitis-1)^[4-6] have provided hope for a successful therapy for acute pancreatitis. TCMP-1 designed for acute edematous pancreatitis (AEP) at our college has been commonly used in clinical practice, but its mechanisms are still not clear. In this study, we investigated the effects of TCMP-1 on the changes of platelet endothelial cell adhesion molecule-1 expression on PMNs in AEP.

Methods

Caerulein acute edematous pancreatitis model

Male and female Wistar rats weighing 250-350 g were provided by the Center of Experimental Animals, Sichuan University, Chengdu, China. The animals were fasted for 12 hours before experiment but had free access to water. They were treated in accordance with the protocols approved by the local Animal Use and Care Com-

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mittees and executed according to the National Animal Welfare Law of China. All of these experimental rats were given subcutaneous injection of caerulein (Sigma Co., USA) 5.5 and 7.5 $\mu\text{g}/\text{kg}$ at 0 and 1 hour after the beginning of experiment respectively, while control rats were given comparable subcutaneous injection of 0.9% saline solution. TCMP-1 treated groups were given TCMP-1 by catheterization from mouth to stomach (20 ml/kg) after first time subcutaneous injection of caerulein. At different time points (2, 4, 6, or 8 hours) after induction of AEP, the animals ($n=10$ at each time point) underwent laparotomy under the anesthesia induced by intraperitoneal injection of 50 mg/kg sodium pentobarbital (Sanofi; Libourne, France). Blood samples were immediately obtained from the splenic vein by retrograde catheterization from the portal vein and inferior vena cava. Samples of the pancreatic head were collected rapidly for measuring the dry/wet weight ratio. The control animals ($n=10$) underwent laparotomy and were sampled in the same manner as animals with AEP.

Prescription of TCMP-1

TCMP-1 is a traditional Chinese prescription for the treatment of AEP and contains *Bupleurum Chinese DC*, *Scutellaria baicalensis Georgi*, *Picrorhiza kurrooa Royle ex Ben*, *Radix paeoniae alba*, *Radix Aucklandiae*, *corydalis humosa Migo*, *Rheum officinale Baill*, and *Natrii sulfas*. The decoction was prepared by the Department of Formula of Traditional Chinese Medicine of this hospital.

Neutrophils preparation

Rat PMNs were isolated using the technique described by Hjorth et al.^[7] Blood was immediately mixed with heparin (50 U/ml) and centrifuged in a discontinuous Percoll gradient to yield a fraction of approximately 97% purity. Cell viability as assessed by trypan blue exclusion was above 96% under all experimental conditions.

Measurement of PECAM-1

After fixation, phycoerythrin (PE)-anti-PECAM-1 monoclonal antibody (BD PharMingen, USA) was added to label PMNs surface PECAM-1 20 minutes at 4 °C. After being washed with PBS, 10^5 - 10^6 cells/ml suspension was prepared for flow cytometry (Elite Esp, Coulter, USA) test.

Measurement of serum amylase

Serum amylase levels were measured at 37 °C by means of an enzymatic assay with a spectrophotometer according to the manufacturer's instructions. All serum samples were assayed in duplicate, and the results were averaged at the end of the experiment.

Measurement of the dry/wet weight ratio

Tissue water content was determined by measuring

the dry weight-to-wet weight ratio of tissue specimens from the pancreatic head. Each specimen was weighed immediately after sampling and oven drying at 80 °C for at least 48 hours.

Statistical analysis

The results were expressed in mean \pm standard deviation, and individual comparisons of group means were made with two-tailed Student's *t* test. A *P* value less than 0.05 was considered significant.

Results

Serum amylase

Compared with the control rats, all experimental groups demonstrated hyperamylasemia ($P < 0.05$) except the TCMP-1 treated groups at AEP8h time point ($P > 0.05$). Compared with the AEP groups, serum amylase levels increased slightly in the TCMP-1 treated groups and significantly at AEP4h time point ($P < 0.05$, Fig).

The dry/wet weight ratio

Subcutaneous injection of caerulein led to a continuous decrease in the dry/wet weight ratio in experimental rats. Compared with the control animals, all AEP groups had a significant decrease in the dry/wet pancreatic weight ratio ($P < 0.05$), but the TCMP-1 treated groups did not change obviously ($P > 0.05$, Table 1).

Expression of PECAM-1 on the surface of PMNs

In the AEP groups, expression of PECAM-1 on PMNs showed no significant difference between pancreatic microcirculation and systemic circulation at AEP2h and AEP4h time points. From AEP4h time point to AEP8h time point, the expression of PECAM-1 was up-regulated in systemic circulation but it was down-regula-

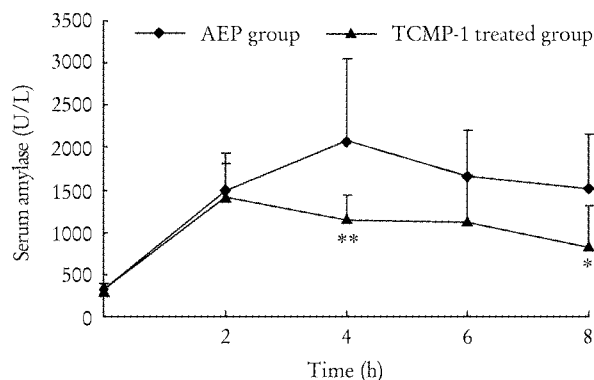


Fig. Serum amylase level in AEP. In the AEP model, blood samples were collected at different time points (2, 4, 6, 8 hours; *: compared with control values, $P > 0.05$; **: compared with AEP group values, $P < 0.05$).

Table 1. The dry/wet pancreatic weight ratio in AEP

Groups	The dry/wet weight ratio (%)
Control	29.6 ± 2.2
AEP2h	26.2 ± 3.1 *
AEP4h	25.5 ± 2.3 *
AEP6h	23.4 ± 2.6 *
AEP8h	24.9 ± 3.8 *
TCMP-1 treated AEP2h	30.0 ± 2.8
TCMP-1 treated AEP4h	27.7 ± 1.7
TCMP-1 treated AEP6h	27.7 ± 2.0
TCMP-1 treated AEP8h	28.5 ± 6.1

In the experimental model, the samples of the pancreatic head were collected at different time points (2, 4, 6, 8 hours; *: compared with control values, $P < 0.05$).

Table 2. The expression of PECAM-1 on the surface of PMNs

Groups	PECAM-1 expression in systemic circulation (mean ± SD, %)	PECAM-1 expression in microcirculation (mean ± SD, %)
Control	100.0 ± 0.0	100.0 ± 0.0
AEP2h	89.5 ± 14.9	91.2 ± 36.3
AEP4h	92.6 ± 22.5	87.8 ± 32.6
AEP6h	106.3 ± 26.2	72.6 ± 21.4
AEP8h	117.5 ± 29.5	78.3 ± 30.4 *
TCMP-1 treated AEP2h	90.1 ± 18.9	59.0 ± 13.2
TCMP-1 treated AEP4h	98.6 ± 17.7	51.5 ± 13.6
TCMP-1 treated AEP6h	75.0 ± 10.6	48.7 ± 11.6
TCMP-1 treated AEP8h	71.9 ± 11.9 **	66.0 ± 19.8

*: compared with systemic circulation values, $P < 0.05$; **: compared with AEP values, $P < 0.05$ (mean ± SD).

tion in pancreatic microcirculation. There was a significant difference between pancreatic microcirculation and systemic circulation at AEP8h time point ($P < 0.05$). In the TCMP-1 treated groups compared with the AEP groups, the expression of PECAM-1 on PMNs decreased in different level between pancreatic microcirculation and systemic circulation. There was a significant difference at AEP8h time point ($P < 0.05$, Table 2).

Discussion

Acute pancreatitis is a common disease of varied severity. Despite perceived advances in the diagnosis and treatment of acute pancreatitis, the etiology of acute pancreatitis remains unclear.^[8] Understanding potential factors initiating acute pancreatitis, mechanisms regulating the local and distant inflammatory response, and possible therapeutic interventions continue to be investigated.

Since the 1980s, extensive research into the curative mechanisms of traditional Chinese medicine have been carried out.^[9-14] Some experimental studies proved that traditional Chinese medicine with effects of “clearing heat and detoxicating” and “activating blood circula-

tion and dispersing stasis” can improve the blood supply of abdominal viscerae and reduce inflammatory exudation.^[15,16] Purgative herbs such as rhubarb root can improve vermiculation and inhibit absorption of bacterial toxin and have a significant protection on the intestinal barrier by reducing the risks of enterogenic infections and enterogenic endotoxemia.^[17]

PECAM-1 is a cell adhesion molecule that belongs to the Ig superfamily which expressed on endothelial cells as well as circulating leukocytes including neutrophils,^[18-20] monocytes and NK cells.^[21,22] The homophilic PECAM-1 interaction of neutrophil or monocyte PECAM-1 with endothelial PECAM-1 is very important to neutrophil and monocyte transendothelial migration as demonstrated in studies in several different laboratories.^[23-27] The interaction between neutrophil or monocyte PECAM-1 and endothelial PECAM-1 may be mediated by interdigitating PECAM-1 molecules from neutrophils or monocytes and endothelial cells, forming a zipper which promotes their adhesion,^[28-30] while facilitating leukocytes transmigration.^[31-34]

Several lines of evidence show that PECAM-1 is required for leukocyte transmigration through endothelial cell monolayers^[35-37] and PMNs play an important role in microcirculatory injury during inflammation.^[38-40] The present study showed that oral treatment of rats with TCMP-1 decoction could significantly down-regulate the expression of PECAM-1 on PMNs in systemic circulation compared with the AEP group at AEP8h time point ($P < 0.01$). Furthermore, down-regulation of PECAM-1 expression on PMNs in systemic circulation may block the interaction between endothelial cells and PMNs and subsequently prevent PMN from transmigrating through the endothelium.

Overall, this study suggested that inhibition of PECAM-1 expression may be one of the mechanisms in the treatment actions of TCMP-1 decoction. Our data may provide a new idea for the development of therapeutic strategies by means of traditional Chinese medicine.

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Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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