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Protective effect of TMP on pancreas function of acute pancreatitis rats

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ABSTRACT

Objective: To explore the protective effect and mechanism of Tetramethy1Pyrazine (TMP) on the pancreas function of acute pancreatitis rats.

Methods: A total of 75 SD rats were randomly divided into three groups (A, B, C) with 25 rats in each group. Group A served as sham operation group. In the groups B and C, AP model was prepared as by injecting taurocholic acid sodium. Group B was model group. After modeling, rats were administrated by intraperitoneal injection of normal saline. Group C was TMP treatment group, which was administrated by intraperitoneal injection of 0.6% TMP after modeling. The rat blood specimens in each group were collected with 1 mL/100 g solution after modeling of 2, 6, 12 and 24 h. Levels of amylase (AMS), blood urea nitrogen (BUN), creatinine (CR), TNF-α and IL-6 were detected, and 5 rats were sacrificed. Histopathological examination was performed in he pancreatic tissue specimens of each group to observe pancreatic tissue damage.

Results: After modeling in each time point, AMS, BUN, CR, TNF-α and IL-6 in groups B and C were significantly higher than that of in group A ($P < 0.05$). After modeling of 2 h, AMS, BUN and CR in group B increased significantly and reached the peak value at 6 h. After modeling of 12 h, serum level of TNF-α and IL-6 were significantly lower than that of in control group, while after 24 h of modeling, serum level of AMS, BUN, CR, TNF-α and IL-6 were significantly lower than that of in control group ($P < 0.05$). The histological observation showed that pancreatic tissue in rats of group A was normal without damage lesions. Massive bleeding, necrosis and serious injury were visible in pancreatic tissue of group B. The rat pancreatic tissue was bleeding in group C with small pieces of necrotic lesions. The degree of inflammatory cell infiltration was lower than group B, and the degree of injury was significantly lower than group B.

Conclusions: TMP can significantly decrease the serum level of TNF-α and IL-6 in AP rats, inhibits inflammatory response of AP, and has significant protective effect on pancreatic tissue and function in AP rats.

1. Introduction

Acute pancreatitis (AP) is the clinical common emergency with urgent onset and fast progress [1–3]. Some studies confirmed that the onset of AP is not only confined to local inflammation of the pancreas but involved in systemic disease of many organs, which can lead to serious complications such as multiple organ dysfunction syndromes, shock and disseminated intravascular coagulation to cause death of the patients [4,5]. Many researchers have long done a number of studies in the onset and treatment of AP and obtained gratifying achievements, but the pathogenesis is still not completely clear [6]. Some researchers believed that anti-inflammatory cytokine, proinflammatory factor, protease inhibitor and protease disequilibrium all involve in the onset of AP [7–10]. AMP is the alkaloid preparation extracted from *Ligusticum wallichii*, which can reduce the calcium concentration of cells, interdict the inflow of extracellular calcium and relieve pancreatic inflammation when calcium flows within the damage to the cells [11]. In order to explore the

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protective effect and mechanism of TMP on the pancreas function, SD rats were selected to prepare for AP model, which were given TMP intraperitoneal injection for treatment. The serum level of amylase (AMS), blood urea nitrogen (BUN), creatinine (CR), TNF-α and IL-6 were observed and performed histopathological examination.

2. Materials and methods

2.1. Animals

A total of 75 female adult SD rats, of clean grade and weighting (320 ± 10) g were selected, which were purchased from Shanghai Laboratory Animal Research Center. Rats were kept at (23 ± 3)°C and given free access to food and water. Experiments on animals were strictly followed the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Drugs and instruments

Ligustrazine injection (Henan Kelun Pharmaceutical Co., Ltd., approved by H20059447), taurocholic acid sodium (Sigma–Aldrich), TNF-α, IL-6 EUSA kits and TXB_2 kits (Nanjing Jiancheng Bioengineering Institute), PBS buffer, SABC kit, DAB color reagent (Shanghai Sen-Xiong Science and Technology Industrial Co., Ltd.), Olympus BH-2 microscope (Japan), BI-2000 Medical Image Analysis System, BL420 multichannel physiologic recorder (Chengdu), automatic biochemical analyzer 717OA (Hitachi Limited), and 722 spectrophotometer (Shanghai Analytical Instrument Factory) were used in the present study.

2.3. Model preparation methods

Animals were anesthetized with subcutaneous injection of 0.5% pentobarbital sodium. A median incision was made in the abdomen. No. 5 skin test needle was used to poke a hole in the avascular area of duodenum mesenteric border. The No. 5 blunted needle was inserted to the opening biliopancreatic duct of duodenal mucosa deep to 1 cm through intestinal wall puncture hole. The porta hepatis biliopancreatic duct and puncture needle were clamped to prevent the infusion liquid flowing to liver and duodenum. About 3.5% sodium taurocholate (the dose 1.6 mL/kg, injection speed 0.2 mL/min) was injected to biliopancreatic duct with constant speed through 1 mL syringe. After injection, the upper and lower end of biliopancreatic duct was interdicted for 5 min. When the pancreas envelope tension increased, the surface swelling became hard with obvious hyperemia edema, and a small amount of bloody ascites was found, it was considered that the animal model was established. Then the microvascular occlusion clamps were removed. After the suture of puncture hole with silk thread, rats were feeding separately.

2.4. Animal group and experimental method

75 experimental rats were randomly divided into three groups: group A, B and C, with 25 rats in each group. Group A served as sham operation group, which was only performed laparotomy without modeling operation. Rats in group B and C were modeling. After modeling, rats in group C were given intraperitoneal injection of 0.6% ligustrazine injection for treatment (1 mL/100 g solution), while rats in group B were given intraperitoneal injection with the same dose of normal saline for treatment after abdominal closure.

2.5. Observation index

After modeling of 2, 6, 12, 24 h, blood specimens of each group were collected. Serums of AMS, BUN, and CR were detected with automatic biochemical analyzer. The detecting procedure of serum of TNF-α and IL-6 was strictly followed with the instruction of kit. Five rats were sacrificed after modeling of 2, 6, 12, 24 h, and the pancreatic tissue of each group was taken, which was fixed with 4% triformol and conventional embedding and cut into slices. After preparation, the slices were put into oven at 60 °C for 30 min, deparaffinized with xylene for twice, washed with absolute ethyl alcohol for twice, dehydrated with gradient ethanol, stained with hematoxylin, differentiated with hydrochloric acid alcohol, washed with tap water to return to blue, re-stained with Eosin, dehydrated with absolute ethyl alcohol, cleared in xylene and sealed. The pancreatic tissue damage of rats in each group was observed under the light microscope.

2.6. Statistical method

The obtained data were analyzed with SPSS13.0 software package. Measurement data were expressed as mean ± sd. One-way ANOVA analysis was used for the compare of multiple

Table 1
Compare of AMS, BUN and CR levels in different time points after modeling.

Group	Time (h)	*n*	AMS	BUN	CR
Group A	2	5	1 049.32 ± 160.44	6.81 ± 1.36	61.71 ± 9.35
	6	5	1 291.24 ± 245.62	6.91 ± 1.77	62.50 ± 11.77
	12	5	1 706.85 ± 389.67	7.24 ± 1.55	70.53 ± 11.93
	24	5	1 337.31 ± 184.53	7.64 ± 1.94	69.84 ± 12.54
Group B	2	5	4 042.54 ± 554.26*	15.46 ± 1.64*	98.15 ± 27.32*
	6	5	10 027.33 ± 2 788.32*	16.22 ± 1.63*	116.22 ± 17.63*
	12	5	6 471.32 ± 839.47*	22.55 ± 2.14*	170.41 ± 20.14*
	24	5	5 638.56 ± 743.75*	24.30 ± 2.54*	203.30 ± 42.54*
Group C	2	5	3 479.38 ± 723.73*	13.73 ± 2.57*	80.14 ± 11.57*
	6	5	9 026.71 ± 1 584.36*	14.88 ± 1.99*	96.03 ± 15.84*
	12	5	5 800.53 ± 846.85*	17.75 ± 1.79*#	120.87 ± 21.88*#
	24	5	4 083.32 ± 649.34*#	18.50 ± 2.14*#	145.34 ± 25.14*#

*$P < 0.05$ indicates the compare with group A in the same period. #$P < 0.05$ indicates the compare with group B in the same period.

Table 2

Compare of the serum levels of TNF-α and IL-6 in different time points after modeling (pg/mL).

Group	Time (h)	*n*	TNF-α	IL-6
Group A	2	5	28.2 ± 3.2	113.3 ± 22.8
	6	5	29.1 ± 3.2	118.2 ± 11.9
	12	5	28.2 ± 2.4	135.7 ± 21.5
	24	5	29.1 ± 4.0	159.4 ± 24.6
Group B	2	5	113.8 ± 17.5*	530.0 ± 54.6*
	6	5	135.7 ± 14.0*	699.8 ± 63.5*
	12	5	185.2 ± 25.0*	833.6 ± 43.2*
	24	5	194.1 ± 27.3*	807.2 ± 41.8*
Group C	2	5	98.8 ± 8.0*	488.5 ± 40.0*
	6	5	122.3 ± 8.7*	627.0 ± 47.7*
	12	5	157.0 ± 11.6*#	697.1 ± 31.0*#
	24	5	138.1 ± 7.8*#	660.2 ± 66.2*#

*$P < 0.05$ indicates the compare with group A in the same period. #$P < 0.05$ indicates the compare with group B in the same period.

sample mean. *q* test and LSD test were used for each mean value. $P < 0.05$ was considered significantly different.

3. Results

3.1. Compare of AMS, BUN, and CR level in different time points after modeling

The serum levels of AMS, BUN and CR of different time points in groups B and C were significantly higher than that of in group A, which had significant difference ($P < 0.05$). The serum levels of AMS, BUN and CR in group B were gradually increased. After modeling, the serum level of AMS of 24 h in group C was significantly lower than that of in group B ($P < 0.05$). After modeling of 12 and 24 h, serum levels of BUN and CR were significantly lower than that of in group B, which had statistical significance among the groups ($P < 0.05$) (Table 1).

3.2. Compare of the serum levels of TNT-α and IL-6 in different time points after modeling

After modeling, the serum levels of TNF-α and IL-6 in different time points in groups B and C were significantly higher than that of in group A, which had statistical significance among the groups ($P < 0.05$). After modeling of 12 and 24 h, the serum levels of TNF-α and IL-6 in group C was significantly lower than that of in group B, which had statistical significance among the groups ($P < 0.05$) (Table 2).

3.3. Histological change

Microscopically, pancreatic tissue structure of group A in different time points were normal. The pathological score of pancreas damage in group B was significantly increased after modeling of 2 h, and was increased gradually along with the prolonged time. After modeling of 24 h, the microscopic image showed large area of necrosis in pancreatic tissue, fuzzy structure of acinous cell, complete destruction of lobular structures, broadening of pancreatic interstitial and obvious hyperemia and edema, where infiltration of a large number of inflammatory cells, and large area of necrosis in pancreatic tissue were visible. After modeling of 12 and 24 h, the pathological score of pancreas damage in group C was significantly lower than that of in group B ($P < 0.05$). Microscopically, after modeling of 12 and 24 h, pancreatic tissue damage and the degree of infiltration

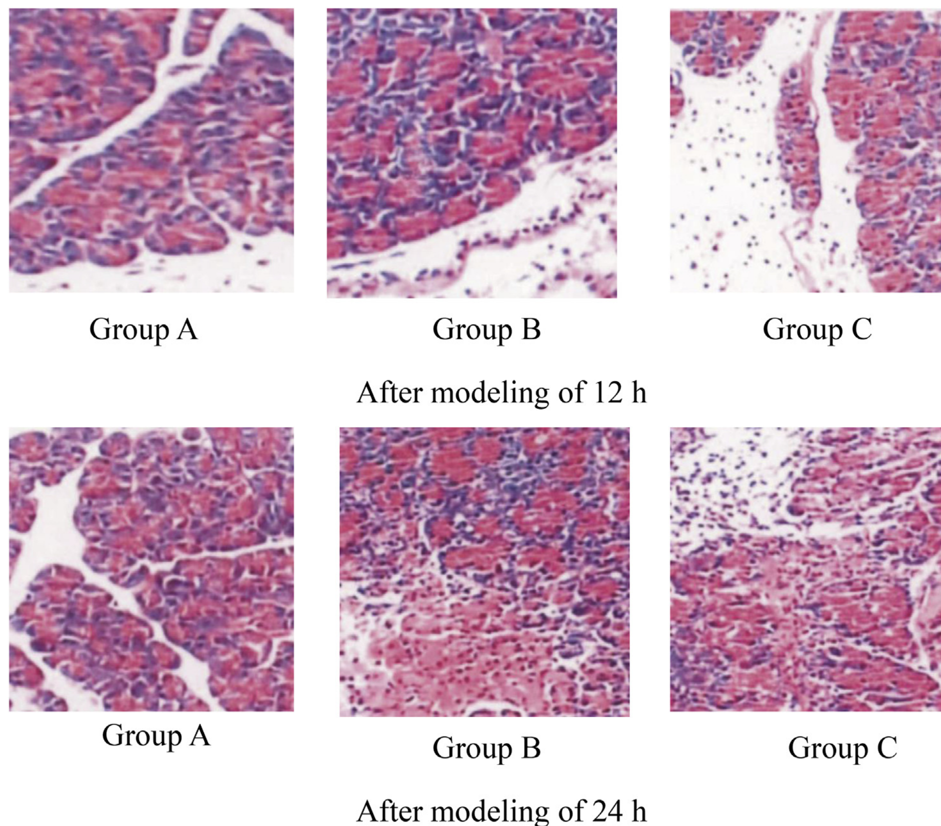

Figure 1. Results of histopathological examination (HE, ×200).

Table 3
Compare of pathological score of pancreas damage in three groups.

Groups	2 h	6 h	12 h	24 h
Group A	0.22 ± 0.13	0.52 ± 0.16	0.43 ± 0.121	0.35 ± 0.13
Group B	6.81 ± 1.21*	8.61 ± 1.32*	10.74 ± 1.52*	12.64 ± 1.14*
Group C	6.32 ± 1.10*	7.34 ± 1.21*	9.05 ± 1.36*#	9.51 ± 1.52*#

*$P < 0.05$ indicates the compare with group A in the same period. #$P < 0.05$ indicates the compare with group B in the same period.

of inflammatory cells were lighter than that of in group B (Figure 1, Table 3).

4. Discussion

AP is the clinical common disease with urgent onset and fast progress of which main clinical manifestations are acute abdominal pain, emesis, nausea, and the increase of hematuria amylase. The pathogenetic basis is that activated pancreatin causes the inflammation process of autodigestion of pancreatic tissue in pancreas. For example, SAP, which is developed by AP, can lead to systemic inflammatory response syndrome and involve in multiple organs with a highly fatality rate [12]. Currently, the study of SAP has obtained gratifying achievements in clinic. The theory of cascade waterfall effect of inflammatory cytokine has been widely accepted by the clinical staff, but the pathogenesis and prevention scheme still need further study [13].

AMP, an alkaloid monomer extracted and isolated from Ligusticum wallichii, has strong effect of dilating blood vessels and improving minicirculation, antagonism of calcium ion, and can decrease blood viscosity and increase splanchnic blood flow, which has been widely used in the treatment of disease of heart head blood-vessel and pulmonary disease. Studies have confirmed that TMP can directly play a role on dilating blood vessels and reducing microcirculation disturbance by adjusting the disequilibrium of TXA2/PGI2 [3,14–16]. Clinically, it has been confirmed that inflammatory mediator plays an important role in the onset process of AP, and also make the same effect to the progress of AP to SAP. After the onset of AP, a large number of inflammatory mediators are relieved to blood to form cascade network activated by proinflammatory factor, which interact each other, thus aggravating the pancreas injury and causing serious complications such as systemic inflammatory response syndrome [17]. TNF-α and IL-6 are a pair of important proinflammatory factors, which play a central role in the onset of AP [18]. Researches have shown that the serums of TNF-α and IL-6 are significantly higher in AP patients than that of in the normal populations [19,20]. Therefore, how to decrease the levels of TNF-α and IL-6 of the patients in the treatment AP is also the hot issue in the clinical research. The present study detected the serum level to evaluate the therapeutic effect of TMP to AP. The results showed that after modeling, the serum levels of TNF-α and IL-6 indifferent time points in group B and C were significantly higher than that of in group A ($P < 0.05$), indicating the important role of TNF-α and IL-6 in the onset of AP, which is in agreement with the literature reports. After modeling of 12 and 24 h, the serum levels of TNF-α and IL-6 in group C were significantly lower than that of in group B ($P < 0.05$), indicating that TMP can effectively decrease the serum levels of TNF-α and IL-6 of AP rats and reduce waterfalls cascade induced by TNF-α and IL-6, thus improving SAP symptoms. Meanwhile, after modeling of 24 h, serum level of AMS in group C was significantly lower than that of in group B ($P < 0.05$); after modeling of 12 and 24 h, serum levels of BUN and CR were significantly lower than that of in group B ($P < 0.05$), which also confirmed that TMP has a definite therapeutic effect on AP. In addition, after modeling of 12 and 24 h, pathological score of pancreas damage in group C was significantly lower than that of in group B ($P < 0.05$). The histological observation showed that after modeling of 12 and 24 h, pancreatic tissue damage and degree of inflammatory cell infiltration were lighter than that of in group B, which indicated that AMP has a significant therapeutic effect on AP rats.

The results confirmed that TMP can significantly decrease the serum levels of TNF-α and IL-6 in AP rats, and inhibit inflammatory response of AP, which has the significant protective effect on the pancreas function of AP rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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