

• BRIEF REPORTS •

Tumor necrosis factor α antibody prevents brain damage of rats with acute necrotizing pancreatitis

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Abstract

AIM: To study the protective effects of tumor necrosis factor α (TNF α) antibody on pancreatic encephalopathy in rats.

METHODS: One hundred and twenty SD rats were randomly divided into normal control group, acute necrotizing pancreatitis group and TNF α antibody treated group. Acute hemorrhage necrotizing pancreatitis model in rats was induced by retrograde injection of 50 g/L sodium taurocholate into the pancreatobiliary duct. Serum TNF α was detected and animals were killed 12 h after drug administration. Changes in content of brain water, MDA and SOD as well as leucocyte adhesion of brain microvessels were measured.

RESULTS: In TNF α antibody treated group, serum TNF α level was decreased. Content of brain water, MDA and SOD as well as leucocyte adhesion were decreased significantly in comparison with those of acute necrotizing pancreatitis group ($P < 0.05$).

CONCLUSION: TNF α antibody can alleviate the brain damage of rats with acute hemorrhage necrotizing pancreatitis.

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INTRODUCTION

Pancreatic encephalopathy, a syndrome of mental retardation induced by severe acute pancreatitis, has been greatly concerned by clinicians. Unfortunately, the pathogenesis and mechanism of pancreatic encephalopathy are still unclear although many factors are thought to be related to it, such as pancreatin, epiphyte infection, electrolyte disturbance, lack of vitamin, alcoholism, hypoxemia^[1-3]. Recent reports have shown that overactivation of leucocytes and overexpression of cytokines play important roles in the pathogenesis of pancreatic encephalopathy. Moreover, the high level of TNF α in patients has a remarkable correlation with pancreatic encephalopathy^[4-9]. In the present study, we attempted to block or relieve pancreatic encephalopathy by using TNF α antibody.

MATERIALS AND METHODS

Animals

One hundred and twenty male Spargue-Dawley (SD) rats

weighing 230 ± 20 g, were obtained from Animal Research Center of Shaanxi Academy of Traditional Medicine, and fed with standard rat chow.

Drugs

Sodium taurocholate (Sigma) was diluted to 50 g/L with saline prior to use. TNF α monoclonal antibody (Jingmei Co.Ltd., Guangdong, China) was diluted at 1:100 with saline prior to use.

Experimental grouping

One hundred and twenty SD rats were randomly divided into three groups: Group I: normal control group ($n = 40$), sham operation was performed and saline was retrograde injected into the pancreatobiliary duct of the rats; Group II: acute necrotizing pancreatitis group ($n = 40$), in which an acute hemorrhage necrotizing pancreatitis model was induced by retrograde injection of 50 g/L sodium taurocholate into the pancreatobiliary duct; Group III: TNF α antibody treated group ($n = 40$), in which 1 mL TNF α antibody (2.0 mg/kg) was injected into the rats through dorsum veins of penis 5 min prior to operation. Blood samples (2 mL) were taken from inferior vena cava of all animals in each group 12 h after operation. Then the rats were killed and samples were obtained for analysis.

Operation

The animals were fasted but free to drink water 12 h before operation. Then the rats were intraabdominally anesthetized by 100 g/L pentobarbital sodium (30 mg/kg), and incised through median incision of the abdomen. After the common bile duct was clamped in hepatoduodenal ligament by small artery clamps, a cannula was inserted into pancreatobiliary duct through mammary papilla from anterior wall of duodenum. Then sodium taurocholate (50 g/L) was injected by the cannula with even speed of 0.1 mL/min, the scatheless vascular clamp was removed 10 min later. Finally, the abdomen incisions were closed and the animals were given gentamicin to prevent infection^[10,11].

Evaluation of TNF α in serum

Evaluation of serum TNF α was performed by sandwich ELISA method with double antibodies. The kit was purchased from Endogen Company (USA) and the procedures were made according to the manufacturer's instructions.

Evaluation of water content in brain

Evaluations of water content in brain of 20 rats in each group were completed by the methods of dry and wet weight estimation. Water content in brain = (wet weight - dry weight) \div wet weight $\times 100\%$.

Evaluation of brain MDA and SOD content

Skulls of 20 rats in each group were opened to get frontal lobe of the brain. Then brain tissues were homogenized and centrifuged. MDA and SOD contents were gained by chemical colorimetry (kit purchased from Bioengineer Institute of Nanjing Jiancheng, China).

Congregation of leucocytes and mural counting in blood capillary

After stained by HE, the whole number of leucocytes in 20 sections

Table 1 Serum TNF α level, content of brain water, MDA and SOD as well as leucocyte mural counting in brain blood capillary in three groups

Group	Serum TNF α level (pg/mL)	Brain water content (%)	Brain MDA content (nmol/mL)	Brain SOD content (nmol/mL)	Mural leucocytes (per 20 capillary)
I ^a	25.17 \pm 2.26	77.09 \pm 0.51	5.32 \pm 1.40	13.40 \pm 2.77	6.12 \pm 1.60
II	264.58 \pm 4.39	83.17 \pm 1.42	17.26 \pm 3.18	35.52 \pm 3.10	62.15 \pm 5.18
III ^c	74.33 \pm 1.78	81.41 \pm 1.52	11.71 \pm 3.26	23.65 \pm 1.93	38.37 \pm 3.43

^a $P < 0.05$ vs II, ^c $P < 0.05$ vs II.

of brain tissues was counted under light microscope to obtain the mean number.

Statistical analysis

The data were expressed as mean \pm SD and analyzed by software of SPSS10.0. $P < 0.05$ was considered statistically significant.

RESULTS

Serum TNF α level

The level of TNF α in blood serum in acute necrotizing pancreatitis group (264.58 \pm 4.39 pg/mL) was increased markedly ($P < 0.05$) compared with that in the normal control group (25.17 \pm 2.26 pg/mL). But the level of TNF α in blood serum in the TNF α antibody treated group (74.33 \pm 1.78 pg/mL) was decreased markedly ($P < 0.05$) compared with that in acute necrotizing pancreatitis group.

Brain water content

Brain water content in the acute necrotizing pancreatitis group (83.17 \pm 1.42%) was significantly higher than that in the normal control group (77.09 \pm 0.51%). But it was significantly lower in the TNF α antibody treated group (81.41 \pm 1.52%) than in the acute necrotizing pancreatitis group ($P < 0.05$).

Brain MDA content

Brain MDA content of in the acute necrotizing pancreatitis group (17.26 \pm 3.18 nmol/mL) was increased remarkably ($P < 0.05$) compared with that in the normal control group (5.32 \pm 1.40 nmol/mL). It was decreased remarkably in the TNF α antibody treated group (11.71 \pm 3.26 nmol/mL) in comparison with acute necrotizing pancreatitis group ($P < 0.05$).

Brain SOD content

Brain SOD content in the acute necrotizing pancreatitis group (35.52 \pm 3.10 nmol/mL) was increased remarkably compared with that in the normal control group (13.40 \pm 2.77 nmol/mL). It was decreased remarkably in the TNF α antibody treated group (23.65 \pm 1.93 nmol/mL) in comparison with acute necrotizing pancreatitis group ($P < 0.05$).

Leucocyte mural counting of brain blood capillary

The number of leucocyte mural counts in blood capillary in every 20 blood capillaries in the TNF α antibody treated group (38.37 \pm 3.43) was significantly lower ($P < 0.05$) than that in the acute necrotizing pancreatitis group (62.15 \pm 5.18).

DISCUSSION

Cytokines, mainly excreted by immunocytes in human body, are soluble peptides, which play important roles in immune activation and inflammatory reactions. In normal conditions, the content of cytokines is very low. However, the expression of cytokines is increased remarkably following multiple stimulations, such as infection and trauma. It is well known that the overactivation of cytokines could induce or even aggravate

tissue damage^[12-16]. Acute severe pancreatitis could always lead to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS)^[17-24]. The present studies have focused on the pathogenesis and therapy of respiratory failure, kidney failure, hepatic failure and cardiovascular function failure complicated by acute severe pancreatitis. However, investigations on mechanism of brain damage and related preventive measures after acute severe pancreatitis remain ineffective.

TNF α is excreted by activated macrophages, endothelial cells and B lymphocytes with multiple functions. It can play important roles in inflammatory reactions. Animal experiments suggested that serum TNF α in patients with acute severe pancreatitis could induce pancreatic and other organs' damages. In the early period of acute severe pancreatitis, TNF α was involved in occurrence and progress of acute severe pancreatitis. On the other hand, TNF α was closely related with the severity and mortality of this disease^[25-31].

In acute severe pancreatitis, the mechanisms of TNF α - induced brain damage included^[32-40]: (1) TNF α could stimulate differentiation of phospholipase A2 or directly activate phospholipase, aggregate and activate leucocytes to release cellular media, thus reinforcing permeability of blood capillary including platelet active factor (PAF), leukotriene B4 (LTB4), nitrogen monoxide (NO), thromboxane A2 (TXA2), prostaglandin (PG) and oxygen-derived free radicals. The media in positive feedback could accelerate the yield of TNF α , and thereby form "waterfall" cascade reaction. (2) TNF α could directly increase the permeability of blood endothelial cells, and also act through leucocytes, up-regulate adhesive factors of endothelial cells, such as ELAM-1 and ICAM-1, thus facilitating adhesiveness and contraction of leucocytes to cause leakage of blood capillary and tissue damage. (3) TNF α could induce inflammatory injuries of myelin sheath by direct toxicity or activating immune cells.

In the present study, we attempted to relieve pancreatic encephalopathy by using TNF α antibody. The results showed that the level of serum TNF α , content of brain water, MDA and SOD as well as mural leucocyte count of the rats in TNF α antibody treated group, reduced significantly compared with those in acute necrotizing pancreatitis group. The present data suggest that TNF α antibody plays an important protective role in pancreatic encephalopathy in rats. But the optimal concentration of TNF α antibody and its influence on body immune system still need further investigation.

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