

Traditional Chinese medicine “Qing Yi Tang” alleviates oxygen free radical injury in acute necrotizing pancreatitis

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Abstract

AIM To observe the changes in oxygen free radical (OFR) and the curative effect of traditional Chinese medicine “Qing Yi Tang” in acute necrotizing pancreatitis (ANP).

METHODS After induction of ANP by injection of sodium taurocholate into pancreatic duct, 16 dogs were randomly divided into control group and Chinese medicine group. Serum amylase, SOD and MDA were determined on postoperative day 1, 2, 4 and 7. The animals were sacrificed on day 7. SOD and MDA in organs were determined, and pathological changes in pancreas were observed.

RESULTS As compared with control group, the serum level of amylase (734 U/L *vs* 2783 U/L) and MDA (7.8 nmol/ml *vs* 14.8 nmol/ml) in Chinese medicine group were decreased on day 7 ($P < 0.05$), while SOD increased significantly (281 nU/ml *vs* 55 nU/ml, $P < 0.01$), and similar changes occurred in MDA and SOD in organs, especially in the pancreas; the pathological changes in the pancreas were alleviated as well.

CONCLUSION “Qing Yi Tang” is effective in clearing OFRs and alleviating pathological changes in ANP.

INTRODUCTION

It has been shown that OFR plays an important role in the mechanism of ANP^[1], and it mediates the earliest and most fundamental pathophysiological changes, leading to injury of tissues and organs, even to multiple organ dysfunction syndrome (MODS). Zhu N^[2] reported that some Chinese medicines had inhibiting effects in peroxidation in acute pancreatitis. In this study, a formula of Chinese medicine “Qin Yi Tang” was used and its curative effect in ANP dog model was observed.

MATERIALS AND METHODS

Animal model

Adult mongrel dogs weighing 15 kg \pm 2 kg were acclimatized in the laboratory for a week before the experiment was started. Laparotomy was performed under general anesthesia with sodium thiopental, 30 mg/kg intravenously. The duodenum was exposed and turned over, and the main pancreatic duct was identified at the mesenteric margin of the duodenum. Acute pancreatitis was induced by injection of 0.5 ml/kg of 5% sodium taurocholate with 3 000U/kg trypsin into the pancreatic duct under a pressure of 7.84kPa (80cmH₂O). During the first 2-3 days after operation, 5% glucose-saline was infused as needed and anti-shock measures were taken when indicated. After induction of ANP, 16 dogs were equally divided in-to two groups at random. Group 1 (ANP, $n = 8$) received no treatment for ANP, group 2 (CM, $n = 8$) was fed with “Qing Yi Tang”, 20 ml/kg \cdot d, by gavage everyday. The ingredients of “Qing Yi Tang” include: rhubarb root, bupleurum root, white peony root, 24 g each; scutellaria root, picrorhiza rhizome, corydalis tuber, aucklandia root, sodium sulphate, 18 g each. All animals were sacrificed on the 7th postoperative day.

Parameters examined

Serum and organ superoxide dismutase (SOD) and malonyldialdehyde (MDA) Blood samples were obtained on d 1, d 2, d 4 and d 7 postoperatively. Tissues of the liver, pancreas, kidney, and ileum were harvested on d 7, weighed and homogenized with phosphate buffered solution. SOD activities were determined by xanthine oxydase method and expressed as nU/ml or mg protein. The levels of

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MDA were quantified according to the reference^[3] and expressed as nmol/ml or mg protein.

Serum GPT and BUN Monarch biochemical analyser (USA) was used for determination.

Serum amylase (Amy) Amy was measured by iodoamylum method and expressed as U/L.

Morphological studies Tissue samples of pancreas were harvested and observed under light microscope and transmission electron microscope.

Statistical analysis All results were expressed as $\bar{x} \pm s$. Data were analyzed by *t* test. *P*-values <0.05 were considered statistically significant.

RESULTS

Morphological changes of pancreas

Macroscopy In ANP group, the pancreas was enlarged, swollen and tough, with grey to dark foci, while in CM group, only uniform edema of pancreas was noticed.

Light microscopy In ANP group, there were multiple areas of hemorrhage and necrosis on a background of inflammatory infiltration. In CM group, the pancreatic acini were basically intact. There was slight interstitial congestion and edema, with mild infiltration of inflammatory cells.

Transmission electron microscopy A significant dilatation of the rough-surfaced endoplasmic reticulum (RER) was revealed in ANP group, and the mitochondria were swollen markedly as well. In CM group, there was mild dilatation of RER, without significant swelling of the mitochondria, and the zymogen granules could be easily seen.

Serum and organ SOD and MDA

In CM group, as compared with ANP group, the serum SOD activities were increased significantly ($P < 0.05$) from second postoperative day, while the serum levels of MDA were decreased significantly ($P < 0.05$, Table 1). At the same time, SOD in organs were increased markedly ($P < 0.05$), and MDA reduced significantly ($P < 0.05$), especially in the pancreas ($P < 0.01$).

Serum GPT and BUN

In CM group, as compared with ANP group, serum levels of GPT and BUN were significantly lower (Table 3).

Serum amylase

Serum amylase levels were markedly elevated after induction of ANP in both groups. But in CM group, amylase levels were significantly lower than those in ANP group on d 4, and returned to normal on d 7 (Table 4).

Table 1 Changes in serum SOD (nU/ml) and MDA (nmol/ml) levels

Group	Parameter	d 1	d 2	d 4	d 7
ANP	SOD	89.87±18.43	78.19±13.39	63.89±11.23	55.87±28.54
	MDA	21.48±3.62	26.20±5.37	20.49±4.53	14.83±2.03
CM	SOD	90.85±15.27	94.72±11.54 ^a	234.83±45.96 ^b	281.59±29.79 ^b
	MDA	20.29±3.44	16.62±2.67 ^a	11.85±2.36 ^a	7.84±1.69 ^a

^a $P < 0.05$, ^b $P < 0.01$, vs ANP.

Table 2 Levels of SOD (nU/mg) and MDA (nmol/mg) in organs

Group	Parameter	Liver	Pancreas	Kidney	Ileum
ANP	SOD	149.53±24.05	146.94±31.48	115.65±18.29	126.25±14.54
	MDA	29.56±8.42	10.41±1.38	25.80±4.49	6.06±2.19
CM	SOD	279.46±38.66 ^a	295.92±53.38 ^a	200.87±31.37 ^a	298.88±22.26 ^a
	MDA	15.83±3.21 ^a	4.52±1.09 ^b	13.37±1.02 ^a	3.24±0.64 ^a

^a $P < 0.05$, ^b $P < 0.01$, vs ANP.

Table 3 Changes in GPT (U/L) and BUN (mmol/L) levels

Group	Parameter	Before operation	d 1	d 2	d 4	d 7
ANP	GPT	22.25±8.25	72.10±12.61	54.88±10.67	41.38±6.75	25.85±8.41
	BUN	3.11±1.13	5.49±2.20	4.26±1.08	3.83±0.99	2.84±0.79
CM	GPT	18.96±4.32	68.56±12.68	34.71±9.46 ^a	28.92±5.28 ^a	24.35±6.44
	BUN	3.13±0.84	5.14±1.89	3.48±0.87 ^a	2.97±0.64 ^a	3.12±0.56

^a $P < 0.05$ vs ANP.

Table 4 Changes in serum amylase levels (U/L)

Group	Before operation	d 1	d 2	d 4	d 7
ANP	825.50±82.94	7363.25±1383.26	7060.75±1135.65	4590.25±1312.44	2783.75±893.42
CM	816.58±74.85	7158.60±1258.82	5673.43±1173.45	1567.43±863.55 ^b	743.68±101.44 ^b

^b*P*<0.01 vs ANP.

DISCUSSION

The tissues of intestine and pancreas are rich in xanthine oxidase (XOD). In healthy tissues, XOD exists as a dehydrogenase which is inactive or minimally active. During ischemia and hypoxia of the intestinal tissue and subsequent reperfusion, a large amount of xanthine dehydrogenase was converted rapidly to active XOD, promoting the oxidation of hypoxanthine which was accumulated in hypoxic tissue. Hence a burst of oxygen free radical (OFR) generation occurred, including O_2^- , O^- , OH^- , 1O and H_2O_2 . OFRs were highly reactive, with a half-life time in μs . Usually MDA, a product of lipid peroxidation, was quantified to reflect the OFR levels^[4]. Dabrowski^[5] showed a decrease of SOD in pancreatic tissue and blood in experimental acute pancreatitis and referred it to the enhanced lipid peroxidation caused by OFR. OFR can react on almost all components of cells such as phospholipids, proteins and DNA, exerting influences on cell metabolism and function, leading to destruction of tissue structure, producing a series of pathophysiologic changes. There has been evidence that OFR can cause dysfunction of acinar cell microtubule of the pancreas, releasing a large amount of abnormal secretion-zymogen granules directly into pancreatic interstitium and bloodstream. OFR-induced reduction of membrane stability may lead to release of acinar cell lysosome and activation of various pancreatic enzymes. OFR can also activate phospholipase A_2 , decompose cell membrane lecithin of the pancreas, bringing about further damage to pancreatic tissue. The long half-life lipid peroxides may reach via blood stream to the remote organs, causing extrapancreatic damage^[6]. It is believed that some severe complications of ANP, especially ARDS and MSOF, are related to OFR injury which may play a

role as a “trigger”. The data of this study demonstrated that in Chinese medicine group, in contrast to the control group, the SOD levels in serum and organs were higher significantly, while the MDA levels decreased markedly, especially in the pancreatic tissues, and the functions of the liver and kidneys were improved remarkably. These results suggested that Chinese medicine “Qing Yi Tang” could reduce OFR generation significantly, thus attenuating the lipid peroxidation injury in ANP. The mechanism of alleviating OFR injury by Chinese medicine in ANP is not clear. The effects of “Qing Yi Tang” might include: ① inhibiting the XOD activity in tissue of the pancreas, attenuating the production of OFR; ② improving the blood perfusion of gastrointestinal mucosa, alleviating its ischemic and hypoxic state, thus inhibiting the OFR generation; ③ reducing the organ damage caused by OFR and their chain reactions; ④ decreasing pancreatic enzyme release; and ⑤ playing a part in regulating the immunologic function of the organism. A large number of clinical reports has indicated that “Qing Yi Tang” has satisfactory therapeutic effects in acute edematous pancreatitis. Our study suggested that “Qing Yi Tang” could also alleviate the pathophysiologic changes in ANP, and might be beneficial to improving the prognosis.

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