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Original Article

Influence of Leptin on Severity of Acute Pancreatitis in Experimental Rat Model

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ABSTRACT

Background and Aim: In acute pancreatitis, hyperproduction of inflammatory mediators, including inflammatory cytokines, exacerbation of tissue injury due to microcirculatory disturbances, secondary activation of inflammatory cells in distant organs, and the cascade are the pathogenic basis of pancreatitis severity. Therefore, inhibition of pancreatic enzyme activity, blocking production, and preventing the activation of inflammatory cells is a practical way to prevent the development of severe acute pancreatitis and multiorgan failure. In our study, we sought to elucidate the effect of leptin on a rat model of severe acute pancreatitis. **Materials and Methods:** We prepared an acute pancreatitis model in rats by intraperitoneal injection of 20μg/kg of leptin and single injection of 0.1mL/100g of bile into a pancreatic duct extending from the duodenal papilla, measured survival, serum amylase activity, MPO activity in pancreatic tissue, SOD activity and MDA content, and observed the histopathological findings of pancreatic tissue. **Results:** Pre-administration of 20μg/kg leptin improved the survival of rats, decreased serum amylase activity, MPO activity in pancreatic tissue, increased MDA content, increased SOD activity, and improved the histopathological features of pancreatic tissue. **Conclusion:** Leptin administration prevented the destruction of pancreatic cells and improved the antioxidant function in rats with severe acute pancreatitis.
Key words: Leptin, Severe acute pancreatitis, Sprague Dawley rats, Oxidative stress, Survival rate.

INTRODUCTION

Acute pancreatitis is an autodigestion of pancreatic tissue by activated pancreatic enzymes, a disease of the digestive system that defines a variety of pathological conditions ranging from mild to shock, multiple organ failure, and severity with infection.[1-2] Severe acute pancreatitis is a multiorgan disorder syndrome that causes extensive pancreatic tissue necrosis by a variety of causes, resulting in explosive production of inflammatory mediators, including activated pancreatic enzymes and cytokines, and rapidly death within 2 weeks after onset of functional failure in distant organs.[1-2]

The protective effect of leptin on multi-organ dysfunction of complicated severe acute pancreatitis is currently well studied. *In vitro* and *in vivo* studies have shown that leptin induces neutrophil activation, migration of monocytes, production of IL-6a, promotes monocyte/macrophage activation and phagocytosis, and increases lymphocyte proliferation.[3] Leptin affects the secretory function of gastric and pancreatic tissues, protecting the gastric mucosa and also contributing to the inflammation of pancreatic tissue. Leptin has a protective effect on pancreatic tissue in severe acute pancreatitis by reducing the inflammatory response of tissues, improving tissue circulation, etc. We sought to determine the effect of leptin on severe acute pancreatitis.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (200-250g) were obtained from the Laboratory Animal Center of Pyongyang University of Medical Sciences. Animals were fed a standard rodent diet and water and bred in a controlled environment with 12-hr light-dark cycles. They were treated as recommended in the Guide for the Care and Use of Laboratory Animals issued by the DPRK Association of Laboratory Animal Care.

Severe Acute Pancreatitis Rat Model

A model of acute pancreatitis was created after anesthetizing rats by injecting 5% thiopental at a dose of 0.1mL/100g intraperitoneally. After anesthesia, a midline incision of the abdomen was made and biliary duct was punctured with a needle, and bile was injected into the pancreatic duct extending from the duodenal papilla at a dose of 0.1mL/100g. After injection of bile the incision was sutured. From the point of completion of postoperative suturing, an index examination was performed according to the time course of acute pancreatitis.

Injection of Leptin

Leptin was injected intraperitoneally at different doses such as 10μg/kg, 20μg/kg and 40μg/kg prior to

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injection of bile to assess the protective effects of leptin in severe acute pancreatitis.

Survival

Survival rate was calculated by counting the number of deaths and according to the time elapsed after bile infusion and by the percentage of live animals per total number.

Serum Amylase Activity

Serum amylase activity was measured by the iodine-starch reaction colorimetric method.

Oxidative Damage of Pancreatic Tissue

The myeloperoxidase (MPO) activity of pancreatic tissue was measured by the benzidine method. MPO activity unit is defined as 1U when absorbancy is changed by 0.01 in unit hour. Superoxide dismutase (SOD) was assayed using the SOD assay kit (Cayman) according to manufacturer instructions. One unit of SOD is defined as the amount of enzyme necessary to have 50% of inhibition. SOD activity was expressed as units/mg of proteins. Thiobarbituric acid reactive substances (TBARS) content was measured by the method of Richard *et al.* Briefly, TBARS contents were expressed in pmol of MDA/mg of proteins.

Histology

Formalin-fixed, paraffin-embedded rat liver specimens were sectioned at 4 μm and stained with HE. The sections were used for histopathology examinations by light microscopy.

Statistical Analysis of Data

Statistical analysis was carried out using SPSS software, version 14.0. Results are expressed as mean and standard deviation. Parameters were analyzed by Student *t*-test. P<0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the dose-dependent effect of leptin on survival in rats with severe acute pancreatitis. Rats with severe acute pancreatitis had a higher survival rate up to 24h at all doses in the study group compared to the model control.

As shown in Table 2, serum amylase activity was significantly decreased in the experimental group compared to the sham group, and above 20μg/kg, it was significantly decreased compared to the 10μg/kg group.

Table 1: Survival percentage in rats with severe acute pancreatitis.

Group	Dose (μg/kg)	Time after injection(h)			
		3	6	12	24
Model	-	7/7 (100%)	5/7 (71.4%)	4/7 (57.1%)	4/7 (57.1%)
Leptin	10.0	7/7 (100%)	6/7 (85.7%)	6/7 (85.7%)	5/7 (71.4%)
	20.0	7/7 (100%)	7/7 (100%)	6/7 (85.7%)	6/7 (85.7%)
	40.0	7/7 (100%)	7/7 (100%)	7/7 (100%)	6/7 (85.7%)

Data was presented as number of rats survived/total number of rats (percentage)

Table 2: Effect of leptin on serum amylase activity.

Group	Dose (μg/kg)	Serum amylase activity
Model	-	3629.3±41.9
Leptin	10.0	3264.5±70.8*
	20.0	2756.4±82.3**△
	40.0	2534.8±89.6**△

Data was expressed as mean±SE.

* indicates comparison with control group; *P<0.05; **P<0.01

△ indicates comparison with 10μg/kg group; △P<0.05

Table 3: Influence of MPO, SOD activity and MDA content in pancreatic tissue.

Group	MPO (U/mg)	SOD (U/mg)	TBARS (pmol/mg)
Sham	6.5±0.33	38.74±2.75	19.63±3.11
Model	19.1±2.4	10.73±1.23	75.93±4.06
Leptin	9.2±3.7*	30.20±2.87*	40.65±5.31*

Data was expressed as mean±SE.

* indicates comparison with control group; *P<0.05

Table 4: Pancreatic degenerative changes in rats with acute pancreatitis by bile infusion.

Group	Edema	Hemorrhage	Necrosis	Cellular infiltration
Model	+++	+++	+++	+++
Leptin	+	+	+	+

+: weak positive, +++: strong positive

In rats with severe acute pancreatitis, the MPO activity and SOD activity at 14h after leptin administration were significantly higher than in the control group, and the MDA content was significantly lower (Table 3).

As shown in Table 4, pancreatic degenerative changes were observed in rats with acute pancreatitis induced by bile infusion, and the changes improved in the leptin infusion group.

DISCUSSION

Severe acute pancreatitis is considered to be one of the refractory diseases without specific treatment due to its multifactorial and multifactorial nature, severe complications such as shock, hypoxia, dyspnea, ascites, hyperglycemia, hypokalemia, multiorgan dysfunction, DIC, rapid progression and high mortality.[1,4]

Since the study of severe acute pancreatitis is growing at the cellular and molecular level, the traditional pancreatic enzyme abnormalities and the "self-digestion hypothesis" cannot fully understand the mechanisms of severe acute pancreatitis, many recent studies have focused on the rationale for treating severe acute pancreatitis by proposing novel pathogenic mechanisms such as pancreatic microcirculation, inflammatory mediators and cytokine release, apoptosis, and ischemia-reperfusion injury.[2] Extensive damage to pancreatic acinar cells due to impaired pancreatic tissue microcirculation, digestion of the pancreas and adjacent organ tissues by pancreatic enzyme activity, absorption of these metabolites and inflammatory cytokines. The activation of leukocytes in distant organs, and the development and progression of SIRS and MODS in a cascade of inflammation underlie the pathogenesis of pancreatitis

severity. As an experimental model for the pathogenesis and treatment of severe acute pancreatitis, cellulase + LPS infusion model, biliary ductal desoxycholic acid, L -arginine, and sodium taurocholate infusion models have been proposed, but there are some differences in dose, method, and severity-related parameters.

It has been reported in many studies that leptin is protective against multiorgan dysfunction complicated by severe acute pancreatitis.[3] *In vitro* and *in vivo* studies, leptin promotes neutrophil activation and monocyte homing, induces IL-6a production, promotes monocyte/macrophage activation and phagocytosis, and increases lymphocyte proliferation.[5-6] Leptin affects the secretory function of gastric and pancreatic tissues to protect the gastric mucosa from damaging harmful substances and also has an effect on pancreatic inflammation.[7] Leptin exerts a protective effect on pancreatic tissue in severe acute pancreatitis, reducing the inflammatory response of tissues and improving tissue blood circulation.[8] In our study, after pre-administration of leptin, the rats with severe acute pancreatitis had a higher survival rate up to 24hr at all doses in the study group compared to the model control, as shown in Table 1. In addition, as shown in Table 2, serum amylase activity was significantly decreased in the experimental group compared to the sham group, and above 20μg/kg, it was significantly decreased compared to the 10μg/kg group. In addition, as shown in Table 3, 14hr after leptin administration, MPO activity and SOD activity were significantly higher in severe acute pancreatitis rats compared to the control group, and MDA content was significantly lower. On the other hand, as shown in Table 4, pancreatic degeneration was observed in rats with acute pancreatitis induced by bile infusion, and it improved in the leptin infusion group.

CONCLUSION

Leptin administration prevented the destruction of pancreatic cells and improved the antioxidant function in rats with severe acute pancreatitis by bile infusion, thus preventing multiorgan dysfunction caused by severe acute pancreatitis.

However, this is only a preliminary study of the action of leptin, and the pathophysiology of acute pancreatitis and its associated multiorgan failure remain to be investigated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SAP: Severe Acute Pancreatitis; **DIC:** Diffuse Intravascular Coagulation; **SIRS:** Systemic Inflammatory Response Syndrome; **MODS:** Multiple Organ Dysfunction Syndrome.

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