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• BASIC RESEARCH •

# A mouse model of severe acute pancreatitis induced with caerulein and lipopolysaccharide

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## Abstract

**AIM:** To establish a non-traumatic, easy to induce and reproducible mouse model of severe acute pancreatitis (SAP) induced with caerulein and lipopolyasccharide (LPS).

**METHODS:** Thirty-two healthy mature NIH female mice were selected and divided at random into four groups (each of 8 mice), i.e., the control group (NS group), the caerulein group (Cn group), the lipopolysaccharide group (LPS group), and the caerulein+LPS group (Cn+LPS group). Mice were injected intraperitoneally with caerulein only, or LPS only, and caerulein and LPS in combination. All the animals were then killed by neck dislocation three hours after the last intraperitoneal injection. The pancreas and exo-pancreatic organs were then carefully removed for microscopic examination. And the pancreatic acinus was further observed under transmission electron microscope (TEM). Pancreatic weight, serum amylase, serum nitric oxide (NO) concentration, superoxide dismutase (SOD) and malondialdehyde (MDA) concentration of the pancreas were assayed respectively.

**RESULTS:** (1) NS animals displayed normal pancreatic structure both in the exocrine and endocrine. In the LPS group, the pancreas was slightly edematous, with the infiltration of a few inflammatory cells and the necrosis of the adjacent fat tissues. All the animals of the Cn group showed distinct signs of a mild edematous pancreatitis characterized by interstitial edema, infiltration of neutrophil and mononuclear cells, but without obvious parenchyma necrosis and hemorrhage. In contrast, the Cn+LPS group showed more diffuse focal areas of nonviable pancreatic and hemorrhage as well as systemic organ dysfunction. According to Schmidt's criteria, the pancreatic histologic score showed that there existed significant difference in the Cn+LPS group in the interstitial edema, inflammatory infiltration, parenchyma necrosis and parenchyma homorrhage in comparison with those of the Cn group, LPS group and NS group ($P<0.01$ or $P<0.05$). (2) The ultrasturcture of acinar cells was seriously damaged in the Cn+LPS group. Chromatin margination of nuclei was present, the number and volume of vacuoles greatly increased. Zymogen granules (ZGs) were greatly decreased in number and endoplasmic reticulum exhibited whorls. The swollen mitochondria appeared, the crista of which was decreased in number or disappeared. (3) Pancreatic weight and serum amylase levels in the Cn +LPS was significantly higher than those of the NS group and the LPS group respectively ($P<0.01$ or $P<0.05$). However, the pancreatic wet weight and serum amylase concentration showed no significant difference between the Cn+LPS group and the Cn group. (4) NO concentration in the Cn+LPS group was significantly higher than that of NS group, LPS group and Cn group($P<0.05$ or $P<0.01$). 5) The SOD and MDA concentration of the pancreas in the Cn+LPS group were significantly higher than those of NS, LPS and Cn groups ($P<0.05$ or $P<0.01$).

**CONCLUSION:** The mouse model of severe acute pancreatitis could be induced with caerulein and LPS, which could be non-traumatic and easy to induce, reproducible with the same pathological characteristics as those of SAP in human, and could be used in the research on the mechanism of human SAP.

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## INTRODUCTION

Severe acute pancreatitis (SAP) is characterized by local pancreatic necrosis as well as systemic organ failure and is still associated with a higher morbidity and mortality despite continuing improvements in critical care[1-9]. Several animal models have been developed for studying the mechanism, recovery, prognosis and treatment of human SAP[10-12]. However, these methods such as retrograde pancreaticduct injection, intraductal infusion of sodium taurocholate, closed duodenal loop, pancreatic duct obstruction are so invasive and complex in operation that the mortality of the animals was high[10-12]. The aim of this study is to establish a SAP animal model induced with caerulein and lipopolyasccharide (LPS), which is non-traumatic, convenient and easy to practise, and reproducible and with the same histopathologic characteristics as those of SAP in human.

## MATERIALS AND METHODS

### *Chemicals*

Caerulein and LPS (*Escherichia coli* 0111:B4) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Assay kits for serum amylase, NO, SOD and MDA concentration were from Nanjing Jiancheng Biological Products Institute (Nanjing, China). Other reagents used were of analytic grade.

### *Animal and grouping*

Thirty-two healthy mature NIH female mice, weighing 22.0-28.0 g, provided by Experimental Animal Center of Zhejiang Academy of Medical Sciences, were selected and divided at random into four groups (each of 8 mice), i.e. the control group (NS group), the caerulein group (Cn group), the

lipopolysaccharides group (LPS group), the caerulein + lipopolysaccharides group ( Cn+LPS group).

### *Establishment of the model of severe acute pancreatitis*

All the female NIH mice were fed with a standard diet and fasted for 18 hours before induction of pancreatitis. They received water ad libitum during the experiments. In the NS group, the animals were injected intraperitoneally by normal saline at a dose of 50 ml/kg at a 1 h interval six times. In the Cn group, acute pancreatitis was induced by six intraperitoneal injections of caerulein administered at a dose of 50 μg/kg at a 1 h interval. This pancreatitis was mild, and all animals survived. In the LPS group, the animals were injected intraperitoneally with normal saline (50 ml/kg) six times every one hour prior to the intraperitoneal infusion of LPS (10 mg/kg). In the Cn + LPS group, the mice were induced by LPS challenge after induction of mild pancreatitis, i.e., the mice received intraperitoneal injections of 10 mg/kg LPS immediately after the sixth caerulein injection. Three hours later, all the animals were killed by neck dislocation and the pancreas was carefully dissected from its attachment to the stomach, duodenum and spleen. Fat and tissue excess were trimmed away. The pancreas was rinsed with saline, blotted on paper and weighed. And then, one part was fixed and embedded in paraffin wax for histological analysis; the other part was immediately frozen in liquid nitrogen and stored at -78 ℃ until the measurement of SOD and MDA concentration. And the liver, kidney and lung were also carefully removed. Blood samples were obtained from femoral artery and were stored at -78 ℃ until use.

### *Histological examination*

For histological examination, the pancreas was fixed in 10 % formaldehyde for 24 hours, embedded in paraffin, and stained with haematoxylin and eosin. According to Schmidt' s standard[13], a pathologist who was blinded to the treatment protocol scored the tissues for edema, inflammatory infiltration, parenchymal necrosis, and hemorrhage in 20 fields. Grading for edema was scaled as: 0, absent or rare; 1, edema in the interlobular space; 2, edema in the intralobular space; and 3, the isolated-island shape of pancreatic acinus. Inflammation was scored as: 0, absent; 1, mild; 2, moderate; and 3, severe. The parenchyma necrosis was as follows: 0, absent; 1, focal (<5 %); 2, and/or sublobular (<20 %); 3, and/or lobular (>20 %). The parenchyma hemorrhage was scored as: 0, absent; 1, mild; 2, moderate and 3, severe. Liver, kidney and lung sections were similarly stained and assessed for histological changes.

Separate experiment was performed to observe the changes of the pancreas. Pancreatic tissues were double fixed in 2.5 % glutaraldehyde and 1 % osmic acid for 2 hours, then stained with 2 % acetic acid U rapidly, dehydrated in a graded series of alcohol and acetone, embedded in Epon 812 and cut with Leica Ultracut UCT ultramicrotome. Specimens were double stained by acetic acid U and lead citrate fluid and examined with Philips Tecnai 10 TEM operated at 80Kv.

### *Measurement of serum amylase, NO concentration, SOD and MDA concentration of the pancreas*

Serum amylase and NO concentration were assayed according to I-starch chromatometry and a copper-coated cadmium reduction method respectively. The pancreas was isolated and was made homogenate. SOD and MDA concentration of the pancreas were measured by the xanthine oxidase method and the sulfo-barbituric acid method respectively.

### *Statistical analyse*

Data were expressed as means ± SD and analyzed by two-tailed Student' s *t* test.

## RESULTS

### *Histological examination*

**Microscopic examination** NS animals displayed normal pancreatic histology both in the exocrine and endocrine. In the LPS group, the pancreas was slightly edemamous (Figure 1), with the infiltration of a few inflammatory cells and the necrosis of the adjacent fat tissues. The animals of the Cn group treated with caerulein, showed distinct signs of a mild edematous pancreatitis characterized by interstitial edema (Figure 2), infiltration of neutrophil and mononuclear cells, but without obvious parenchyma necrosis and hemorrhage. The organs, except for the pancreas, showed normal histological characteristics. However, the animals of the Cn+LPS group, which was induced with caerulein and aggravated by subsequent LPS injection, showed the features of a severe form of acute pancreatitis characterized by expansion of interlobular and intralobular spaces caused by moderate to severe interstitial edema, extensive infiltration with inflammatory cells, more diffuse focal areas of nonviable pancreatic parenchyma (Figure 3). Necrosis of peripancreatic fat was also a distinct feature in these animals. In addition, renal cells were swollen (Figure 4A); the lobules of liver was disorganized, with the vacuolization of liver cells (Figure 4B) and a lot of erythrocyte and inflammatory cells also infiltrated in the cavity of pulmonary alveolus (Figure 4C).

According to Schmidt' s criteria, the histological score showed that there existed significant difference in the Cn+LPS group in the interstitial edema, inflammatory infiltration, parenchyma necrosis and parenchyma homorrhage as compared with those of the Cn group, the LPS group and the NS group ($P<0.01$ or $P<0.05$) (Table 1).

**Table 1** Comparison of the pancreas lesion between groups ($n=8$)

| Groups | Interstitial edema | Inflammatory infiltration | Parenchyma necrosis | Parenchyma hemorrhage |
|---|---|---|---|---|
| NS | 0.0 | 0.0 | 0.0 | 0.0 |
| Cn | $1.95\pm0.26^{b}$ | $1.12\pm0.22^{bc}$ | $0.63\pm0.09^{bd}$ | 0 |
| LPS | $0.25\pm0.03^{ad}$ | $0.25\pm0.43^{bd}$ | 0 | 0 |
| Cn+LPS | $2.75\pm0.43^{bc}$ | $2.88\pm0.33^{bc}$ | $2.75\pm0.33^{bd}$ | $1.25\pm0.26^{bd}$ |

$^{a}P<0.05$, $^{b}P<0.01$, *vs* NS group; $^{c}P<0.05$, $^{d}P<0.01$, *vs* Cn group.

**Electron microscope examination of the pancreatic acinus** The ultrastructure of acinar cells was normal in the NS group. In the cytoplasm of the acinar cells, a plenty of rough endoplasmic reticulum (RER) and ribosome and a great deal of ZGs appeared. After LPS stimulation, a few cytoplastic vacuoles formed in acinar cells (Figure 5). In the Cn group, a few cytoplastic vacuoles in acinar cells also appeared, and ZGs were decreased in number. The RER and the mitochondria was slightly swollen (Figure 6). However, in the Cn+LPS group, the morphological alterations of mouse pancreatic acinar cells were observed under transmission electron microscope. Chromatin margination of nuclei was present (Figure 7A), the number of vacuoles greatly increased and their volume also greatly increased (Figure 7B). ZGs were greatly decreased in number and endoplasmic reticulum exhibited whorls (Figure 7C). The swollen mitochondria appeared, the crista of which was decreased or disappeared (Figure 7D).

### *Comparison of pancreatic weight and serum amylase*

Subsequent experiment showed that pancreatic weight and serum amylase in the Cn +LPS and the Cn group were significantly higher than those in the NS group and the LPS group respectively ($P<0.01$ or $P<0.05$). However, the pancreatic wet weight and serum amylase showed no significant difference between the Cn+LPS and the Cn groups (Table 2).

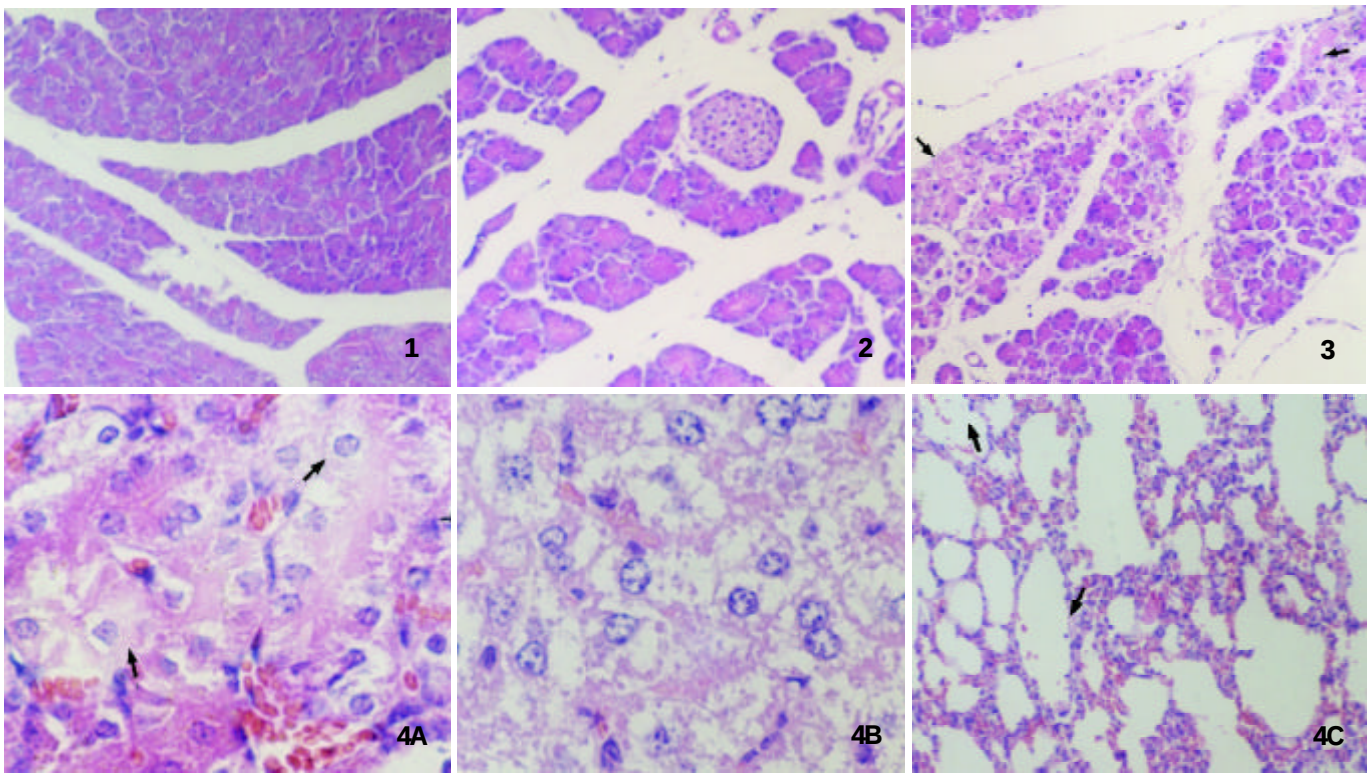


**Figure 1** In the LPS group, the pancreas was slightly edemamous. ×100.
**Figure 2** Microscopic section of the pancreas from the Cn group, showing the features of acute edematous pancreatitis notably interstitial edema. ×100.
**Figure 3** In the Cn+LPS group, more diffuse focal areas of nonviable pancreatic parenchyma appeared obviously. ×100.
**Figure 4** The histological change of the exo-pancreatic organs in the Cn+LPS group. A: Renal cells were swollen; ×100; B: The vacuolization of liver cells; ×400; C: A lot of erythrocyte and inflammatory cells also infiltrated in the cavity of pulmonary alveolus. ×100.

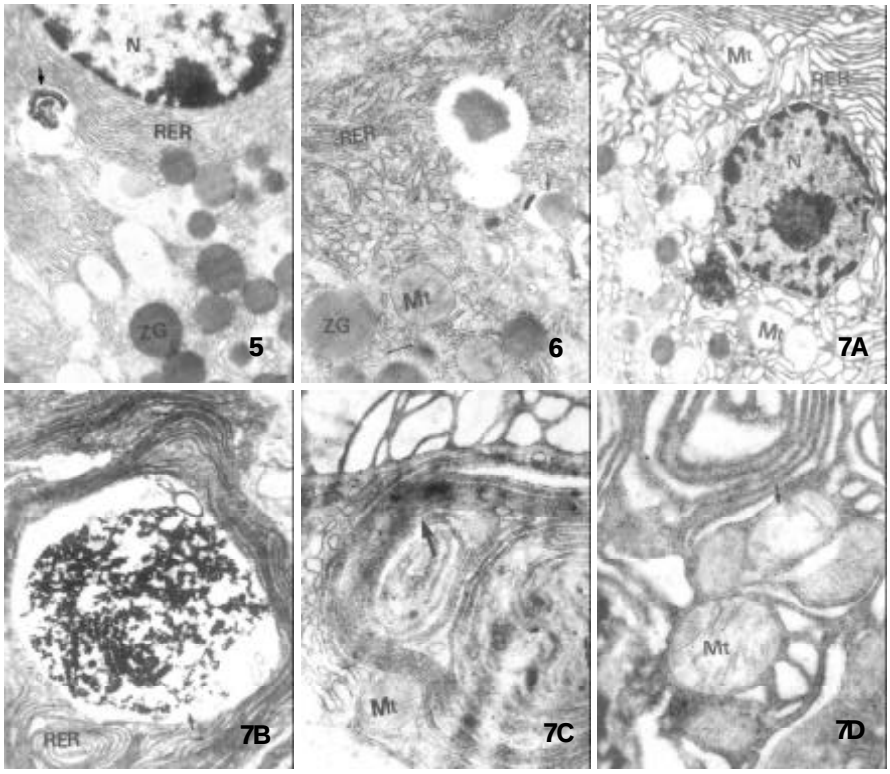


**Figure 5** A few cytoplastic vacuoles formed in acinar cells in the LPS group. ×10 000.
**Figure 6** In the Cn group, a few cytoplastic vacuoles in acinar cell appeared, and ZGs were decreased in number. The RER and the mitochondria was slightly swollen. ×10 000.
**Figure 7** The ultrastructure change of the pancreatic acinus in the Cn+LPS group. A: Chromatin margination of nuclei was present, the swollen mitochondria appeared; ×7 000; B: the number of vacuoles greatly increased and their volume also greatly increased; ×7 000; C: the endoplasmic reticulum exhibited whorls; ×14 000; D: The crista of mitochondria was decreased or disappeared. ×20 000.

**Table 2** Comparison of pancreatic wet weight and serum amylase between groups ($\bar{x}\pm s$)

| Groups | Pancreatic weight (mg) | Serum amylase (U/L) |
|---|---|---|
| NS | 247.70±30.20 | 1861.35±303.36 |
| Cn | 337.20±50.90[bc] | 11042.32±528.03[bd] |
| LPS | 290.10±39.70[a] | 2385.50±73.60[b] |
| Cn+LPS | 380.00±32.00[bc] | 10031.70±906.19[bd] |

[a]$P$<0.05, [b]$P$<0.01, *vs* NS group; [c]$P$<0.05, [d]$P$<0.01, *vs* LPS group.

### *Comparison of NO concentration*

There was significant difference in the concentration of NO among the groups (Figure 8). The results showed that the concentration of NO in the Cn+LPS group was significantly higher than that in the NS, LPS and Cn groups ($P$<0.01).

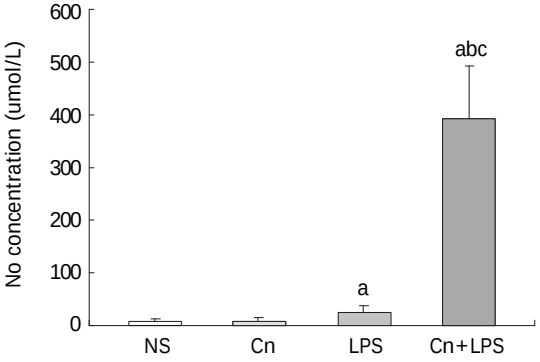


**Figure 8** Comparison of NO concentration between groups ($\bar{x}\pm s$) ($n$=8). [a]$P$<0.01, *vs* NS group; [b]$P$<0.01, *vs* LPS group; [c] $P$<0.05, *vs* Cn group.

### *SOD and MDA concentration of the pancreas*

The concentration of SOD and MDA in the pancreas showed significant difference between groups (Table 3). SOD concentration of the pancreas in the Cn+LPS group decreased significantly as compared with that of the NS, LPS and Cn groups. However, MDA concentration of the pancreas in the Cn+LPS group increased significantly compared with that of the NS, LPS and Cn groups.

**Table 3** Comparison of SOD and MDA concentration between groups ($\bar{x}\pm s$) ($n$=8)

| Groups | SOD concentration (NU/mgprot) | MDA concentration (nmol/mgprot) |
|---|---|---|
| NS | 151.67±10.74 | 0.74±0.34 |
| Cn | 135.73±10.87[a] | 0.79±0.31 |
| LPS | 136.05±17.25[a] | 0.97±0.29 |
| Cn+LPS | 85.13±11.19[bcd] | 1.22±0.24[bd] |

[a]$P$<0.05, [b]$P$<0.01, *vs* NS group; [c]$P$<0.01, *vs* LPS group; [d]$P$<0.01, *vs* Cn group.

## DISCUSSION

Acute pancreatitis may be classified histologically as interstitial edematous or necrotizing according to the inflammatory changes in the pancreatic parenchyma[14-19]. As for a mild edematous form, the pancreas observed under light microscope showed interstitial edema, interstitial hyperemia and inflammatory cell infiltration and occasionally punctate fat necrosis, but without obvious parenchyma necrosis and hemorrhage[20-24]. However, a severe necrotizing form is characterized by extensively coagulative necrosis and hemorrhage as well as systemic organ dysfunction[25-34]. In the present study, the results showed that the animals of the Cn group, which was treated with caerulein only, showed distinct signs of a mild edematous pancreatitis, indicating that the pancreatic weight and serum amylase became gradually higher than those of the NS group and the LPS group. The microscopic examination showed interstitial edema and inflammatory cell infiltration in the pancreas, and slight parenchyma necrosis and hemorrhage. And the organs except the pancreas, had a normal histological feature. Furthermore, transmission electron microscopic observation of the acinus cells displayed a few cytoplastic vacuoles in acinar cell, and ZGs were found decreased in number and the RER and the mitochondrion were slightly swollen. In the LPS group which was treated with LPS only, serum amylase and pancreatic weight had an increasing tendency, but the pancreas showed interstitial edema and the formation of a few vacuoles in the cytoplasm of the acinar cells. LPS could not induce acute pancreatitis, which further confirmed Jaworek' s findings[35]. In the Cn+LPS group, which was induced by caerulein and aggravated by subsequent LPS injection, the level of serum amylase and pancreatic weight increased and the pancreas became edematous and the inflammatory cells infiltrated and necrosis and hemorrhage appeared obviously in the pancreas, the ultrastructure of the acinus was destroyed, the organs except the pancreas was lesioned to a different extent. Moreover, in the histological score, there existed significant difference in the Cn+LPS group in the interstitial edema (2.75±0.43), inflammatory infiltration (2.88±0.33), parenchyma necrosis (2.75±0.33) and parenchyma homorrhage (1.25±0.26) in comparison with those of carerulein only (1.95±0.26; 1.12±0.22; 0.63±0.09; 0), those of LPS only (0.25±0.03; 0.25±0.43; 0; 0) and the NS group (0; 0; 0; 0) ($P$<0.01 or $P$<0.05). It is thus clear that, when induced with caerulein and LPS in combination, the pancreas was destroyed so severely as to result in inflammatory reaction in the body and systemic organ dysfunction. Moreover, the model induced with caerulein and LPS was almost stable under the condition of duplications. Therefore, the mouse model induced with caerulein and LPS was non-traumatic, convenient, easily replicating and bearing the same histological changes as those of human SAP.

Caerulein is a kind of ten-peptide substances, the analog of cholecystokinin, and possesses different biological activities[36,37]. Caerulein can stimulate the acinar cells to excrete a large amount of digestive enzyme and pancreatic fluid, resulting in a mild edematous pancreatitis characterized by a higher serum amylase level, interstitial edema, leukocyte infiltration and the vacuolation of acinar cells[36,37]. LPS is a kind of endotoxin, which could activate the mononuclear cell system to release cytokines to switch on systemic inflammation reaction[38]. Clinically, the endotoxin level was related to the severity of the illness. Therefore, the mechanism to develop severe acute pancreatitis and organ failure with caerulein and LPS might be that caerulein could activate the pancreatin to destroy the pancreas, and detonate inflammatory cells to release inflammation mediators and subsequently LPS challenged the reaction of inflammation medium, thus developing local pancreatitis into severe inflammation reaction in the body[39-50].

Recent evidence indicated that these cytokines from the inflamed pancreas can activate the production of the inducible nitric oxide (NO) synthase, resulting in overproduction of NO, which acts as a key final cellular and intercellular mediator[51-53]. In this study, the concentration of NO was significantly higher in the Cn+LPS group, as compared with that of caerulein alone or LPS alone ($P$<0.01). NO as an endothelium-derived relaxing factor (EDRF) and a highly reactive free radical, is produced from the amino acid L-arginine by a family of isoenzymes, the nitric oxide synthases (NOS). Two broad groups can be

identified: constitutive (cNOS) and inducible (iNOS). cNOS is present predominantly as a normal constituent of healthy endothelial cells (endothelial isoform, eNOS) and synthesizes NO in small amounts in response to physical or receptor stimulation. iNOS is not a normal cellular constituent, but can be expressed in a wide variety of cells and generates large amounts of NO in a sustained and largely uncontrolled manner. Excessive production of NO causes vasodilatation and hypotension that is refractory to vasoconstriction, together with increased microvascular permeability and extravascular third spacing. The physiological inability to correct these adverse responses results in end organ hypoperfusion, oedema, initiation of anaerobic metabolism, and end organ dysfunction. Moreover, the reaction of NO with superoxide causes the formation of peroxynitrite, which is a powerful oxidant and cytotoxic agent and may play an important role in the cellular damage associated with the overproduction of NO. The spontaneous reaction of peroxynitrite with proteins makes the nitration of tyrosine residues to form nitrotyrosine, which is a specific nitration product of peroxynitrite and a marker for peroxynitrite induced oxidative tissue damage. It is thus evident that the increase of NO concentration is related to the lesion of pancreas and other organs. In addition, the concentration of SOD as an antioxidant significantly lowered and that of MDA as the lipid peroxide significantly increased, which further indicated that the free radical reaction and oxidation response could be intensified with caerulein and LPS so that a mild edematous form could change subsequently into a severe necrotizing form.

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