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• BASIC RESEARCH •

Effects of octreotide on acute necrotizing pancreatitis in rabbits

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Abstract

AIM: To assess the role of oxygen-derived free radicals and cytokines in the pathogenesis of taurocholic acid-induced acute pancreatitis, and to evaluate the preventive effects of octreotide towards the development of acute pancreatitis.

METHODS: Acute pancreatitis was induced in male New Zealand white rabbits by retrograde injection of 0.8 mL/kg·b.m. of 50 g/L sodium taurocholate (NaTC) in the pancreatic duct. Sham-operated animals served as control. Octreotide 1 mg/kg·b.m. was administered subcutaneously before the induction of pancreatitis. Blood was taken from the jugular vein before and at 1, 3, 6, 12 and 24 h after pancreatitis induction. Serum activities of amylase, IL-6 and TNF-α and levels of malonyl dialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), catalase and superoxide dismutase (Mn-, Cu-, and Zn-SOD) in pancreatic tissue were measured.

RESULTS: Serum TNF-α and IL-6 levels increased significantly 3 h after the onset of pancreatitis, and then returned to control level. The tissue concentration of MDA was significantly elevated at 24 h, while the GSH level and GP-x, catalase, Mn-SOD, Cu-, Zn-SOD activities were all significantly decreased in animals with pancreatitis as compared to the control. Octreotide pretreatment significantly reversed the changes in cytokines and reactive oxygen metabolites. Octreotide treatment did not alter the serum amylase activity and did not have any beneficial effects on the development of histopathological changes.

CONCLUSION: Oxygen-derived free radicals and proinflammatory cytokines are generated at an early stage of NaTc-induced acute pancreatitis in rabbits. Prophylactic octreotide treatment can prevent release of cytokines and generation of reactive oxygen metabolites, but does not have any beneficial effects on the development of necrotizing pancreatitis.

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INTRODUCTION

Acute pancreatitis is clinically classified into mild and severe forms. Mild or edematous acute pancreatitis is a self-limiting disease with a low complication and mortality rate. However, severe necrotizing pancreatitis has an unacceptably high morbidity and mortality rate. Multiple therapeutic modalities have been suggested for acute pancreatitis, but none has been unambiguously proven to be effective yet. The major problem is that the pathophysiology of the disease is not fully understood and hence, there is no specific casual treatment yet. The treatment of acute pancreatitis to date is essentially supportive[1-3].

Theories on the pathogenesis of acute pancreatitis suggest that autodigestion of the gland and peripancreatic tissues by activated digestive enzymes is a key component[4,5]. Furthermore, stimulation of exocrine pancreatic secretion in experimental acute pancreatitis has been demonstrated to worsen the disease. Prevention of release and activation of enzymes by inhibition of pancreatic exocrine secretion has been therefore suggested as a specific treatment. Somatostatin and its long-acting analogue octreotide are potent inhibitors of pancreatic secretion[6, 7]. The efficacy of somatostatin and octreotide in the management of acute pancreatitis has been studied for decades, yet the data still remain inconclusive. Some experimental[8,9] and clinical[10] studies have shown beneficial results, but others[11-14] demonstrated no benefit[15,16].

Somatostatin and octreotide increase the tone of the sphincter of Oddi, which can be reversed by administration of glyceryl trinitrate[17,18]. Furthermore, octreotide may trigger acute pancreatitis and worsen the disease[19].

We studied the effects of octreotide on necrotizing pancreatitis in rabbits. In these animals the pancreatic duct enters the duodenum at its distal part, and is completely separated from the common bile duct. Therefore, we can exclude the effect of octreotide on the sphincter of Oddi. The present study was to assess the roles of oxygen-derived free radicals and cytokines in the pathogenesis of taurocholic acid-induced acute pancreatitis, and to evaluate the preventive effects of octreotide on the development of acute pancreatitis.

MATERIALS AND METHODS

Animals

New Zealand white rabbits weighing 2.5-3.5 kg were used. The animals were kept at a constant room temperature of 27 ℃, and had free access to water and a standard laboratory chow [LATI, Gödöllö, Hungary]. The experimental protocol followed the principles of Laboratory Animal Care of the National Institute of Health, USA.

Experimental protocol

Overnight fasted animals were anesthetized with an intravenous

injection of pentobarbital 20 mg/kg and urethane 1 g/kg, and supplemented when needed. Four groups of animals were prepared through a midline incision; the pancreatic duct was cannulated transduodenally with a polyvinyl catheter. Acute pancreatitis was induced by retrograde intraductal infusion of 0.8 ml/kg· b.m. of 50 g/L sodium taurocholate (NaTc) (Reanal, Budapest) dissolved in 0.15 mol/L NaCl under steady manual pressure over a period of 30 s (Group I)[20]. After infusion the catheter was removed, and the abdomen was closed in two layers. In control animals, laparotomy was performed with visualization of the pancreatic duct before closure of the abdomen [Group II]. Animals in which pancreatitis was induced by administration of NaTc were injected subcutaneously with 1 mg/kg· b.m. octreotide (SANDOSTATIN Novartis, Basel, Switzerland) before pancreatitis induction (Group III). In Group IV animals were treated with saline before induction of pancreatitis. The body temperatures and weights of the animals were measured every 6 h. Blood was taken from the jugular vein before and at 1, 3, 6, 12 and 24 h after pancreatitis induction. Physiologic saline was injected into the jugular vein during the experiment in order to avoid severe hypovolemia. Twenty-four hours after the abdominal operation, the animals were sacrificed by aorta exsanguination. The pancreas was removed.

Assays

Serum amylase activity was measured by the Phadebas test method[21]. TNF-α levels were titrated in a bioassay on the WEHI-164 cell line[22]. IL-6 concentrations were measured via their proliferative action on the IL-6-dependent mouse hybridoma cell line B-9[23]. The activities were calibrated against recombinant TNF [Genzyme, Cambridge, UK] and recombinant IL-6 [Sigma-Aldrich, Munich, Germany].

The pancreata were homogenized. The homogenates were centrifuged at 3 000 r/min for 10 min and the supernatants were used for measurements.

Lipid peroxide MDA level was measured after reaction with thiobarbituric acid, according to the method of Placer *et al*.[24], and was corrected for the protein content of the tissue.

Superoxide dismutase (SOD) activity was determined on the basis of the inhibition of epinephrine-adrenochrome autoxidation[25]. Mn-SOD activity was measured by the autoxidation method in the presence of 5×10^{-3} mol/L KCN[26]. Cu-, Zn-SOD activity resulted from the deduction of Mn-SOD from SOD activity.

Catalase activity was determined spectrophotometrically at 240 nm by the method of Beers *et al*.[27] and expressed in Bergmeyer units (BU) (1 BU=decomposition of 1 g H_2O_2/min at 25 ℃).

Glutathione (GSH) level was measured spectrophotometrically with Ellman' s reagent from the supernatant, and was corrected for the protein content of the tissue[28]. Glutathione peroxidase (GPx) activity was determined according to the ' chemical' method, using cumene hydroperoxide and reduced GSH as substrates of GPx[29].

Protein concentration of the pancreatic tissue was determined by the method of Lowry *et al*.[30].

Histologic examination

A portion of the pancreas was fixed overnight in a 6% neutral formaldehyde solution and embedded in paraffin. Tissue slices were subjected to hematoxylin and eosin staining and histologic study by light microscopy. Slides were coded and examined blind by the pathologist for the grading of histologic alterations. Grading of intestinal edema, vacuolization, inflammation, hemorrhage and acinar cell necrosis was performed on a scale of 1 to 4.

Statistical analysis

Results are expressed as mean±SE. Experiments were evaluated statistically with two-way analysis of variance (ANOVA). *P* value less than 0.05 was statistically significant.

RESULTS

Seven animals died (2 within 1 h, 3 at 1 h, and 2 at 6 h after pancreatitis induction). Two of them were in the octreotide pretreatment (Group III), 2 in the saline pretreatment (Group IV), and 3 in the acute pancreatitis group (Group I). Thus, the results were the data on the surviving 32 animals (8 rabbits in each group). The results of Group III did not statistically differ from those of Group IV, therefore they were not depicted in figures and tables.

The body weights of the animals in the 3 groups did not change significantly during the experiments, indicating that the parenteral fluid supplementation during the observation period was sufficient.

The serum amylase activity was already increased significantly at 1 h, and increased gradually up to 24 h after the induction of pancreatitis in Group I as compared with the control group (5 678±881 *vs* 517±99 U/L) (Figure 1). Pretreatment of pancreatitic animals with octreotide (Group III) did not alter the serum amylase activity as compared with Group I at any time point. Since the amylase, TNF-α and IL-6 levels in Group IV did not differ significantly from those in Group III, they were not shown.

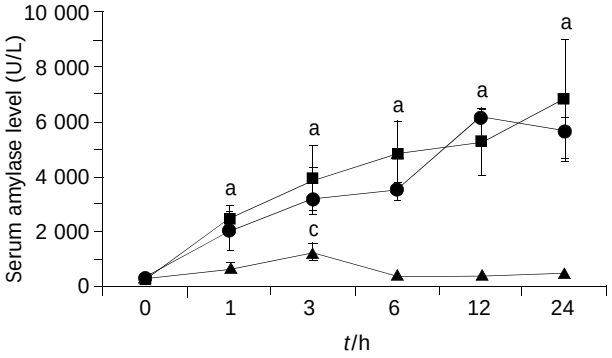

Figure 1 Effects of octreotide on serum amylase levels in rabbits with NaTc-induced acute pancreatitis. Group I: ●; Group II as control: ▲; Group III treated with octreotide: ■. $^aP<0.05$ *vs* Group II; $^cP<0.05$ *vs* Group I.

The serum TNF-α level increased significantly 3 h after the onset of pancreatitis in Group I (3 120±340 *vs* 85±15 U/L in Group II), and returned to the control level by 6 h (Figure 2). There was no significant serum TNF-α level elevation in the octreotide treated group.

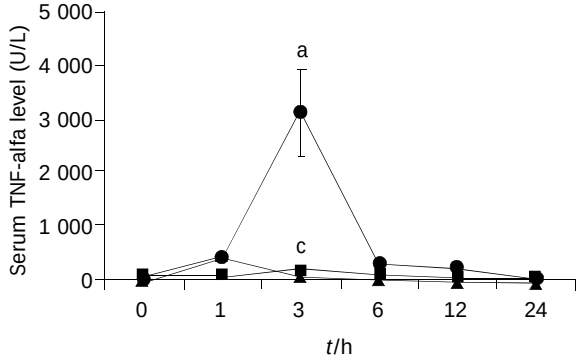

Figure 2 Effects of octreotide on serum TNF-α levels in rabbits with NaTc-induced acute pancreatitis. Group I:●; Group II as control: ▲; Group III treated with octreotide: ■. $^aP<0.05$ *vs* Group II; $^cP<0.05$ *vs* Group I.

Table 1 Effects of octreotide on pancreatic level of MDA and endogenous scavengers in rabbits with NaTc-induced acute pancreatitis

	Control	NaTc	NaTc+octreotide
MDA (nmol / mg protein)	2.24±0.51	7.52±1.05[a]	4.14±0.86[a,c]
GSH (nmol / mg protein $\times 10^{-2}$)	2.76±0.45	1.68±0.24[a]	2.23±0.48[a,c]
GPx (U / mg protein $\times 10^{-2}$)	1.18±0.34	0.53±0.08[a]	0.82±0.16[a,c]
Catalase (U / mg protein $\times 10^{-4}$)	3.08±0.54	2.79±0.41[a]	3.16±0.14[a,c]
Mn-SOD (U / mg protein)	1.03±0.22	0.43±0.08[a]	0.53±0.08[a]
Zn-,Cu-SOD (U / mg protein)	4.96±0.76	3.34±0.56[a]	4.12±0.84[a,c]

[a]$P<0.05$ *vs* control group; [c]$P<0.05$ *vs* NaTc treated group.

Table 2 Effects of octreotide on histologic score in rabbits with NaTc-induced acute pancreatitis

Group	Vacuolization	Edema	Necrosis	Inflammation	Congestion
Control	0	0	0	0	0
NaTc	1.12±0.16	2.00±0.35	2.37±0.42	3.15±0.48	1.57±0.33
NaTc+ somatostatin	1.57±0.45	2.14±0.32	2.43±0.58	3.05±0.36	1.50±0.25

The serum IL-6 level increased significantly 3 h after the onset of pancreatitis in Group I (690±88 *vs* 25±15 U/L in Group II), and returned to the control level by 24 h (Figure 3). Octreotide pretreatment attenuated the increase in IL-6 level throughout the study.

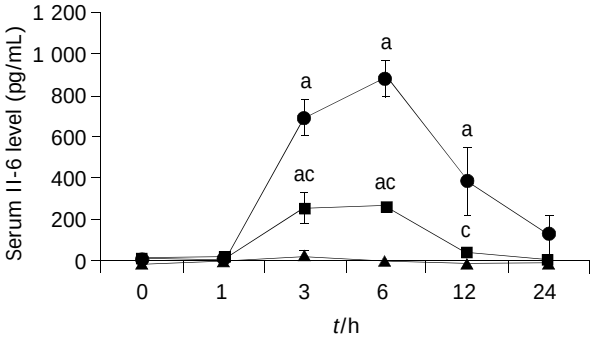

Figure 3 Effects of octreotide on serum IL-6 levels in rabbits with NaTc-induced acute pancreatitis. Group I: ●; Group II as control: ▲; Group III treated with octreotide: ■. [a]$P<0.05$ *vs* Group II; [c]$P<0.05$ *vs* Group I.

The tissue concentration of MDA was elevated significantly at 24 h as compared to the control. Octreotide pretreatment prevented the increase in MDA level. The GSH level and the activities of endogenous scavengers (GPx, catalase, Mn-SOD and Cu-, Zn-SOD) were all decreased significantly in pancreatitis animals in comparison with the control. Octreotide treatment significantly reversed the decrease of GPx, catalase and Cu-, Zn-SOD, but not Mn-SOD activity in comparison with Group I (Table 1).

Histological examination revealed acinar cell necrosis, hemorrhage, inflammatory cell infiltration and edema. Octreotide pretreatment did not exert any beneficial effect on the histological score (Table 2).

DISCUSSION

The results of our study can be summarized as follows. In our model, a short-lasting infusion of taurocholic acid in the pancreatic duct of rabbits produced a rapidly evolving necrotizing pancreatitis with mortality, as described earlier[20]. Proinflammatory cytokines were generated early in NaTc-induced acute pancreatitis. Tissue imbalance of the offense system represented by MDA and the defense system represented by GSH, SOD, GPx and catalase was observed. Prophylactic octreotide treatment prevented the release of cytokines, the increase in MDA and the decrease in GSH, SOD, GPx and catalase activities, but did not reduce the serum amylase level, and did not have beneficial effects on the development of histopathological changes.

Oxygen-derived free radicals have been reported to play an important role in the pathogenesis of acute pancreatitis[31,32]. In the present study, oxidative stress was assessed by measuring the MDA, a product of lipid peroxidation, the intracellular antioxidant GSH, and the endogenous scavengers SOD, GPx and catalase in pancreatic tissues, in order to elucidate the participation of free radicals in the process of NaTc-induced pancreatitis in rabbits. In agreement with previous studies made in other animals[31,32], this study revealed that the elevation of MDA as an offense system, and lowered GSH, SOD, GPx and catalase activities as a defense system, might be one cause of pancreatic injury induced by NaTc in rabbits.

Published data suggest that activated pancreatic macrophages can release proinflammatory cytokines in response to local tissue damages. These cytokines may act locally to aggravate acute pancreatitis, and both locally and systemically to increase the capillary permeability and to promote leukocyte adherence and extravasation[33, 34]. TNF-α, a major proinflammatory cytokine, is considered to be important in orchestrating the early events in the inflammatory cascade and contributes to the induction of systemic inflammatory response syndrome (SIRS) which is responsible for multiple organ failure in severe acute pancreatitis. IL-6 is the most potent inductor of acute-phase protein synthesis in the liver, and it has been shown that its level reflects the severity of acute pancreatitis[35]. This study confirmed the observations of others in rabbits that TNF-α and IL-6 were released in the early phase of NaTc-induced pancreatitis.

There is a growing evidence that somatostatin could inhibit the production of different cytokines, especially TNF-α and IL-6[36-38] and reduce the local generation of free radicals[39]. Nuclear factor-κB (NF-κB) plays a pivotal role in inducing the expression of multiple genes involved in immune and inflammatory responses, such as cytokines. NF-κB transcription pathway has been found to be activated by reactive oxygen species[39]. Therefore, somatostatin may also reduce indirectly the production of cytokines by inhibiting the generation of reactive oxygen metabolites. Furthermore, somatostatin could exert a direct cyto- and organoprotective

action in several models of toxic organ injury and stimulate on the reticulo-endothelial system[40-43].

In our study, octreotide completely reversed the pancreatitis-induced changes in cytokines, MDA, GSH, SOD, GPx and catalase activities. However, we were unable to detect a significant improvement both in the serum amylase level and in the histologic score in octreotide-treated group. This could be explained by severe chemical and mechanical destruction of the pancreatic gland induced by taurocholic acid. The severity of this local injury exceeded the pancreatic damage induced by inflammatory mediators. Overproduction of cytokines can lead to the development of SIRS, which is responsible for the mortality of the disease. However, reduction of cytokine production in octreotide-treated group did not result in a significant decrease in the morbidity or mortality. This suggests that liberation of other vasoactive and toxic mediators [e.g. phospholipase A2, platelet activating factor, nitric oxide, leukotrienes *etc*] by the necrotizing process plays an essential role in the development of SIRS. Therefore, blockade of one particular part of this complex inflammatory process is not beneficial.

Octreotide has been suggested for the treatment of acute pancreatitis on the basis of its inhibitory effect on pancreato-biliary secretion. However, a number of experimental studies have demonstrated that pancreatic enzyme secretion is almost abolished after induction of acute pancreatitis[44-46]. Therefore, the use of drugs inhibiting exocrine pancreatic secretion does not have a beneficial effect on the progression and outcome of the disease. In contrast, the basal pancreatic fluid secretion was greatly increased during the early stages of acute experimental pancreatitis[45, 46]. This fluid hypersecretion was resistant to cholecystokinin, secretin and cholinergic antagonists, and was probably caused by acinar cell proliferation[46]. Fluid hypersecretion can wash out inflammatory mediators and activated pancreatic enzymes from the pancreas, thereby serving as a natural host protective mechanism after a pancreatic injury. Accordingly, it seems unnecessary to attempt to suppress the basal pancreatic enzyme secretion further since it is already blocked. If the fluid hypersecretion is interrupted after the onset of acute pancreatitis, this might exacerbate the inflammatory process.

Furthermore, somatostatin could reduce splanchnic blood flow and the impairment of pancreatic microcirculation could lead to further deterioration in acute pancreatitis[47, 48].

Octreotide might worsen acute pancreatitis and even cause the disease by increasing the contractility of the sphincter of Oddi[18,19]. The most commonly used animals to study the effects of octreotide in acute pancreatitis were rats, whereas rabbits have never been used. However, rabbits seem to be the excellent animals for this research. Since the pancreatic duct enters the duodenum at its distal part, and is completely separated from the common bile duct, we may avoid the harmful effect of octreotide on the sphincter of Oddi. However, we can not exclude the effect of octreotide on pancreatic sphincter.

In conclusion, proinflammatory cytokines are generated early in NaTc-induced acute pancreatitis in rabbits. Tissue imbalance of the offense system represented by MDA and the defense system represented by GSH, SOD, GPx and catalase was detected in the pancreas. Prophylactic octreotide treatment can prevent the release of cytokines, the increase in MDA and the decrease in GSH, SOD, GPx and catalase activities, but does not have any beneficial effects on the development of necrotizing pancreatitis. The pancreatic duct injection model in rabbit is a useful model to exclude the effect of sphincter of Oddi on the course of acute pancreatitis.

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