Turk J Med Sci
32 (2002) 463-468

Abdulkadir BEDİRLİ[1]
Erdoğan M. SÖZÜER[1]
Recep SARAYMEN[2]
Nail ERSÖZ[1]
Eser KILIÇ[2]

Protective Effect of Nitric Oxide in Lung Injury Associated with Cerulein-Induced Acute Pancreatitis*

Received: March 15, 2002

Departments of [1]General Surgery and [2]Biochemistry, Faculty of Medicine, Erciyes University, Kayseri - Turkey

Abstract: The aim of this study was to investigate the role of NO in the pathogenesis of lung injury associated with pancreatitis, and the relation between malondialdehyde (MDA) and myeloperoxidase (MPO). Forty Wistar male rats were divided into control or pancreatitis groups, and treated with saline, L-arginine (100 mg/kg i.v.), NO donor, or N-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg i.v.), an inhibitor of NO synthase. Pancreatitis was induced in rats intravenously via the administration of cerulein (10 µg/kg/h) for 4 h). Eight hours after the induction of acute pancreatitis (AP), amylase and lipase in plasma significantly increased in all animals in which mild pancreatitis was induced in comparison with the control group. Cerulein induced gross pancreatic and lung edema as reflected by an increase in the wet/dry ratio. L-arginine significantly decreased pancreatic and lung edema. L-arginine treatment prevented the increase of MDA levels in the pancreas (760 ± 252 nM/g tissue) and lung (520 ± 188 nM/g tissue). In contrast, L-NAME increased MDA levels in the pancreas (3280 ± 474 nM/g tissue) and lung (2650 ± 411 nM/g tissue). Compared with the AP group, MPO activity was reduced by L-arginine (17.2 ± 3.7 U/g pancreas versus 6.2 ± 2.2 U/g pancreas; 28.4 ± 3.3 U/g lung versus 11.1 ± 3.0 U/g lung). In contrast, L-NAME treatment significantly increased MPO levels in the pancreas (32.8 ± 4.7 U/g pancreas) and lung (43.7 ± 7.1 U/g lung). In conclusion, L-arginine treatment improved the course of mild pancreatitis and prevented lung injury. Lung injury was aggravated by the administration of the NO inhibitor L-NAME.

Key Words: Acute pancreatitis, nitric oxide, lung injury, malondialdehyde, myeloperoxidase

Introduction

Acute pancreatitis is an autodigestive inflammatory process. Following initial tissue injury, neutrophils migrate to the area, become activated and initiate a series of events. Neutrophils and alveolar macrophages release mediators such as platelet-activating factor, leukotrienes, nitric oxide (NO), tumor necrosis factor (TNF) and interleukin (IL)s, which play a role in the evolution of the local pancreatic inflammation to systemic complications culminating in multiple organ failure (1-4). Although NO was originally discovered in the vascular endothelium, it is synthesized in a variety of cells including macrophages, platelets and pancreatic cells by the enzyme nitric oxide synthase (NOS) from the amino acid L-arginine. Various pharmacological agents may suppress NO synthesis by inhibiting NOS. Arginine analogs such as N^G-monomethyl-L-arginine (L-NMMA), N^G nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine-methylester (L-NAME) strongly block NO synthesis (5). Because NO participates in the modulation of vascular tone and leukocyte function, its role in acute pancreatitis has been investigated. NO has been shown to have protective effects by preventing neutrophil adhesion and regulating microcirculation in experimental pancreatitis in rats (6-8). However, the role of NO in the most frequent systemic complication, lung injury, is unclear. In studies on lung damage in acute pancreatitis, the secretory agent cerulein is usually used because the model, which is characterized by edema and hyperamylasemia, is very appropriate (9). The lung injury induced is very similar to the early stages of the adult respiratory distress syndrome (ARDS) (1,10). In this experimental study, we aimed to investigate the still controversial role of NO in lung injury due to cerulein-induced pancreatitis.

* Presented, in part, at the 4th World Congress International Hepato-Pancreato-Biliary Association, May 28-June 1, 2000, Brisbane, Australia

Materials and Methods

Animal preparation. Experiments were performed in 40 male Wistar rats weighing 250 to 300 g. All animals were fasted overnight before the experiments and had free access to water. Care was provided in accordance with the procedures outlined in the "Guide for Care and Use of Laboratory Animals" (National Institutes of Health Publication no. 85-23, 1985). The study was approved by the subcommittee on animal research at our institution. Surgical anesthesia was induced by subcutaneous administration of ketamine hydrochloride 100 mg/kg body weight. Polyethylene catheters (0.20 mm internal diameter) were inserted by cutdown into the right jugular vein. The catheters were tunneled subcutaneously to the dorsal cervical region and exited through a steel tether, which permitted free movement. The animals were randomly divided between a control group (n = 10) and groups at various treatment of acute pancreatitis. Acute mild edematous pancreatitis was induced by continuous individual i.v. infusions of cerulein (Sigma Chemical, St. Louis, MO) at 10 µg/kg/h over 4 h. Acute pancreatitis groups were treated with saline (AP group, n = 10); L-arginine, NO donor, (L-arginine group, n = 10); and L-NAME, an NOS inhibitor, (L-NAME group, n = 10). L-arginine and L-NAME were administered 30 min after the initiation of cerulein infusion. L-arginine (100 mg/kg bolus, Merck & Co., NJ) and L-NAME (10 mg/kg bolus, Sigma Chemical, St. Louis, MO) were infused intravenously. Animals were killed using an intra-arterial pentobarbital overdose (200 mg/kg) 8 h after the induction of pancreatitis, and a 5 ml blood sample from the heart was obtained. The pancreas and lungs were rapidly excised, weighed, and processed for individual assays.

Amylase and Lipase Measurement. Plasma amylase and lipase were determined by using commercial kits from Boehringer-Mannheim (Mannheim, Germany) according to the supplier's specifications.

Assessment of Pancreatic and Lung Edema. Pancreatic water content was determined by calculating the ratio (wet/dry ratio) from the initial weight of the pancreas (wet weight) to its weight after incubation at 160 °C for 24 h (dry weight). The wet/dry ratio of lung was determined in the same fashion.

Malondialdehyde Assay. Serum malondialdehyde (MDA) was estimated using the thiobarbituric acid method described by Ohkawa et al. (11).

Myeloperoxidase Assay. The previously frozen pancreas and lung tissues were homogenized for 30 sec in 4 mL of 20-mmol/L potassium phosphate buffer, pH 7.4 and centrifuged for 30 min at 40,000 g at 4 °C. The pellet was resuspended in 4 mL of 50-mmol/L potassium phosphate buffer, pH 6.0, containing 0.5 g/dL of hexadecyltrimethyl ammonium bromide. Samples were sonicated for 90 sec at full power, incubated in a 60 °C water bath for 2 h, and centrifuged. The supernatant (0.1 mL) was added to 2.9 mL of 50 mmol/L potassium phosphate buffer, pH 6.0, containing 0.167 mg/mL o-dianisidine and 0.0005% hydrogen peroxide. Absorbance of 460 nm of visible light (A460) was measured for 3 min. Myeloperoxidase (MPO) activity is expressed as units per gram of tissue.

Statistical Analysis. All results are expressed as mean ± SD. Differences between groups were compared using a one-way analysis of variance. The Mann-Whitney test was performed to evaluate significant differences between the groups. Differences were assumed to be significant at $p < 0.05$.

Results

The plasma amylase and lipase levels are shown in Table 1. The induction of acute pancreatitis was confirmed by the rise in amylase and lipase levels ($p < 0.001$). L-arginine treatment reduced plasma amylase and lipase levels but did not approach statistical significance. L-NAME administration did not cause significant changes in plasma amylase or lipase levels ($p > 0.05$). The wet weight/dry weight ratio of the pancreas and the lungs in the pancreatitis groups were significantly higher in comparison with the control group (Figs. 1 and 2). Although L-arginine and L-NAME treatments decreased the degree of edema, the change reached statistical significance only in the L-arginine group ($p < 0.001$ for pancreas, $p < 0.005$ for lung).

MDA, a quantitative marker of lipid peroxidation, was measured in the pancreas and lung. Induction of pancreatitis caused significant increases in the tissue levels of MDA (Table 2). L-arginine treatment decreased tissue MDA levels to the control levels, whereas L-NAME aggravated lipid peroxidation. The decrease in the tissue MDA levels in the L-arginine group was statistically significant ($p < 0.001$).

Table 1. Plasma amylase and lipase levels.

	Control	AP	L-arginine	L-NAME
Plasma amylase (U/L)	234 ± 52	2502 ± 370*	2128 ± 452*	2744 ± 434*†
Plasma lipase (U/L)	14 ± 6	471 ± 35*	433 ± 44*	502 ± 51*†

*p < 0.001 vs. control
†p < 0.01 vs. L-arginine

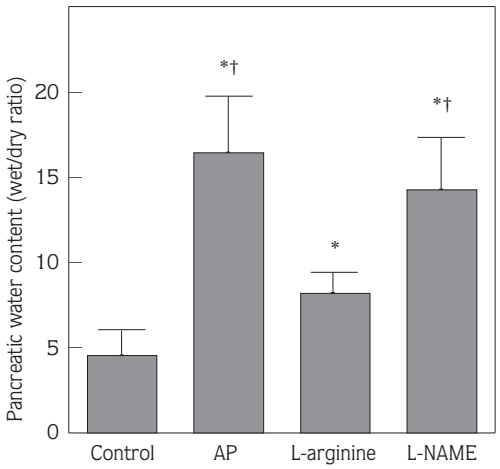

Figure 1. Pancreatic water content.
* p < 0.001 vs. control
† p < 0.001 vs. L-arginine

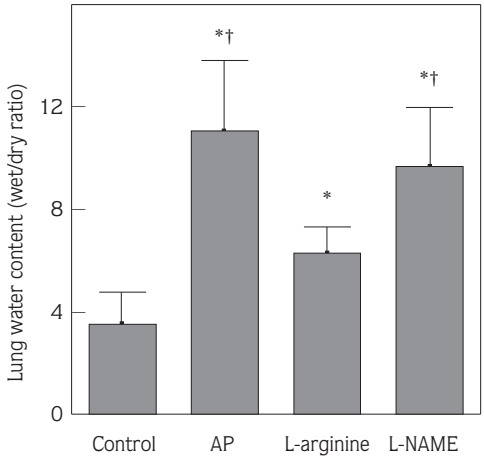

Figure 2. Lung water content.
* p < 0.001 vs. control
† p < 0.005 vs. L-arginine

Table 2. Malondialdehyde levels in pancreatic and lung tissue.

	Control	AP	L-arginine	L-NAME
Pancreas (nM/g tissue)	427 ± 187	2184 ± 578*†	760 ± 252*	3280 ± 474*†
Lung (nM/g tissue)	287 ± 125	1950 ± 675*†	520 ± 188*	2650 ± 411*†

*p < 0.01 vs. control
†p < 0.001 vs. L-arginine

The degree of neutrophil infiltration in the pancreas and lung is shown in Table 3. As expected, induction of pancreatitis causes significant increases in neutrophil infiltration in both organs. In comparison with the control group, lung MPO levels were increased in all rats with acute pancreatitis ($p < 0.05$). L-arginine treatment significantly suppressed the increase in MPO levels in the pancreas and lung ($p < 0.001$). In contrast, L-NAME treatment significantly increased MPO levels in the pancreas and lung ($p < 0.001$)

Discussion

Acute pancreatitis is a pathophysiological process initiated by acinar cell damage due to released proteolytic enzymes. In response to acinar cell damage, cytokines are released and the complement system is activated. These molecules cause further releases of cytokines, free radicals and vasoactive substances such as NO (1,2,12). In addition to local effects such as edema and necrosis in the pancreas, these mediators induce systemic effects such as hypotension, tachycardia, capillary leakage and hypoxia. They may also trigger apoptosis and necrosis (2). Most studies have attempted to elucidate the relationships in this series of events and modulate the pancreatitis cascade. NO, which has a regulatory role in many organs, is a reactive free radical that is produced excessively by an endogenous inducible form of NOS (iNOS) during inflammatory processes such as acute pancreatitis. In an experimental model of acute pancreatitis in rats, it has been shown that increased NO levels due to induction of iNOS aggravate oxidative damage and this is associated with arterial hypotension (13). Satoh et al. have emphasized that iNOS response varies between

Table 3. Myeloperoxidase activity in the pancreas and lung.

	Control	AP	L-arginine	L-NAME
Pancreas (U/g tissue)	4.75 ± 2.1	17.2 ± 3.7*†	6.2 ± 2.2	32.8 ± 4.7*†
Lung (U/g tissue)	8.3 ± 2.4	28.4 ± 3.3*†	11.1 ± 3.0*	43.7 ± 7.1*†

* $p < 0.05$ vs. control
† $p < 0.001$ vs. L-arginine

pancreatitis models and reported that, in necrotizing pancreatitis, various soluble factors activate the macrophage/monocyte system and cause increased NO production by iNOS (14). NO donors and inhibitors have been used in experimental studies to investigate the role of NO in acute pancreatitis. Masamune et al. have argued that the role of NO in acute pancreatitis is limited to endothelial activation and inhibition of leukocyte activation (15). In another study on lipopolysaccharide-induced pancreatitis, NO caused a fall in TNF-α levels whereas L-NNA caused a rise; these results suggest a protective role of NO (16). Other studies have shown that NO plays an important role in the preservation of microcirculation (6,7). Werner et al. have reported beneficial effects of NO donors on pancreatic perfusion, leukocyte-endothelium interaction, platelet aggregation and acinar cell damage in acute pancreatitis (17). Activated leukocytes play a significant role in the aggravation of pancreatitis and development of complications. There is an acute release of oxygen free radicals from activated neutrophils (1,2,18). MDA is an end product of lipid peroxidation induced by free oxygen radicals. Increased plasma and tissue MDA levels reflect increased free radical production and give information on the magnitude of tissue damage. In our study, we investigated the role of NO in cerulein-induced acute pancreatitis. The increased plasma amylase and lipase levels as well as edema in pancreatic tissue confirmed the applicability of the model. Pancreatic tissue MDA levels, which reflect tissue damage, were significantly increased in all rats with acute pancreatitis. L-arginine treatment decreased pancreas MDA levels, whereas L-NAME increased pancreas MDA levels. In order to evaluate leukocyte infiltration in pancreatic tissue, we used a standard marker MPO. Leukocyte infiltration was significantly more severe in acute pancreatitis. L-arginine suppressed the leukocyte infiltration, whereas L-NAME had a reverse effect.

Although acute pancreatitis is a local inflammation, it has significant systemic effects, the most important of which is lung injury. Organ dysfunction develops in approximately one fourth of all cases and 60% of the deaths in the first week are due to pulmonary complications (19). However, the pathogenesis of pulmonary complications in acute pancreatitis remains unclear. Several factors have been implicated: mechanical, alteration in capillary permeability, and pulmonary microembolization. Guice et al. have reported that in a hypersecretion model of acute pancreatitis the increased capillary permeability and interstitial edema in acute pancreatitis are histologically indistinguishable from ARDS (10). Neutrophils release specific proinflammatory mediators and play a definitive role in ARDS. Although neutrophil activation and aggregation in the lungs comprise the final common pathway leading to lung damage, the initial triggering event is controversial. Complement system activation and proinflammatory factors from alveolar macrophages are putative candidates. Alveolar macrophages have the capacity to mobilize large numbers of leukocytes and secrete cytokines, arachidonic acid metabolites and NO (20). The protective anti-inflammatory role of L-arginine or NO in the lung has been evaluated in a number of studies. Previous investigations have demonstrated that L-arginine or NO decreases alveolar macrophage proinflammatory cytokine (TNF-α, IL-1β) production (21) and prevents alveolar edema (22). Sheridan et al. found that L-arginine decreases lipopolysaccharide-induced polymorphonuclear neutrophil (PMN) accumulation and attenuates acute lung injury in a rat model of endotoxemia (23). The capacity of alveolar macrophages to mobilize a large amount of PMN and to release secretory products such as cytokines, arachidonic acid metabolites, and NO after their activation in the course of different pulmonary inflammatory diseases suggests that these cells could be involved in the lung damage associated with acute pancreatitis. The activation of alveolar macrophages seems to be regulated by cytokines and inflammatory mediators, which are reportedly generated during the course of acute

pancreatitis. In our study, we found that L-arginine treatment attenuated both MDA and MPO production in the lung tissue, whereas L-NAME had the opposite effect. Our findings corroborate those of a previously reported study (24) regarding the effects of NO pulmonary neutrophil reflux and the prevention of lung damage in acute pancreatitis-induced lung injury.

In conclusion, this experimental study shows the protective role of L-arginine in a model of acute pancreatitis associated with lung injury. The provision of L-arginine early in the course of acute pancreatitis may act to downregulate the inflammatory response via a NO-dependent mechanism before the lung damage that occurs as the result of PMN accumulation and free radical production.

Correspondence author:
Abdulkadir BEDİRLİ
Department of General Surgery,
Faculty of Medicine, University of Erciyes,
38039, Kayseri - TURKEY

References

1. Guice KS, Oldham KT, Caty MG, Johnson KJ, Ward PA. Neutrophil-dependent, oxygen-radicals mediated lung injury associed with acute pancreatitis. Ann Surg 210: 740-7, 1989.
2. Denham W, Yang J, Norman J. Evidence for an unknown component of pancreatic ascites that induced adult respiratory distress syndrome through an interleukin-1 and tumor necrosis factor-dependent mechanism. Surgery 122: 295-302, 1997.
3. Tsukahara Y, Horita Y, Anan K, Morisaki T, Tanaka M, Torisu M. Role of nitric oxide derived from alveolar macrophages in the early phase of acute pancreatitis. J Surg Res 66: 43-50, 1996.
4. Closa D, Sabater L, Fernandez-Cruz L, Prats N, Gelpi E, Rosello-Catafau J. Activation of alveolar macrophages in lung injury associated with experimental acute pancreatitis is mediated by the liver. Ann Surg 229: 230-6, 1999.
5. Moncada S, Palmer RMJ, Higgs AE. Nitric oxide. Physiology, pathophysiology and pharmacology. Pharmacol Rev 43: 109-42, 1991.
6. Dobosz M, Hac S, Wajda Z. Does nitric oxide protect from microcirculatory disturbances in experimental acute pancreatitis in rats. Int J Microcirc Clin Exp 16: 221-6, 1996.
7. Liu X, Nakano I, Yamaguchi H, Ito T, Goto M, Koyanagi S, Kinjoh M, Nawata H. Protective effect of nitric oxide on development of acute pancreatitis in rats. Dig Dis Sci 40: 2162-9, 1995.
8. Werner J, Fernandez-del Castillo C, Rivera JA, Kollias N, Lewandrowski KB, Rattner DW, Warshaw AL. On the protective mechanisms of nitric oxide in acute pancreatitis. Gut 43: 401-7, 1998.
9. Banejee AK, Galloway SW, Kingsnorth AN. Experimental models of acute pancreatitis. Br J Surg 81: 1096-103, 1994.
10. Guice KS, Oldham KT, Johnson KJ, Kunkel RG, Morganroth ML, Ward PA. Pancreatitis-induced acute lung injury. Ann Surg 208: 71-7, 1988
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-8, 1979.
12. Karne S, Gorelick FS. Etiopathogenesis of acute pancreatitis. Surg Clin North Am 79: 699-710, 1999.
13. Al-Mufti RA, Williamson RC, Mathie RT. Increased nitric oxide activity in a rat model of acute pancreatitis. Gut 43: 564-70, 1998.
14. Satoh A, Shimosegawa T, Kimura K, Moriizumi S, Masamune A, Koizumi M, Toyota T. Nitric oxide is overproduced by peritoneal macrophages in rat taurocholate pancreatitis: the mechanism of inducible nitric oxide synthase expression. Pancreas 17: 402-11, 1998.
15. Masamune A, Shimosegawa T, Satoh A, Fujita M, Sakai Y, Toyota T. Nitric oxide decreases endothelial acivation by rat experimental severe pancreatitis-associated ascitic-fluids. Pancreas 20: 297-304, 2000.
16. Jaworek J, Jachimczak B, Bonior J, Kot M, Tomaszewska R, Karczewska E, Stachura J, Pawlik W, Konturek SJ. Protective role of endogenous nitric oxide (NO) in lipopolysaccharide-induced pancreatic damage/a new experimental model of acute pancreatitis. J Physiol Pharmacol 51: 85-102, 2000.
17. Werner J, Rivera J, Fernandez-del Castillo C, Lewandrowski K, Adrie C, Rattner DW, Warshaw AL. Differing roles of nitric oxide in the pathogenesis of acute edematous versus necrotizing pancreatitis. Surgery 121: 23-30, 1997.
18. Groos V, Leser HG, Heinisch A, Scholmerich J. Inflammatory mediators and cytokines-new aspect of the pathophysiology and assessment of severity of acute pancreatitis? Hepato-Gastroenterology 40: 522-30, 1993.
19. Mann DV, Hershmann MJ, Hittinger R, Glazer G. Multicentre audit of death from acute pancreatitis. Br J Surg 81: 890-3, 1994.
20. Driscoll KE. Macrophage inflammatory proteins: biology and role in pulmonary inflammation. Exp Lung Res 20: 437-90, 1994.
21. Meldrum DR, McIntyre RC, Sheridan BC, Cleveland JC Jr, Fullerton DA, Harken AH. L-arginine decreases alveolar macrophage proinflammatory monokine production during acute lung injury by a nitric oxide synthase-dependent mechanism. J Trauma 43: 888-893, 1997.

22. Hinder F, Stubbe HD, Van Aken H, Waurick R, Booke M, Meyer J. Role of nitric oxide in sepsis-associated pulmonary edema. Am J Respir Crit Care Med 159: 252-257, 1999.

23. Sheridan BC, McIntyre RC Jr, Meldrum DR, Fullerton DA. L-arginine prevents lung neutrophil accumulation and preserves pulmonary endothelial function after endotoxin. Am J Physiol Lung Cell Mol Physiol 274: L337-L342, 198.

24. O'Donovan DA, Kelly CJ, Abdih H, Bouchier-Hayes D, Watson RW, Redmond HP, Burke PE, Bouchier-Hayes DA. Role of nitric oxide in lung injury associated with experimental acute pancreatitis. Br J Surg 82: 1122-6, 1995.