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Organ Microcirculatory Disturbances in Experimental Acute Pancreatitis. A Role of Nitric Oxide

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Summary

Microcirculatory disturbances are important early pathophysiological events in various organs during acute pancreatitis (AP). The aim of the study was to investigate an influence of L-arginine (nitric oxide substrate) and N^G-nitro-L-arginine (L-NNA, nitric oxide synthase inhibitor) on organ microcirculation in experimental acute pancreatitis induced by four consecutive intraperitoneal cerulein injections (15 μg/kg/h). The microcirculation of pancreas, liver, kidney, stomach, colon and skeletal muscle was measured by laser Doppler flowmeter. Serum interleukin 6 and hematocrit levels were analyzed. AP resulted in a significant drop of microperfusion in all examined organ. L-arginine administration (2x100 mg/kg) improved the microcirculation in the pancreas, liver, kidney, colon and skeletal muscle, and lowered hematocrit levels. L-NNA treatment (2x25 mg/kg) caused aggravation of edematous AP to the necrotizing situation, and increased IL-6 and hematocrit levels. A further reduction of blood perfusion was noted in the stomach only. It is concluded that L-arginine administration has a positive influence on organ microcirculatory disturbances accompanying experimental cerulein-induced AP. NO inhibition aggravates the course of pancreatitis.

Key words

Acute pancreatitis • Microcirculation • Nitric oxide

Introduction

Hemodynamic shock is one of the initial events accompanying acute pancreatitis. Although the impairment of macrohemodynamic functions (cardiac output, mean arterial pressure) can easily be normalized by vigorous fluid replacement (Knol *et al.* 1987), persistent microcirculatory dysfunction may be detrimental in organs vulnerable to failure during shock, such as the liver, lung and kidney (Mulder *et al.* 1994), and may be a key element in the development of the pancreatitis-associated multiorgan dysfunction syndrome (Foitzik *et al.* 2000). The microcirculatory disturbances within the pancreatic capillary bed is believed to be a crucial factor in the evolution of pancreatitis from edema to necrosis (Zhou and Chen 2002, Strate *et al.* 2003).

Various vasoactive mediators, such as bradykinin, endothelin, thromboxane, the platelet activating factor, and nitric oxide participate in the development of microcirculatory failure (Zhou and Chen

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2002). In the last decade, the beneficial effect of therapeutic strategies in acute pancreatitis, affecting vasoactive mediators, has been confirmed in several experimental studies. Recent evidence suggests that nitric oxide, due to its vasodilatory, anti-inflammatory, antiadhesive and anticoagulant properties (Werner *et al.* 1998a,b), appears to have a beneficial influence on the course of acute pancreatitis. The aim of this study was to evaluate the impact of L-arginine (a substrate for NO synthase) and N^G-nitro-L-arginine (L-NNA, NO synthase inhibitor) on splanchnic malperfusion in experimental cerulein-induced acute pancreatitis.

Material and Methods

The study was carried out in 46 male Wistar rats weighing 180-200 g, kept on standard rat chow and fasted overnight before the experiment with water allowed *ad libitum.* Acute pancreatitis was induced by four intraperitoneal injections of cerulein (Cn) – (Sigma, St. Louis, USA) (15 μg/kg) in 1 ml of saline at 1-hour intervals: at the beginning, and consecutively after the first, second and third hour of the experiment. Five hours after the first cerulein injection, rats were anesthetized with pentobarbital sodium (40 mg/kg). Following anesthesia, a laparotomy was performed, and a fiber optic probe of laser Doppler flowmeter (Periflux 4001, Perimed Jarfalla, Sweden) was positioned against the surface of the pancreas, liver, kidney, stomach, colon and skeletal muscle of the thigh in order to investigate organ perfusion. Blood flow was measured in three different portions of each organ, the mean values were calculated and expressed as percentage basal values obtained in control rats (100 %). After the measurements, blood was aspirated from the inferior cava vein for hematocrit estimation and interleukin 6 functional assay, as depicted previously (Dobosz *et al.* 1999), the pancreas was removed for microscopic evaluation, and the animals were exsanguinated.

The animals were randomly allocated into four groups: Group I (n=10) – control, Group II (n=12) – Cn-induced pacreatitis without treatment, Group III (n=12) – Cn-induced pancreatitis treated with L-arginine (Calbiochem, Lucerna) 2x100 mg/kg, given in the 1st and 2nd hour after the first Cn injection, Group IV (n=12) – Cn-induced pancreatitis treated with L-NNA (Calbiochem, Lucerna) and 2x25 mg/kg given in the 1st and 2nd hour after the first Cn injection.

Statistical analysis

Data are presented as means ± standard deviation (S.D.). The differences between the groups were analyzed by means of the ANOVA test. $P<0.05$ values were considered significant.

Results

Four intraperitoneal cerulein injections resulted in marked pancreatic edema with the collection of peritoneal exudates in all the animals. The microscopic examination revealed an edematous form of acute pancreatitis, with inter- and intralobular edema, vacuolization of parenchymal cells, and leukocyte infiltration within the pancreatic gland. No parenchymal necrosis was noticed. In the group of animals treated with L-arginine, besides vacuolization, glandular edema, and leukocyte infiltration, small foci of parenchymal necrosis were detected in two rats. In rats with AP receiving L-NNA, an aggravation of microscopic alterations, including necrosis and hemorrhages were within the pancreatic gland observed.

Microcirculatory values

Cerulein-induced acute pancreatitis resulted in a significant drop of pancreatic microperfusion to 37±4 % of basal values. Administration of L-arginine significantly improved the microcirculation of the pancreas up to 72±10 %, but L-NNA did not lower the pancreatic blood flow (Table 1). Hepatic perfusion in rats with pancreatitis receiving no treatment was decreased to 57±6 %, L-arginine injection raised this value to 76±7 %, L-NNA had no effect on hepatic capillary flow. Renal blood flow in group with pancreatitis was diminished to 45±6 %, which was improved significantly with L-arginine treatment up to 64±5 %, L-NNA administration did not influence renal perfusion. Microcirculatory values of stomach blood flow in group II with AP were reduced to 65±8 %, L-arginine treatment had no effect on this parameter, but L-NNA significantly decreased the stomach perfusion to 46±7 %. Cerulein-induced AP diminished the colonic blood flow to 70±6 % which was augmented with L-arginine to 85±10 %, L-NNA injection had no effect on colonic microcirculation in AP. Skeletal muscle perfusion in animals with pancreatitis was significantly ameliorated after L-arginine administration from 59±3 % to 82±6 %, L-NNA did not change the value of skeletal muscle blood flow (Table 1).

Table 1. Microcirculatory values of pancreas, liver, kidney, stomach, colon, skeletal muscle.

Group	Pancreas (%)	Liver (%)	Kidney (%)	Stomach (%)	Colon (%)	Muscle (%)
Control	100±7	100±9	100±6	100±13	100±10	100±6
Acute pancreatitis	36.9±4 [a]	56.6±6 [a]	45.1±6 [a]	65.2±8 [a]	69.8±6 [a]	59.2±6 [a]
AP + L-arginine	71.9±10 [b]	76.1±7 [b]	63.7±5 [b]	62.2±16	84.6±14 [b]	81.6±16 [b]
AP+L-NNA	34.5±8	53.3±9	43.0±9	45.6±7 [b]	66.9±16	60.0±13

Mean values ± SD. [a] P<0.05 in comparison to the control group; [b] P<0,05 in comparison to the acute pancreatitis group.

Serum interleukin 6

Cn-induced acute pancreatitis caused a significant increase of serum IL-6 activity from 38±21 U/ml in control animals up to 359±66 U/ml. L-arginine administration had no effect on the IL-6 level, but L-NNA significantly increased this parameter to 409±44 U/ml (Table 2).

Hematocrit

In group with cerulein-induced pancreatitis the hematocrit was significantly increased to 53±4 % compared to 41±3 % in controls. L-arginine administration diminished hematocrit to 48±3 %, while L-NNA injection increased it to 56±5 % (Table 2).

Table 2. Interleukin 6 and hematocrit values in experimental groups.

Groups	Interleukin 6 (µ/ml)	Hematocrit (%)
Control	37.7±21	41±3
Acute pancreatitis	359±66[a]	53±4[a]
AP + L-arginine	352±59	48±3[b]
AP + L-NNA	409±44[b]	56±5

Mean values ± SD. [a] P<0.05 in comparison to control group; [b] P<0.05 in comparison to the acute pancreatitis group

Discussion

In the present study, four intraperitoneal cerulein injections caused an edematous form of acute pancreatitis. The consequence of AP in rats was the reduction of capillary blood flow in the pancreas, measured by a laser Doppler flowmeter. The pancreatic microcirculatory disturbances which accompany experimental acute pancreatitis were confirmed by other authors, in both a mild edematous form of the disease and a severe necrotizing one (Konturek *et al.* 1994, Liu *et al.* 1995, Schmidt *et al.* 2002, Strate *et al.* 2003).

The disturbances of microcirculation in acute pancreatitis are not only confined to the pancreatic capillary bed, but are also observed in other organs (Skoromnyi and Starosek 1998, Foitzik *et al.* 2002). It was suggested that diffused microcirculatory disorders may play a crucial role in the development of the pancreatitis-associated multiorgan dysfunction syndrome, some authors even define severe AP as a systemic dysfunction syndrome (Foitzik *et al.* 2000).

The present study confirms these data. Besides the pancreas, reduced capillary perfusion was observed in the liver, kidney, stomach, colon, and skeletal muscle, however, the drop in perfusion of other organs was not so pronounced as in the pancreas. This suggests that in pancreatitis, the pancreatic gland is especially susceptible to microcirculatory disorders. Kinnala *et al*. (2002) found that splanchnic malperfusion begins with pancreatic hypoperfusion before disturbances in gut microcirculation. On the other hand, Hotz *et al.* (1998) noted that in mild pancreatitis, pancreatic capillary perfusion remained unchanged, whereas mucosal and subserosal colonic capillary blood flow was significantly reduced. They also demonstrated that severe pancreatitis was associated with a marked reduction in both pancreatic and colonic capillary perfusion.

Microcirculatory disturbances in AP comprise many components: decreased capillary blood flow and capillary density, increased capillary permeability, and enhanced leukocyte-endothelial interaction (Foitzik *et al.* 2000). It is still not clear which of these factors is the initiating one or the most important. It seems to be logical that any effort to improve the microcirculation may be beneficial for all organs, irrespective of the underlying triggering mechanism.

Several studies documented a positive impact of various therapeutic agents on AP course, improving tissue perfusion: dextran (Klar *et al.* 1993), pentoxifylline

(Gomez-Cambronero *et al.* 2000), heparin (Dobosz *et al.* 1999), bovine hemoglobin (Strate *et al.* 2003), ICAM-1 monoclonal antibodies (Werner *et al.* 1998a,b), and endothelin receptor antagonist (Plusczyk *et al.* 2003). In the current study, the intraperitoneal L-arginine administration (substrate for NO synthase) significantly augmented capillary blood perfusion of all the examined organs, except the stomach. The improvement of pancreatic microperfusion should have a positive influence on microscopic alterations within the pancreas. Contrary to other observations (Konturek *et al.* 1994, Liu *et al.* 1995), we observed focal pancreatic necrosis in rats receiving L-arginine. In a recent study using the same model, we analyzed microscopic alterations within the pancreas by means of histological grading (Dobosz *et al.* 1999). The scoring revealed slightly higher vacuolization rate of acinar cells, leukocyte infiltration and necrosis, although the differences were not significant in comparison to acute pancreatitis group without treatment. The deterioration of morphological changes of pancreatic parenchyma in rats receiving L-arginine could be explained by the intraperitoneal drug administration which might result in an excessive local NO concentration and cytotoxic peroxynitrite production (Beckman *et al.* 1990). This phenomenon could also explain why we did not observe a decrease of IL-6 concentration in spite of improved pancreatic blood flow.

It was shown that a significant number of adherent leukocytes had been observed in hepatic microcirculation two hours after AP induction (Chen *et al.* 2001). The L-arginine administration, due to the antiadhesive properties of NO (Werner *et al.* 1998a,b), could prevent neutrophil adhesion to hepatic capillaries and improve hepatic perfusion noted in our study. It was suggested in another study that hepatic microcirculatory improvement ameliorated phagocytic Kupffer cell function in the liver (Forgacs *et al.* 2003).

The pathophysiology of renal insufficiency, which is an often observed complication of acute pancreatitis, is heterogeneous. The improvement of renal blood flow observed after L-arginine treatment in rats with pancreatitis could prevent this complication. It was found in severe acute pancreatitis that endothelin receptor blockade, besides the enhancement of pancreatic perfusion, also improved renal function (Foitzik *et al.* 2000).

Decreased capillary blood flow in the colonic mucosa is associated with impaired gut barrier function and increased translocation of live bacteria through the morphologically intact colonic wall (Foitzik *et al.* 1997). The present study revealed that L-arginine treatment in the group with pancreatitis significantly improved the altered microperfusion of the colonic wall. This suggests that nitric oxide may play a role in the prevention of secondary pancreatic infection. It was shown that NO substrates limit bacterial translocation and pancreatic inflammation associated with AP, probably by their bactericidal actions and ability to improve pancreatic blood flow (Cevikel *et al.* 2003).

Besides organ microcirculatory improvement, L-arginine administration diminishes the hematocrit level. Beneficial influence of L-arginine on hematocrit levels in group III, may also suggest that nitric oxide restricts capillary permeability not only in the pancreatic gland and prevents fluid escape into the extracellular space. Therefore, nitric oxide therapy may allow to avoid vigorous fluid resuscitation observed in patients with AP. It was shown that L-arginine concentrations are depleted in the serum of patients with acute pancreatitis (Sandstrom *et al.* 2003), so that this kind of therapy seems to be justified.

Protective effect of nitric oxide in acute pancreatitis has also been observed in other recent studies. It was suggested that NO protects against tissue injury in AP, acts indirectly *via* microcirculatory changes, including inhibition of leukocyte activation and preservation of capillary perfusion (Werner *et al.* 1998b). A protective role of endogenous NO against oxidative damage to subcellular fractions was noted by Sanchez-Bernal *et al.* (2004). Other authors found that glyceryl trinitrate and L-arginine treatment significantly attenuated damage of the pancreatic gland and augmented cell proliferation after AP (Jurkowska *et al.* 1999).

Although the microcirculatory values in rats with AP after L-NNA injection became significantly decreased in the stomach only, inhibition of NO synthase in the current study resulted in aggravation of the course of acute pancreatitis. Microscopic examination revealed the development of a severe necrotizing form of the disease, serum interleukin 6 and hematocrit levels being increased. Similar observations concerning a negative impact of NOS suppression were made by other authors, including reduction in pancreatic blood perfusion, decrease pancreatic tissue oxygenation, deterioration of inflammatory changes and growth of proinflammatory cytokine levels (Konturek *et al.* 1994, Liu *et al.* 1995, Werner *et al.* 1998 a,b).

In summary, these data suggest that in the early period of AP, nitric oxide, maintaining the splanchnic

microcirculation, plays an important role in the pathophysiological events of the disease. However, the short time of observation does not allow to conclude clearly about the beneficial effect of L-arginine on AP. The inhibition of NO seems to be deleterious and enhances the progression of the disease.

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