Transpl Int (2004) 17: 208–214
DOI 10.1007/s00147-004-0702-y

ORIGINAL ARTICLE

R. Obermaier
E. von Dobschuetz
O. Muhs
T. Keck
O. Drognitz
L. Jonas
W. Schareck
U. T. Hopt
S. Benz

Influence of nitric oxide on microcirculation in pancreatic ischemia/reperfusion injury: an intravital microscopic study

Received: 10 June 2003
Revised: 30 September 2003
Accepted: 22 October 2003
Published online: 30 April 2004

R. Obermaier (✉) · E. von Dobschuetz
T. Keck · O. Drognitz · U. T. Hopt
S. Benz
Department of Surgery,
University of Freiburg,
Hugstetter Strasse 55,
79106 Freiburg, Germany
E-mail: robert.obermaier@web.de
Tel.: +49-761-2702806
Fax: +49-761-2702804

O. Muhs · W. Schareck
Department of General, Vascular,
Thoracic and Transplantation Surgery,
University of Rostock,
Rostock, Germany

L. Jonas
Department of Pathology,
University of Rostock,
Rostock, Germany

Abstract Recently, protective effects of nitric oxide donors in pancreatic ischemia/reperfusion (IRI) injury have been described. Their role in post-ischemic microcirculation was previously not investigated. Ischemia reperfusion was induced in an isolated pancreatic tail segment in situ. Animals were randomized to four experimental groups ($n = 7$ animals/group), the control group (CO) received saline as placebo. Treatment groups received either sodium nitroprusside (SN) 5 min before until 2 h after reperfusion, L-arginine (LA) 30 min before reperfusion until 2 h after reperfusion or sodium nitroprusside and L-arginine (SNLA) together. After induction of ischemia (2 h) post-ischemic microcirculation was observed for 2 h by intravital-fluorescence microscopy. Functional-capillary density (FCD), leukocyte adherence in post-capillary venules (LAV) and histological damage were analysed. After reperfusion FCD decreased in all groups ($P < 0.05$). FCD was significantly restored in all groups with administration of nitric oxide donors after reperfusion ($P < 0.05$) as compared to CO without significant difference between the individual nitric oxide donor groups. Leukocyte adherence was significantly increased 1 h and 2 h after reperfusion ($P < 0.001$) as compared to baseline, which was lower in all nitric oxide donor groups. Histological damage in the pancreatic tail-segment was significantly reduced in nitric oxide donor groups ($P < 0.01$). Administration of nitric oxide donors might be useful in ischemia-reperfusion injury of the pancreas by its protective effect on microcirculation and inflammatory reaction.

Keywords Nitric oxide · Ischemia/reperfusion · Pancreas · Intravital microscopy · Microcirculation

Introduction

In the treatment of Type 1 diabetes mellitus with end-stage renal disease, simultaneous pancreas-kidney transplantation is the treatment of choice [1]. After enormous progress in handling problems concerning immunocompatibility, factors like brain death [2] and ischemia/reperfusion injury (IRI) [3, 4] are becoming the center of interest of many investigations with the aim to improve outcome after solid-organ transplantation. IRI plays an important role in the development of graft pancreatitis [5], which is still an unsolved problem in pancreas transplantation. Graft pancreatitis can cause local and systemic complications [5] with an incidence of around 30%, which is responsible for up to 7% of early organ losses [6]. It may also be a trigger of increased immunogenicity of the graft. The current approach to avoid these problems is very strict donor selection

leading to considerable organ shortage [7]. To date the complex pathophysiology of graft pancreatitis is not completely understood. Experimental [8, 9] and clinical [10] data show evidence, that microcirculatory disorders in the early phase after reperfusion can decisively influence the development of IRI and thus the degree of graft pancreatitis. We previously reported that impairment of microcirculation in the reperfusion period leads to recurrent tissue hypoxia [11] with further depletion of high-energy stores, breakdown of transmembranal ion transport and finally irreversible cell damage [12]. Reperfusion of ischemic tissue with oxygenated blood also leads to generation of free radicals, release of local mediators (e.g. prostaglandins and leukotrienes) that have a direct toxic and inflammation-promoting effect [13]. Nitric oxide is also released during reperfusion, however, it is still under debate whether this is a protective or an aggravating factor. Intravascular nitric oxide was thought to be protective because of its leukocyte inhibition and vasodilatory effect. In contrast, interstitial nitric oxide was thought to be harmful due to its radical properties and peroxynitrite formation. We hypothesized that administration of a direct nitric oxide donor (e.g. sodium nitroprusside) that releases nitric oxide independently from nitric oxide synthases and their local distribution may have differential effects on post-ischemic pancreatic microcirculation compared to the administration of L-arginine, the substrate of nitric oxide-synthases. Therefore we investigated the effect of L-arginine and sodium nitroprusside in a rat model of normothermic in situ IRI [8] on post-ischemic microcirculatory events and tissue damage.

Material and methods

Anaesthesia and monitoring

The experiments were carried out in accordance with German legislation for the protection of animals. For experiments male Wistar rats (250–330 g, Tierzuchtanlage Schönwalde, Schönwalde, Germany) were used. Anaesthesia was induced with pentobarbital (60 mg/kg b.w.), and a tracheostomy was performed for mechanical ventilation (O_2 70%, N_2O 30%). Continuous intra-arterial blood pressure monitoring (right carotid artery) and intravenous access (left jugular vein) were achieved by placed catheters (PE-50, 0.58 mm inner diameter, Portex, Hythe, Kent, UK). The venous catheter was rinsed by a continuous infusion of an electrolyte–glucose infusion (3–8 ml/h; contents: Na 49.1 mmol/l, K 24.9 mmol/l, Mg 2.5 mmol/l, Cl 49.1 mmol/l, H_2PO_4 9.9 mmol/l, lactate 20.0 mmol/l, glucose 50 g/l; Jono-Steril Bas; Fresenius, Bad Homburg, Germany) for volume replacement, the arterial catheter with 0.5 ml/h heparinized saline (1,000 IU heparin/100 ml NaCl 0.9%). Repeated blood gas measurements were done (BGA 288 BGS; Ciba Corning, Fernwald, Germany). According to this, ventilation parameters (volume controlled ventilation, Kleintierbeatmungspumpe: TSE, Bad Homburg, Germany) were adapted during baseline conditions: pH 7.4 ± 0.1, apO_2 110–190 mmHg, $apCO_2$ 25–45 mmHg.

Animal model

Preparation of the abdominal situs was performed as previously described in detail [8]. The upper abdomen was opened by transverse laparotomy. The stomach was turned up cranially and fixed on the skin. The prepared pancreatic tail segment was isolated and pedunculated on the splenic vessels, a micro-catheter was then inserted into the left gastric artery for organ flushing. Followed by a stabilization period (~10 min) the organ was flushed (0.5 ml NaCl 0.9%) via the catheter in the left gastric artery and an ischemic period of 2 h was induced by clamping the splenic vessels. After reperfusion the animals were observed for 2 h. To keep the temperature of the exteriorized pancreas constant (35–38°C) the pancreatic tail segment was perfused with warmed saline.

Experimental protocol

Animals were randomly assigned to one of the four experimental groups ($n = 7$ animals/group). Animals assigned to the control group (CO) received as placebo NaCl 0.9% i.v. (12.8 ml/h/Kg b.w. for 30 min before reperfusion and with 3.2 ml/h/Kg b.w. for 240 min after reperfusion) and i.a. (1 ml /Kg b.w. beginning 5 min before reperfusion until 2 h after reperfusion). The animals received -nitroprusside (SN) (Na-Nitroprussid, Schwarz Pharma, Germany) 1 µg/kg/min starting 5 min until 2 h after reperfusion (i.a. catheter in left gastric artery) and the i.v. placebo (see CO). Animals received L-arginine (LA) (Pharmacy, University of Rostock) 200 mg/kg b.w. for 30 min before reperfusion and 200 mg/kg b.w. for 2 h after reperfusion and i.a. placebo (see CO). In the group with a combination of SN and LA (SNLA) nitric oxide donors were utilized as described in SN and LA groups.

Intravital microscopy and quantification of microcirculation

Microcirculatory measurements were done by intravital fluorescence microscopy. For contrast enhancement of microvessels 0.2 ml of 0.4% bovine-albumin labelled with fluorochrome fluorescein-isothiocyanate (FITC-

albumin, Sigma-Aldrich, Deisenhofen, Germany) and for in-vivo staining of leukocytes 0.1 ml 0.01% Rhodamine 6G (Sigma) were administered intravenously. Intravital fluorescent microscopy was performed, using a modified Nikon-Eclipse E600-FN epifluorescence microscope with mercury vapour lamp HB-10103AF-Hg 100 Watt (Nikon, Düsseldorf, Germany). Filter blocks for fluorescein (excitation 465–495 nm, emission > 515 nm) and G-2A (excitation 510–560 nm, emission > 590 nm) were used for epi-illumination (Nikon). A universal immersion objective (water and oil, X20/0.45, Plan Flour, Nikon) provided a magnification of approximately X650 on the video screen (WV-BM1700, Panasonic, Osaka, Japan). Observations were recorded by means of a charge-coupled device video camera (RS-170 Monochrome CCD Camera, Cohu, San Diego, USA) and stored on a video tape (video recorder AG-4700EY, Panasonic, Osaka, Japan) for off-line evaluation. A time-code generating interface was installed between the camera and video recorder (TCI 70, Alpermann/Velte, Supernal, Germany). Assessment of microcirculatory changes was done by quantification of functional capillary density (FCD) and the number of leukocytes adherent in postcapillary venules (LAV). Measurements were performed before ischemia (baseline), 60 and 120 min after reperfusion. FCD, the length of red blood cell-perfused capillaries per defined area (cm/cm^2), and LAV, the number of leukocytes adherent to the vessel wall for at least 30 s, were assessed. FCD was quantified by off-line analysis of the video tapes, as previously described [8]. For FCD ten randomly chosen areas (350×200 μm) of the pancreas (distance to the organ border > 5 mm) were evaluated at each time point. Leukocyte adherence in post-capillary venules (LAV) was quantified by analysing three postcapillary venules (diameter of > 20 μm and length of < 80 μm) at each time point. Adherent leukocytes ($cells/mm^2$) were defined as leukocytes sticking to the vessel wall for at least 30 s.

Histology

At the end of the experiment the pancreatic tail segment was removed for histological investigations. Samples from the pancreatic tail segments were taken from the central parts, with a minimum distance of 5 mm to the edge of dissection area and to the ligated spleen, and were immediately fixed in 4% neutral buffered formalin. After dehydration and embedding in paraffin the samples were cut ~4 μm (Biocut 2035, Lexica, Munich, Germany) and stained with hematoxylin and eosin. Tissue samples for cryo-sections were blocked onto a metal stamp and immediately frozen in liquid nitrogen. They were cut (~6 μm) and subjected to an antibody-specific staining procedure (APAP method, granulocyte specific antibody RK4, Dianova, Hamburg, Germany). Histomorphological characteristics were evaluated blinded accordingly to a prescribed semi-quantitative score including edema, vacuolization, PMN-infiltration and necrosis (at least five high power fields were investigated) [4].

Tissue edema

To quantify pancreatic edema, determination of water content was performed after weighing in a wet state and incubation for 20 h at 100°C in a heating box (Memmert, Schwabach, Germany). Data are demonstrated as dry/wet ratio calculated with the formula:

$$\text{Edema } [\%] = \frac{\text{wet pancreas} - \text{dry pancreas}}{\text{wet pancreas}} \times 100$$

Statistics

Data are presented as mean values ± SEM (normally distributed) and mean values ± SD (non-normally distributed). After normal distribution testing was performed, data at each time point were subjected to the ANOVA one-way analysis of variance performing the Bonferroni post-test. Within each individual group one-way repeated-measures ANOVA followed by the Bonferroni post-test. Histological damage scores were compared using Mann–Whitney U test using a Prism Graph Pad (Graph Pad Software, San Diego, USA). Significance was defined as a P value less than 0.05.

Results

Macrocirculation

Under baseline measurements and in the course of the experiment there were no significant changes in heart rate (HR) and mean arterial pressure (MAP) in and between all experimental groups (Table 1). Rinsing of the exteriorized pancreas with warmed saline maintained the temperature of the pancreatic tail segment constant (35.5–37.5°C), while the body temperature did not differ significantly between the experimental groups.

Microcirculatory data

There were no significant differences in functional capillary density (FCD) under baseline conditions between all groups. The loss of FCD was significant in all experimental groups after reperfusion ($P < 0.01$). Com-

Table 1 Heart rate (HR) and mean arterial pressure (MAP). Values are means ± SEM

Variable		Baseline	60 Min reperfusion	120 Min reperfusion
HR (per min)	CO	396●7	427±11	419±10
	SN	383±10	410±13	396±16
	LA	396±11	421±16	432±19
	SNLA	413±17	409±19	412±16
MAP (mmHg)	CO	105±5	99±3	94±4
	SN	101±2	96±2	91±3
	LA	100±2	90±5	89±4
	SNLA	102±7	97±5	93±5

pared to CO animals FCD was significantly higher in all groups with administration of nitric oxide donors 1 h and 2 h after reperfusion ($P<0.05$). Between the different nitric oxide donor groups there were no significant differences in FCD after reperfusion (Fig. 1, Table 2).

Leukocytes adherent in postcapillary venules

There were no significant differences in LAV under baseline conditions between all experimental groups. Reperfusion in CO animals the increase of LAV was significant after 2 h ($P<0.001$) and there were significantly lower LAV values in nitric oxide donor groups as compared to controls ($P<0.001$). Between the different nitric oxide donor groups there were no significant differences in LAV after reperfusion (Fig. 2).

Light microscopy

Histological damage in the pancreas tail segment subjected to 2 h of normothermic in situ ischemia was highest in CO animals (9.1 score points ±0.8 SD). In all other groups administration of nitric oxide donors could ameliorate the histological damage significantly (SN 6.7±1.3 $P=0.003$, LA 5.6±1.4 $P=0.003$, SNLA 5.1±2.2 $P=0.004$). There was no significant difference between nitric oxide donor groups (Fig. 3).

Dry/wet ratio

Tissue fluid content at the end of the experiment showed no significant difference between all four experimental groups (CO 87.1%±1 SD, SN 87.3%±1, LA 83.6%±3, SNLA 85.3%±1).

Discussion

There has been no systematic intravital-microscopic investigation on the effect of exogenic and endogenic nitric oxide donors in pancreatic ischemia/reperfusion injury. In the present study we demonstrated a significant protective effect of nitric oxide donors (sodium nitroprusside, L-arginine and their combination) on post-ischemic nutritive tissue perfusion, local tissue inflammation and histological damage in a model of normothermic in situ IRI by means of intravital microscopy.

The pancreas is very susceptible to ischemia and hypoxia. The ischemic period is characterized by the depletion of tissue energy stores with a continuous loss of cellular function and integrity [3]. Reperfusion of the ischemic pancreas activates a variety of pathophysiological cascades that lead to functional and morphological changes and disturbances. Microcirculatory disturbances caused by ischemia/reperfusion injury (IRI) are the crucial hallmarks of pancreatitis following pancreas transplantation [10]. A decrease of nutritive tissue perfusion (FCD) and elevated local inflammatory tissue

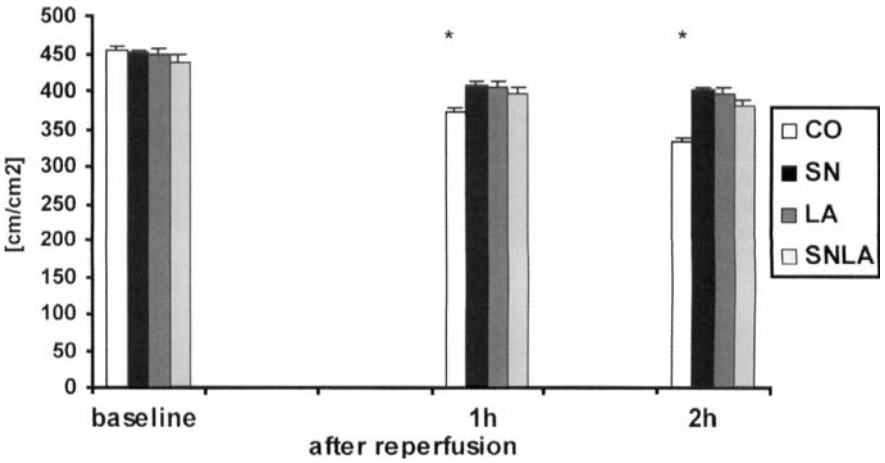

Fig. 1 Functional capillary density (cm/cm^2). Microcirculatory disorders after reperfusion following a period of 2 h normothermic in situ ischemia. Significant decrease of FCD in all groups. Improvement of the microcirculation by nitric oxide donors after reperfusion. *$P<0.01$ CO vs nitric oxide donor groups 1 h and 2 h after reperfusion

Table 2 Microcirculatory data for FCD and LAV. Values are means ± SEM

Variables		Baseline	60 Min reperfusion	120 Min reperfusion
FCD (cm/cm^2)	CO	455 ± 4^a	372 ± 5	33 ± 6
	SN	451 ± 10^a	409 ± 8^b	403 ± 10^c
	LA	448 ± 4^a	405 ± 5^b	396 ± 4^c
	SNLA	448 ± 12^a	397 ± 9^b	380 ± 8^c
LAV ($cells/mm^2$)	CO	22 ± 7^d	83 ± 15	283 ± 47
	SN	27 ± 11	98 ± 40	125 ± 41^d
	LA	35 ± 11	40 ± 13	63 ± 2^d
	SNLA	43 ± 15	39 ± 14	60 ± 32^d

[a] $P < 0.01$ vs 60 min and 120 min after reperfusion
[b] $P < 0.05$ vs CO 60 min reperfusion
[c] $P < 0.05$ vs CO 120 min reperfusion
[d] $P < 0.001$ vs CO 120 min after reperfusion

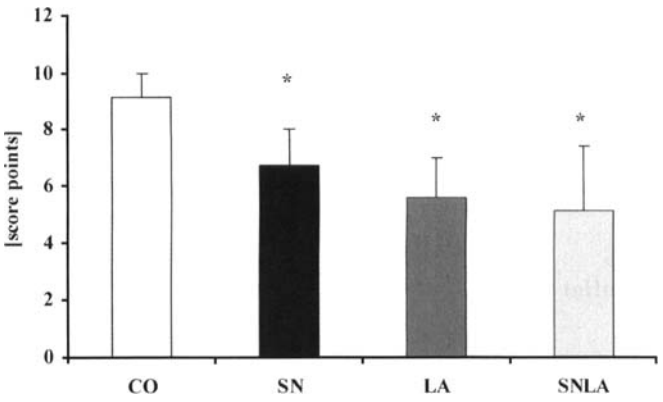

Fig. 3 Histological damage, semi-quantitative score including edema, vacuolization, PMN infiltration, necrosis (score points) at the end of the experiment. Significant amelioration of histological damage in nitric oxide donor groups at the end of the experiment. *$P < 0.01$ vs CO

response (LAV) are important determinants of tissue damage after reperfusion of the ischemic pancreas [8].

The role of nitric oxide in pancreatic IRI is still not clearly understood. Nitric oxide is important in physiological regulation of blood pressure via vascular smooth muscle tone [14]. It is also crucially involved in the physiological regulation of microcirculation. Furthermore nitric oxide inhibits neutrophil-endothelial interaction by inhibition of leukocyte activation and expression of adhesion molecules, it also interferes with platelet aggregation [15, 16]. All these effects are thought to be mediated by the activation of the soluble guanylate cyclase and consecutive modulation of ion channels by cGMP [14].

Negative effects are believed to be related to the interaction of nitric oxide with the superoxide anion generating toxic peroxynitrite [17], in combination with the stimulation of exocrine secretion [18] and induction of apoptosis [17]. All of these effects have been described to be detrimental in pancreatic IRI [19].

For other organs contradictory effects have been described, e.g. in the small intestine nitric oxide is clearly protective [20]. In kidneys higher lipid-peroxidation [21] after administrating L-arginine could have a toxic effect on tubular cells [22] but publications also see a protective effect of L-arginine and sodium nitroprusside [23], e.g. in the liver [24]. In the heart protective effects are described both for nitric oxide administration and inhibition of nitric oxide-synthesis [25] but also direct toxic effects of L-arginine on cardiomyocytes were seen [26]. In CNS detrimental effects of nitric oxide administration predominate [27].

In pancreatic IRI contradictory effects of nitric oxide have also been described. In experimental pancreas transplantation in rats, amelioration of post-ischemic microcirculatory disorders has been shown by the administration of L-arginine [28]. In a pig model of in situ IRI we could demonstrate the improvement of histological damage, tissue oxygenation, blood flow and lipase activity by the exogenic nitric oxide donor sodium nitroprusside [29]. Similar histological effects were seen for nitric oxide in experimental acute pancreatitis [30].

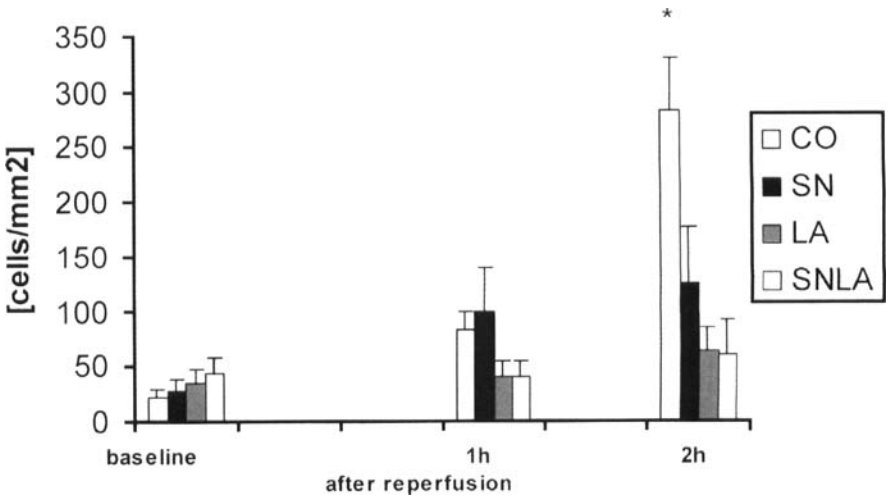

Fig. 2 Leukocytes adherent in postcapillary venules ($cells/mm^2$). Elevated inflammatory tissue response after reperfusion. Significant higher values in CO animals 2 h after reperfusion compared to nitric oxide donor groups. *$P < 0.001$ vs CO baseline, CO 1 h and vs SN, La and SNLA 2 h after reperfusion

For inhibition of nitric oxide-synthesis influence on morphological changes but a reduction of post-ischemic edema and lipase activity was found [31]. In a model of incomplete pancreatic ischemia sodium nitroprusside caused lower systemic lipase levels and an amelioration of histological damage [32].

In our experiments the supply of nitric oxide improves pancreatic microcirculation (FCD) during reperfusion. The viability of the post-ischemic microcirculation is a very sensitive parameter [33]. As well as the reduction of pro-inflammatory mediators of the activated leukocytes, relaxation of the arterial tone could be another way of promoting improved reperfusion [13]. There are hints that inhibition of platelet function ameliorates IRI [16]. Whether or not the anticoagulatory effect of nitric oxide contributes to the protective effect cannot be deduced from our data.

The other important finding of our experiments is the attenuation of leukocyte/endothelium interaction (LAV) with a significant reduction of adherent leukocytes in post-capillary venules 2 h after reperfusion. Thus, administration of nitric oxide leads to a significant reduction of local inflammatory tissue response. This is of special interest because apart from impairment of microcirculation neutrophil infiltration is another major pathogenetic factor in the development of acute pancreatitis [34] and graft pancreatitis [35]. Furthermore it could be speculated that the reduced inflammatory response could also bring lower initial immunogenicity this could be important for later rejection episodes [36]. One important goal of this paper was to define the different effects of direct and indirect nitric oxide supplementation; however, our data showed identical effects on the parameters assessed. The reason why a combined administration of LA and SN did not increase the efficacy remains speculation; a rationale could be a ceiling effect. Probably the high local (intra-arterial) administration SN could prevent further protective effects of LA. Nitric oxide donors are known to cause hypotension. In pilot dose-finding experiments we could define doses of SN and LA effectively ameliorating IRI without depression of MAP (data not shown). In clinical studies LA was used in the same dose but had no effect on MAP and heart rate, neither did it have any other side effects [37]; thus administration of LA seems feasible. Sodium nitroprusside however obviously has a stronger nitric oxide-hypotensive effect in humans and should not be used for clinical studies as it has no advantage over L-arginine.

In summary we demonstrate a clearly protective effect of direct (sodium nitroprusside), indirect nitric oxide administration (L-arginine) and the combination of both. Protective effects were seen for the two most important pathogenetic factors in the development of graft pancreatitis, which are post-ischemic microcirculatory disorders (FCD) and local inflammatory tissue response (LAV). The combination of both nitric oxide donors showed no superiority as compared to L-arginine or sodium nitroprusside alone. Focusing therapeutic efforts on improvement of post-ischemic microcirculation and reducing inflammatory tissue response may be an important step towards reducing morbidity after pancreas transplantation. We postulate that administration of L-arginine should be considered for clinical studies in pancreas transplantation.

References

1. Busing M, Martin D, Riege R, et al. Combined pancreas/kidney transplantation as standard procedure in therapy of type I diabetic patients in renal failure. Chirurg 1996; 67:1002.
2. Pratschke J, Wilhelm MJ, Kusaka M, et al. Brain death and its influence on donor organ quality and outcome after transplantation. Transplantation 1999; 67:343.
3. Benz S, Schnabel R, Morgenroth K, et al. Ischemia/reperfusion injury of the pancreas: a new animal model. J Surg Res 1998; 75:109.
4. Obermaier R, Benz S, Kortmann B, et al. Ischemia/reperfusion induced pancreatitis in rats: a new model of complete normothermic in situ ischemia of a pancreatic tail segment. Clin Exp Med 2001; 1:51.
5. Benz S, Pfeffer F, Buesing M, et al. Lokale- und systemische Komplikationen nach kombinierter Nieren/-Pankreas Transplantation. Chir Gastroenterol 1996; 12 (suppl).
6. Grewal HP, Garland L, Novak K, et al. Risk factors for post-implantation pancreatitis and pancreatic thrombosis in pancreas transplant recipients. Transplantation 1993; 56:609.
7. Benz S, Obermaier R, Wiessner R, et al. Effect of nitric oxide in ischemia/reperfusion of the pancreas. J Surg Res 2002; 106:46.
8. Obermaier R, Benz S, von Dobschuetz E, et al. Characterisation of microcirculatory disturbance in a novel model of pancreatic ischemia-reperfusion using intravital fluorescence-microscopy. Pancreas 2002; 25:142.
9. Benz S, Pfeffer F, Adam U, et al. Impairment of pancreatic microcirculation in the early reperfusion period during simultaneous pancreas-kidney transplantation. Transpl Int 1998; 11 Suppl 1:433.
10. Benz S, Bergt S, Obermaier R, et al. Impairment of microcirculation in the early reperfusion period predicts the degree of graft pancreatitis in clinical pancreas transplantation. Transplantation 2001; 71:759.

11. Benz S, Wiessner R, Obermaier R, et al. Microcirculatory events in ischemia/reperfusion injury defined by continuous tissue oximetry. Transpl Int 2002; 15:173.
12. Heemann U, Szabo A, Hamar P, et al. Lipopolysaccharide pretreatment protects from renal ischemia/reperfusion injury: possible connection to an interleukin-6-dependent pathway. Am J Pathol 2000; 156:287.
13. Sakorafas GH, Tsiotos GG, Sarr MG. Ischemia/reperfusion-induced pancreatitis. Dig Surg 2000; 17:3.
14. Moncada S, Radomski MW, Palmer RM. Endothelium-derived relaxing factor. identification as nitric oxide and role in the control of vascular tone and platelet function. Biochem Pharmacol 1988; 37:2495.
15. Moilanen E, Vuorinen P, Kankaanranta H, et al. Inhibition by nitric oxide donors of human polymorphonuclear leucocyte functions. Br J Pharmacol 1993; 109:852.
16. Radomski MW, Palmer RM, Moncada S. Modulation of platelet aggregation by anL-arginine-nitric oxide pathway. Trends Pharmacol Sci. 1991; 12:87.
17. Bosca L, Hortelano S. Mechanisms of nitric oxide-dependent apoptosis: involvement of mitochondrial mediators. Cell Signal 1999; 11:239.
18. Molero X, Guarner F, Salas A, et al. Nitric oxide modulates pancreatic basal secretion and response to cerulein in the rat: effects in acute pancreatitis. Gastroenterol 1995; 108:1855.
19. Hoffmann TF, Uhl E, Messmer K. Protective effect of the somatostatin analogue octreotide in ischemia/reperfusion-induced acute pancreatitis in rats. Pancreas 1996; 12:286.
20. Kubes P, Kurose I, Granger DN. NO donors prevent integrin-induced leukocyte adhesion but not p-selectin-dependent rolling in post-ischemic venules. Am J Physiol 1994; 267:H931.
21. Cristol JP, Thiemermann C, Guerin MC, et al.L-arginine infusion after ischaemia-reperfusion of rat kidney enhances lipid peroxidation. J Lipid Mediat Cell Signal 1996; 13:9.
22. Tome LA, Yu L, de Castro I, et al. Beneficial and harmful effects ofL-arginine on renal ischaemia. Nephrol Dial Transplant 1999; 14:1139.
23. Lopez-Neblina F, Paez AJ, Toledo-Pereyra LH. Modulation of neutrophil infiltration through nitric oxide in the ischemic rat kidney. Transplant Proc 1995; 27:1883.
24. Liu P, Xu B, Hock CE, et al. NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R. Am J Physiol 1998; 275:H2191.
25. Amrani M, Chester AH, Jayakumar J, et al.L-arginine reverses low coronary reflow and enhances post-ischaemic recovery of cardiac mechanical function. Cardiovasc Res 1995; 30:200.
26. Takeuchi K, McGowan FX, Danh HC, et al. Direct detrimental effects ofL-arginine upon ischemia- reperfusion injury to myocardium. J Mol Cell Cardiol 1995; 27:1405.
27. Verrecchia C, Boulu RG, Plotkine M. Neuroprotective and deleterious effects of nitric oxide on focal cerebral ischemia-induced neurone death. Adv Neuroimmunol 1995; 5:359.
28. Vollmar B, Janata J, Yamauchi JI, et al. Attenuation of microvascular reperfusion injury in rat pancreas transplantation byL-arginine. Transplantation 1999; 67:950.
29. Benz S, Schnabel R, Weber H, et al. The nitric oxide donor sodium nitroprusside is protective in ischemia/reperfusion injury of the pancreas. Transplantation 1998; 66:994.
30. Werner J, Rivera J, Fernandez-del Castillo C, et al. Differing roles of nitric oxide in the pathogenesis of acute edematous versus necrotizing pancreatitis. Surgery 1997; 121:23.
31. Hotter G, Closa D, Pi F, et al. Nitric oxide and arachidonate metabolism in ischemia-reperfusion associated with pancreas transplantation. Transplantation 1995; 59:417.
32. Tanaka S, Kamiike W, Kosaka H, et al. Detection of nitric oxide production and its role in pancreatic ischemia-reperfusion in rats. Am J Physiol 1996; 271:G405.
33. Nolte D, Messmer K. Tissue protection by anti-ischemic drugs. Minerva Cardioangiol 1995; 43:485.
34. Klar E, Endrich B, Messmer K. Microcirculation of the pancreas: a quantitative study of physiology and changes in pancreatitis. Int J Microcirc Clin Exp 1990; 9:85.
35. Benz S, Bergt S, Pfeffer F, et al. Pathophysiologische Veränderungen in der frühen Reperfusionsphase humaner Pankreastransplantate. Transplantationsmedizin 1999; 11:41.
36. Büsing M. Morphologie und Pathophysiologie der Transplantatpankreatitis nach kombinierter Pankreasduodenal-/Nierentransplantation. Habilitationsschrift, Ruhr Universität, Bochum 1994.
37. Zimmermann C, Haberl RL.L-arginine improves diminished cerebral CO2 reactivity in patients. Stroke 2003; 34:643.