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ORIGINAL ARTICLE

Oliver Drognitz
Xuemei Liu
Robert Obermaier
Hannes Neeff
Ernst von Dobschuetz
Ulrich Theodor Hopt
Stefan Benz

Ischemic preconditioning fails to improve microcirculation but increases apoptotic cell death in experimental pancreas transplantation

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O. Drognitz (✉) · X. Liu
R. Obermaier · H. Neeff
E. von Dobschuetz · U. T. Hopt · S. Benz
Department of General and Digestive Surgery, University of Freiburg, Hugstetter Strasse 55, 79106 Freiburg im-Bresigau, Germany
E-mail: oliverdrognitz@web.de
Tel.: +49-761-2702806
Fax: +49-761-2702804

Abstract Brief periods of warm ischemia and subsequent short reperfusion before either long-term cold or warm ischemic insult (ischemic preconditioning, IPC) have proven to ameliorate ischemia/reperfusion (I/R) injury in various organs, such as the liver and lung. The aim of this study was to examine the effect of IPC on pancreatic cell apoptosis and microcirculatory impairments in experimental pancreas transplantation. Male Lewis rats served as donors and recipients of heterotopic syngeneic pancreaticoduodenal transplantation. Recipient animals were divided into two experimental groups: group Tx ($n = 7$) received grafts without IPC, group Tx&IPC received grafts with IPC. Animals that had not undergone transplantation but whose pancreata had been exteriorized served as controls ($n = 5$). All pancreatic grafts were preserved in University of Wisconsin solution for 6 h at 4°C. IPC was induced by interruption of the arterial blood flow for 10 min followed by 10 min of reperfusion. One and two hours after reperfusion, graft microcirculation was assessed by means of intravital microscopy (IVM). Rats were immediately killed after the second measurement and DNA breaks of acinar cells were detected by in situ terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate digoxigenin nick end-labelling (TUNEL) assay and gel electrophoresis (laddering). The apoptotic index (AI) was defined as the number of apoptotic cells per high-power field. Analysis of both groups of transplanted grafts showed a significant decrease in functional capillary density (FCD) and a significant increase in leukocyte sticking to postcapillary venules (LAV) at 1 h and 2 h of reperfusion, compared with animals that had not undergone transplantation ($P < 0.01$). In parallel, AI was significantly increased in transplanted grafts compared to the controls ($P < 0.01$). Grafts subjected to IPC showed no significant differences, neither for FCD nor LAV, at both time points if compared with grafts of group Tx. However, IPC resulted in a significant increase in AI ($P < 0.05$). We can conclude that IPC has no effect on pancreatic microcirculation but enhances acinar cell apoptosis in experimental pancreas transplantation. These results indicate that IPC might increase I/R injury after pancreatic cold ischemia.

Keywords Pancreas transplantation · Ischemic preconditioning · Ischemia–reperfusion injury · Intravital microscopy · Microcirculation · Apoptosis

Introduction

Ischemic preconditioning (IPC) is a process in which one or repeated brief periods of vascular occlusion confer a state of organ protection against subsequent long-term ischemia and sustained reperfusion injury. IPC was first discovered by Murry et al. in a canine model of regional myocardial ischemia [1]. In addition to numerous reports on the heart [2], experimental studies on the liver [3], lung [4], small intestine [5], kidney [6, 7], skeletal muscle [8] and even the brain [9] have demonstrated that IPC is capable of ameliorating ischemia–reperfusion (I/R) injury in different models of warm ischemia. Moreover, the preconditioning effect has been reproduced in human tissue [10, 11, 12, 13]. Despite reports on the beneficial effects of IPC in organ transplantation [14, 15, 16] there is less evidence with regard to IPC in cold I/R injury.

It has long been assumed that ischemia/reperfusion mainly induces typical cell necrosis. However, recent studies have emphasized the contribution of apoptosis to organ cell death after ischemia–reperfusion injury in several organs, such as the kidney [17], heart [18], liver [19], lung [20] and small bowel [21]. Thus, increased apoptotic cell death might be an unspecific marker for I/R injury. Moreover, there are data indicating that IPC attenuates apoptosis formation in different models of warm ischemia [7, 22, 23]. For pancreatic tissue, acinar cell apoptosis has been shown to occur during rejection of human pancreas allografts [24] and in experimental pancreatic warm and cold I/R injury [25, 26].

At present, no reports focusing on the effect of IPC in pancreas transplantation are available. Our aim was, therefore, to determine whether IPC is capable of attenuating microcirculatory impairments and pancreatic cell apoptosis in experimental pancreas transplantation.

Material and methods

All animal experiments were in conformity with current German law on the protection of animals and were approved by the local Government Animal Care and Use Committee.

Surgical procedure

Highly inbred male Lewis rats weighing 290–350 g (Department of Pathology, University of Rostock, Rostock, Germany) were used as donors and recipients. After overnight fasting, but free access to water, the animals were anesthetized by intraperitoneal injection of 60 mg/kg body weight pentobarbidal together with administration of 5 μg atropine subcutaneously. Anaesthesia was maintained with 0.2%–0.6% by volume of isoflurane (Forene, Abbott, Wiesbaden, Germany) in N_2O–O_2 (35% oxygen), by use of a Sulla 808 vaporizer (Draeger, Lübeck, Germany). All operations were performed by a single surgeon (O. D.). The animals were placed in the supine position on a heating pad that maintained the body temperature between 35.5°C and 37.0°C. Polyethylene catheters (i.d. 0.50 mm, Portex, Hythe, UK) were inserted into the left carotid artery and right jugular vein of both the donor and the recipient for continuous monitoring of arterial blood pressure (Sirecust 1261, Siemens, Munich, Germany), for continuous substitution of saline solution (4 ml/h) and for intravenous injection of fluorescent dyes after transplantation. Organ harvest and heterotopic pancreaticoduodenal transplantation were performed using a modification of the technique described by Lee et al. [27]. In short, after meticulous preparation, the pancreaticoduodenal graft was isolated on a segment of the aorta including the celiac axis and the superior mesenteric artery. The venous outflow was provided by the portal vein. After intravenous injection of 100 IU heparin, the pancreatic grafts were flushed with and stored in cold (4°C) University of Wisconsin (UW) solution (ViaSpan, DuPont Pharmaceuticals, Bad Homburg, Germany). Heterotopic transplantation with systemic venous drainage was performed by end-to-side anastomosis between the donor portal vein and the recipient infrarenal caval vein. The aortic segment of the graft was anastomosed end-to-side to the recipient infrarenal aorta. Both anastomoses were performed with running sutures of 9-0 nylon (Serag Wiessner, Naila, Germany). Afterwards, the clamp on the donor portal vein was released first, followed by the arterial clamp. All grafts immediately returned to their normal pink colour, with the arterial stump pulsating. The pancreatic tail was gently exteriorized, and the adjacent spleen was fixed on a specially designed stage for intravital microscopy. To exclude exposure to ambient air and to prevent the graft from drying, we covered the pancreatic surface with an oxygen-impermeable transparent film and also constantly superfused the graft with 37°C Ringer's lactated solution, to avoid temperature changes. The temperature of the pancreatic tail was continuously measured by a temperature-sensitive probe (LICOX, GMS, Kiel, Germany). All measurements were carried out only on recipient animals with a systolic arterial blood pressure above 90 mmHg. Short episodes of hypotension immediately after reperfusion were treated by bolus substitution of saline solution. Animals that bled explicitly from anastomosis or from haemorrhages of the pancreatic graft were excluded from further analysis. In all experimental groups, transplanted grafts were observed for 2 h after reperfusion.

Experimental protocol

A total of 14 recipient animals was randomly assigned to two different groups: group Tx ($n=7$) received grafts without IPC and group Tx&IPC ($n=7$) grafts with IPC. All pancreatic grafts were preserved in UW solution for 6 h at 4°C. In addition, animals that had not undergone transplantation but whose pancreata had been exteriorized ($n=5$) served as controls. IPC was induced in group Tx&IPC by the selective clamping of the coeliac axis and superior mesenteric artery for 10 min, followed by 10 min of reperfusion, before the graft was flushed with cold UW solution.

Intravital fluorescence microscopy

Intravital fluorescence microscopy (IVM) was performed with a modified Nikon Eclipse E600-FN epifluorescence microscope (Nikon, Düsseldorf, Germany) with a 100-W mercury-vapour lamp HB-10103AF-Hg. Filter blocks FITC (excitation 465–495 nm, emission > 515 nm) and G-2A (excitation 510–560 nm, emission > 590 nm) were used for epi-illumination. A universal immersion objective (water and oil, X2010.45, Plan Fluor, Nikon) provided a magnification of approximately ×650 on the video-screen (WV-BM1700, diagonal 41 cm, Panasonic, Osaka, Japan). Observations were recorded by means of a charge-coupled device video-camera (RS-170 Monochrome CCD Camera, Cohu, San Diego, Calif., USA) and transferred to a S-VHS video-recorder (AG-4700EY, Panasonic) for off-line evaluation. A time-code generating interface (TCI 70, Alpermann + Velte, Wuppertal, Germany) was installed between the camera and the video-recorder. For contrast enhancement of microvessels, 0.2 ml of 0.4% bovine-albumin labelled with FITC (FITC-albumin, Sigma, Taufkirchen, Germany), and for in vivo staining of leukocytes, 0.2 ml 0.01% rhodamine 6G (Sigma), were administered. Each observation field was recorded for 30 s.

Assessment of microcirculation

Quantitative analysis of microcirculation of pancreatic exocrine tissue included the determination of functional capillary density (FCD) as well as the measurement of the number of leukocytes sticking in postcapillary venules (LAV). As described by Hoffmann et al. [28], randomly selected non-overlapping regions of the exposed pancreatic tail were scanned at 1 h and 2 h after the onset of reperfusion. FCD, defined as the length of all red blood cell-perfused capillaries per observation area (centimetres per square centimetre), was determined by off-line analysis of ten randomly chosen exocrine observation areas (350 μm×200 μm) per time point, according to the method described by Schmid-Schoenbein et al. [29]. A square-type grid system (7×4 squares, square side representing 50 μm) was superimposed on the video-screen. FCD was calculated by counting the number of intersections between the grid and the capillaries as follows: FCD (cm/cm^2) = $\pi/2$ × (number of points of intersection/(2 × number of squares of the grid system × length of the edge of the grid). LAV was quantified by analysing three postcapillary venules (diameter > 20 μm and length < 80 μm) at each time point. Adherent leukocytes (number per square millimetre) were defined as leukocytes sticking to the vessel wall for at least 30 s. LAV was calculated in accordance with the equation: LAV ($number/mm^2$) = number sticking/π× length of the vessel × diameter of the vessel.

Tissue preparation

Animals were killed after 2 h of reperfusion. Samples of pancreatic tissue were fixed overnight in 10% buffered formaldehyde and then embedded in paraffin. Tissue sections (4 μm) were subjected to haematoxylin and eosin (H&E) staining for histological examination and to in situ terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate digoxigenin nick end-labelling (TUNEL) staining for identification of pancreatic cell apoptosis. For examination of DNA fragmentation by agarose-gel electrophoresis, tissue samples of the pancreatic tail were snap frozen in liquid nitrogen and stored at −80°C.

In situ nick-end labelling of fragmented DNA

Identification of apoptotic cells by detection of DNA strand breaks was performed using an in situ cell death detection kit (TUNEL POD, Roche, Mannheim, Germany). The method uses terminal deoxynucleotidyl transferase (TdT) for in situ nick end-labelling of free 3′-OH ends in genomic DNA with deoxyuridine triphosphate (dUTP) (TUNEL assay). The TUNEL reaction was performed in accordance with the manufacturer's instructions.

Briefly, after deparaffinization, the tissue sections were digested by incubation with 20 μg/ml proteinase K (Roche) for 15 min at room temperature. After being washed with PBS, sections were covered with 3% peroxidase block solution for 5 min at room temperature to inactivate endogenous peroxidase. After two rinses in PBS, tissue sections were immersed on ice in 0.1% Triton X-100 in 0.1% sodium citrate for 2 min. Thereafter, the sections were incubated in the TUNEL reaction mixture containing TdT enzyme at 37°C in a 5% CO_2 humidified chamber for 60 min. The reaction was

terminated by a 30-min incubation in a stop/wash buffer. For visualization of incorporated digoxigenin-11-dUTP, sections were incubated with peroxidase-conjugated anti-digoxigenin for 30 min at room temperature. Visualization was achieved with DAB substrate solution. Finally, the sections were counterstained with haematoxylin. Positive controls were treated with DNase (100 μg/μl, Boehringer, Mannheim, Germany). Negative controls were obtained by omission of the TdT in the reaction mixture. Apoptotic cells were counted from > 50 fields under a light microscope with a magnification of ×400 by an observer blind to the source of the sections. Apoptotic cells were counted only after identification of typical morphological criteria, such as chromatin condensation and cell fragmentation. An apoptotic index (AI) was defined as the number of apoptotic cells counted per high-power field (hpf).

DNA fragmentation analysis by gel electrophoresis

DNA fragmentation analysis by gel electrophoresis is based on the observation that DNA fragments of apoptotic cells subjected to gel electrophoresis reveal a distinctive ladder pattern of multiples of an approximately 180 bp subunit. Briefly, frozen pancreatic tissue was homogenized, and DNA was extracted by means of an apoptotic DNA ladder kit (Roche). DNA concentration was determined by ultraviolet absorbency at 260 nm. An equal quantity of DNA (15 μg) from each tissue sample was mixed with the loading buffer and subjected to electrophoresis on a horizontal 1.5% agarose gel, together with a positive control. The gel was stained with ethidium bromide and photographed under an ultraviolet trans-illumination lamp (302 nm).

Statistical analysis

All data were analysed using the GraphPad Prism software (version 3.00). Results were expressed as mean values ± standard error of the mean (SEM). Values of $P < 0.05$ were considered significant. All data were first proven to fit the assumption of normality. Differences between groups were tested by an analysis of variance (ANOVA), followed by a post-hoc comparison using the Newman–Keuls method.

Results

Reperfusion was successful in all animals with a systolic arterial blood pressure above 90 mmHg. None of the transplanted grafts exhibited signs of graft thrombosis. FCD (in centimetres per square centimetre) of control animals was 441 ± 13 at 1 h and 461 ± 19 at 2 h of reperfusion (Fig. 1a), whereas LAV (in cells per square mm) was 5 ± 5 and 0 ± 0 at 1 h and 2 h of reperfusion (Fig. 1b), respectively. In both groups of animals that had undergone transplantation FCD was significantly lower ($P < 0.05$) and LAV was significantly higher ($P < 0.05$) than the values of the controls. However, in grafts subjected to IPC, we found no significant difference, neither for FCD (352 ± 9 vs 364 ± 10 at 1 h, $P > 0.05$; 342 ± 13 vs 313 ± 22 at 2 h reperfusion, $P > 0.05$) nor for LAV (119 ± 25 vs 107 ± 21 at 1 h, $P > 0.05$; 127 ± 57 vs 191 ± 30 at 2 h reperfusion, $P > 0.05$), if compared with animals of group Tx.

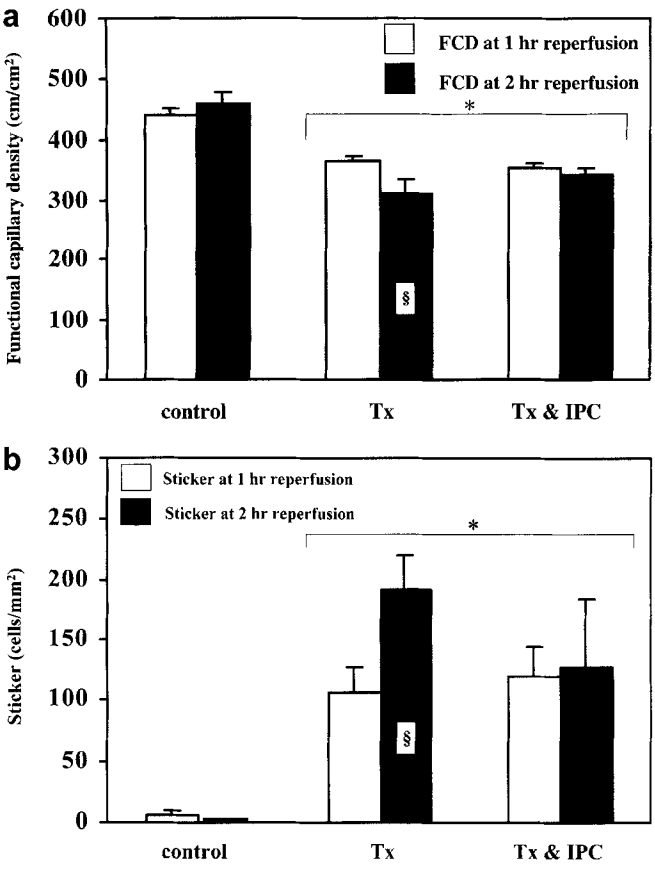

Fig. 1a,b Quantitative analysis of the microcirculation of exocrine pancreatic tissue after whole-pancreas transplantation, assessed by means of intravital fluorescence microscopy according to different transplant settings. Group Tx received pancreatic grafts without IPC ($n = 7$). Group Tx&IPC received pancreatic grafts subjected to IPC by 10 min of warm ischemia followed by 10 min of reperfusion before long-term ischemia and sustained reperfusion ($n = 7$). Animals that did not undergo transplantation but underwent only exteriorization of the pancreas ($n = 5$) served as controls. **a** Functional capillary density; **b** permanently (> 30 s) adherent leucocytes (*sticker*) in postcapillary venules (LAV) at 1 h and 2 h reperfusion. Data presented as means ± SEM; ANOVA and Newman-Keuls multiple post-hoc comparison test. *$P < 0.05$ compared with controls. Student's *t*-test for paired samples (§$P < 0.05$ compared within groups at 1 h reperfusion)

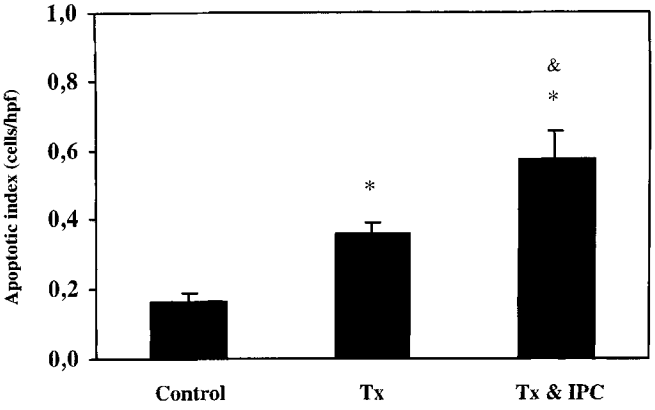

Fig. 2 Apoptotic index (apoptotic cells per high-power field, ×400) of acinar cells in pancreatic tissue. Grafts were harvested after 2 h of reperfusion. Group Tx received pancreatic grafts without IPC ($n=7$). Group Tx&IPC received pancreatic grafts subjected to IPC, with 10 min of warm ischemia followed by 10 min of reperfusion before long-term ischemia and sustained reperfusion ($n=7$). Animals that did not undergo transplantation but underwent only exteriorization of the pancreas ($n=5$) served as controls. Apoptotic cells were identified by typical morphological signs after in situ staining using the TUNEL assay method. Data presented as means ± SEM; ANOVA and Newman–Keuls multiple comparison test (*$P<0.05$ compared with controls and $P<0.05$ compared with group Tx)

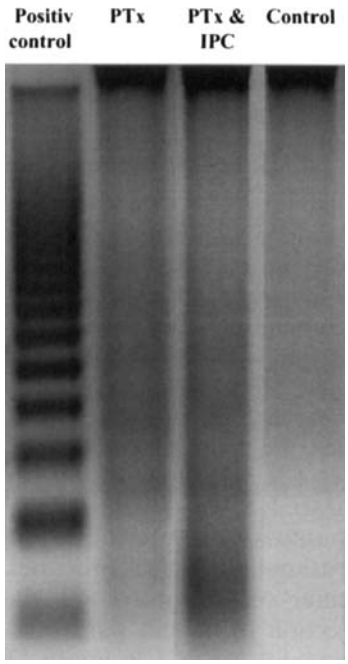

Fig. 3 Pancreatic tissue subjected to 6 h of cold ischemia and subsequent 2 h of reperfusion displayed a characteristic laddering pattern of 180-bp DNA fragments suggestive of apoptosis (*lanes 3 and 4*). The laddering pattern was highest expressed in pancreatic tissue subjected to IPC prior to transplantation (*lane 3*), whereas banding of grafts of group Tx without IPC was less pronounced. No DNA laddering was found in pancreatic tissue of animals that did not undergo transplantation (*lane 2*). Positive control (lane 1)

Using the TUNEL method (Fig. 2), we noted a low level of background apoptosis (AI, cells/hpf) in control animals (0.16 ± 0.03). Pancreatic cold ischemia and subsequent reperfusion was associated with a significant increase in AI for both groups of transplanted grafts (control group vs group Tx, $P<0.01$; control group vs group Tx&IPC; $P<0.01$). However, grafts subjected to IPC showed significantly higher levels of AI than grafts of group Tx (0.57 ± 0.08 vs 0.36 ± 0.03, $P<0.05$). In all groups investigated, apoptosis was detected only in acinar cells, not in islet cells or duct cells.

DNA laddering

DNA was extracted from pancreatic tissue subjected to I/R injury and from controls. DNA laddering (fragments in multiples of an approximately 180-bp subunit) (Fig. 3) was evident only in pancreatic tissue undergoing I/R injury. The laddering pattern was highest expressed in grafts of group Tx&IPC. In contrast, no DNA laddering was detected in control animals.

Histology

Histological examination of pancreatic tissue showed only slight interstitial oedema in control animals. Grafts of group Tx displayed light interstitial oedema and vacuolization in acinar cells, with no signs of cell necrosis. Similar results were found for grafts subjected to IPC. Histological examination revealed no significant leukocyte infiltration in any of the groups investigated. However, cell necrosis was found to occur marginally more frequently among grafts of group Tx&IPC than among grafts of group Tx.

Discussion

Various ways of organ preconditioning to ameliorate the harmful effects of I/R injury have been investigated during the past two decades [30]. One strategy is to expose organs to one or repeated short episodes of warm ischemia and reperfusion before long-term ischemia/reperfusion injury (ischemic preconditioning). Since the introduction of IPC by Murry et al. in a canine model of regional myocardial ischemia [1], its beneficial effects have been confirmed for different organs [31]. Various mechanisms have been suggested to explain the effects of IPC, such as activation of adenosine, adrenergic, bradykinin, and opioid receptors, activation of intracellular mediators such as protein kinase C, tyrosine kinases and the adenosine triphosphate (ATP)-sensitive

potassium channels in the mitochondrial membrane, expression of heat shock proteins, synthesis of antioxidant enzymes, and many others [30, 31, 32]. However, there is still an ongoing discussion as to whether these effects represent causes, effects, or just epi-phenomena [32].

There is a high level of evidence that microcirculatory derangement caused by I/R injury plays a pivotal role in the pathogenesis of pancreatitis [33, 34, 35, 36, 37]. Structural and functional damage in I/R injury results in leukocyte accumulation and adherence to venules, platelet aggregation, vasoconstriction and deterioration of capillary perfusion, which can be quantified by intravital fluorescence microscopy [38]. Recent studies have determined the contribution of apoptosis to total cell death after I/R injury in several organs, such as kidney [17], heart [18], liver [19], lung [20] and small bowel [21]. Apoptosis is involved in a variety of physiological and pathophysiological processes. For pancreatic tissue, acinar cell apoptosis was shown to occur during the rejection of human pancreas allografts [24] and in experimental warm I/R injury [25]. In a cold ischemia model, we were the first to correlate acinar cell apoptosis with the severity of microcirculatory impairments measured by intravital fluorescence microscopy (manuscript accepted in American Journal of Transplantation). Therefore, apoptosis seems to be an unspecific marker of I/R injury in experimental pancreas transplantation.

In the present study, we hypothesized that IPC would diminish I/R injury after pancreas transplantation. That assumption is based on the observation that IPC attenuates apoptosis formation in different models of warm ischemia [7, 22, 23]. However, our data suggest that IPC worsens I/R injury in experimental pancreas transplantation by an acceleration of apoptotic cell death, and it has no beneficial effect on microcirculation. In consequence, the question arises as to whether IPC is unsuitable for diminishing pancreatic ischemia/reperfusion injury in general or if our observation is related to certain features of our experimental setting.

For example, studies support the theory that the effect of IPC depends on an appropriate time frame. The relevance of a precise sequence and design of the different steps involved in IPC has been emphasized for the heart [2]. Each stage of IPC, e.g. preconditioning ischemia, intervening reperfusion, test ischemia and final reperfusion, has important limitations for the total effect derived from IPC. The classic time frame of induction of IPC to the myocardium consists of several short ischemic episodes followed by an intervening period of less than 1 h to 2 h. However, small alterations in the sequence of the steps might decrease protection and result in an infarct size not significantly different from that of the time-matched controls [2]. Since there are no previous reports on IPC with regard to the pancreas, our time frame was derived from studies on the liver, where a preconditioning ischemia of 10 min, followed by intervening reperfusion also of 10 min, was found to have superior effects when compared with other procedures [10, 14].

In addition, most studies on IPC focused on models of warm ischemia. For this scope there is a lot of evidence, even in humans [10, 11, 12, 13]. Despite reports of amelioration of I/R injury in lung [39] and liver [14, 15] transplantation, there is less compelling evidence that IPC is a universal tool for protection of transplanted organs subjected to cold ischemia. Although rarely investigated, temperature dependence might be an important factor in the pathogenesis of I/R injury. The first systematic investigation on the effect of organ temperature on I/R injury was published in 2001. In a model of warm liver ischemia, with a constant core body temperature, it has been shown that the lowering of the organ temperature to 26°C significantly ameliorates microcirculatory impairment, whereas a further reduction, even to 4°, does not increase protection [40]. In addition, minor changes of core body temperature result in a more pronounced liver I/R injury [41, 42]. These data indicate that the extent of I/R injury in IPC models might also be influenced by temperature adjustments. However, the relevance of temperature dependence on microcirculation and apoptotic cell death in IPC has yet to be determined.

In ischemia/reperfusion-induced pancreatitis it has also been reported that sensory and, in part, vagal, nerve ablation counteract the increase of pancreatic blood flow and the reduction in plasma interleukin-1β levels derived from IPC. These findings suggest that the effect of IPC in pancreatic I/R injury might be related, at least in part, to the innervation of the pancreas [43]. Therefore, the total loss of organ innervation early after pancreas transplantation might account for our findings. Accordingly, it has been shown that acute brain death abolishes the cardioprotective effects of ischemic preconditioning in the rabbit [44]. However, this effect was related to norepinephrine release [45], whereas the effect of loss of organ innervation has not been determined.

In summary, our study shows that IPC deteriorates I/R injury in experimental pancreas transplantation by increasing apoptotic cell death. Experimental data suggest that protective effects derived from IPC might strongly depend on the type of organ, the time frame and the experimental setting. Further investigations are necessary to determine the relevance of IPC in pancreatic I/R injury.

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References

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74:1124.
2. Birnbaum Y, Przyklenk K, Kloner RA. Time frame of ischemic preconditioning: is it clinically relevant? J Cardiovasc Pharmacol Ther 1996; 1:339.
3. Nilsson B, Friman S, Gustafsson BI, Delbro DS. Preconditioning protects against ischemia/reperfusion injury of the liver. J Gastrointest Surg 2000; 4:44.
4. Li G, Chen S, Lu E, Hu T. Protective effects of ischemic preconditioning on lung ischemia reperfusion injury: an in-vivo rabbit study. Thorac Cardiovasc Surg 1999; 47:38.
5. Sola A, Hotter G, Prats N, Xaus C, Gelpi E, Rosello-Catafau J. Modification of oxidative stress in response to intestinal preconditioning. Transplantation 2000; 69:767.
6. Lee HT, Emala CW. Protective effects of renal ischemic preconditioning and adenosine pretreatment: role of A(1) and A(3) receptors. Am J Physiol Renal Physiol 2000; 278: F380–F387.
7. Chien CT, Chen CF, Hsu SM, Lee PH, Lai MK. Protective mechanism of preconditioning hypoxia attenuates apoptosis formation during renal ischemia/reperfusion phase. Transplant Proc 1999; 31:2012.
8. Zahir KS, Syed SA, Zink JR, Restifo RJ, Thomson JG. Ischemic preconditioning improves the survival of skin and myocutaneous flaps in a rat model. Plast Reconstr Surg 1998; 102:140.
9. Glazier SS, O'Rourke DM, Graham DI, Welsh FA. Induction of ischemic tolerance following brief focal ischemia in rat brain. J Cereb Blood Flow Metab 1994; 14:545.
10. Clavien PA, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. Ann Surg 2000; 232:155.
11. Ikonomidis JS, Weisel RD, Mickle DA. Ischemic preconditioning: cardioprotection for cardiac surgery. J Card Surg 1994; 9:526.
12. Ikonomidis JS, Tumiati LC, Weisel RD, Mickle DA, Li RK. Preconditioning human ventricular cardiomyocytes with brief periods of simulated ischemia. Cardiovasc Res 1994; 28:1285.
13. Cheng MH, Chen HC, Wei FC, Su SW, Lian SH, Brey E. Devices for ischemic preconditioning of the pedicled groin flap. J Trauma 2000; 48:552.
14. Yin DP, Sankary HN, Chong AS, et al. Protective effect of ischemic preconditioning on liver preservation-reperfusion injury in rats. Transplantation 1998; 66:152.
15. Totsuka E, Fung JJ, Urakami A, et al. Influence of donor cardiopulmonary arrest in human liver transplantation: possible role of ischemic preconditioning. Hepatology 2000; 31:577.
16. Li G, Chen S, Lou W, Lu E. Protective effects of ischemic preconditioning on donor lung in canine lung transplantation. Chest 1998; 113:1356.
17. Schumer M, Colombel MC, Sawczuk IS, et al. Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. Am J Pathol 1992; 140:831.
18. Freude B, Masters TN, Robicsek F, et al. Apoptosis is initiated by myocardial ischemia and executed during reperfusion. J Mol Cell Cardiol 2000; 32:197.
19. Kohli V, Selzner M, Madden JF, Bentley RC, Clavien PA. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation 1999; 67:1099.
20. Fischer S, Cassivi SD, Xavier AM, et al. Cell death in human lung transplantation: apoptosis induction in human lungs during ischemia and after transplantation. Ann Surg 2000; 231:424.
21. Ikeda H, Suzuki Y, Suzuki M, et al. Apoptosis is a major mode of cell death caused by ischemia and ischemia/reperfusion injury to the rat intestinal epithelium. Gut 1998; 42:530.
22. Zhang C, Rosenbaum DM, Shaikh AR, et al. Ischemic preconditioning attenuates apoptotic cell death in the rat retina. Invest Ophthalmol Vis Sci 2002; 43:3059.
23. Yadav SS, Sindram D, Perry DK, Clavien PA. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a caspase-dependent pathway. Hepatology 1999; 30:1223.
24. Boonstra JG, Wever PC, Laterveer JC, et al. Apoptosis of acinar cells in pancreas allograft rejection. Transplantation 1997; 64:1211.
25. Fujimoto K, Hosotani R, Wada M, et al. Ischemia-reperfusion injury on the pancreas in rats: identification of acinar cell apoptosis. J Surg Res 1997; 71:127.
26. Drognitz O, Liu X, Benz S, et al. Ischemia/reperfusion injury induces acinar cell apoptosis in experimental pancreas transplantation. Transplant Proc 2002; 34:2361.
27. Lee S, Tung KS, Koopmans H, Chandler JG, Orloff MJ. Pancreaticoduodenal transplantation in the rat. Transplantation 1972; 13:421.
28. Hoffmann TF, Leiderer R, Waldner H, Arbogast S, Messmer K. Ischemia reperfusion of the pancreas: a new in vivo model for acute pancreatitis in rats. Res Exp Med (Berl) 1995; 195:125.
29. Schmid-Schoenbein GW, Zweifach BW, Kovalcheck S. The application of stereological principles to morphometry of the microcirculation in different tissues. Microvasc Res 1977; 14:303.
30. Raeburn CD, Cleveland JC, Jr., Zimmerman MA, Harken AH. Organ preconditioning. Arch Surg 2001; 136:1263.
31. Cleveland JC, Jr., Raeburn C, Harken AH. Clinical applications of ischemic preconditioning: from head to toe. Surgery 2001; 129:664.
32. Bulkley GB. Preconditioning for protection from ischemic injury: discriminating cause from effect from epiphenomenon. Ann Surg 2000; 232:163.
33. Obermaier R, Benz S, Kortmann B, Benthues A, Ansorge N, Hopt UT. 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.
34. Kusterer K, Poschmann T, Friedemann A, Enghofer M, Zendler S, Usadel KH. Arterial constriction, ischemia-reperfusion, and leukocyte adherence in acute pancreatitis. Am J Physiol 1993; 265: G165-G171.
35. Knoefel WT, Kollias N, Warshaw AL, Waldner H, Nishioka NS, Rattner DW Pancreatic microcirculatory changes in experimental pancreatitis of graded severity in the rat. Surgery 1994; 116:904.
36. Plusczyk T, Rathgeb D, Westermann S, Feifel G. Somatostatin attenuates microcirculatory impairment in acute sodium taurocholate-induced pancreatitis. Dig Dis Sci 1998; 43:575.

37. Kerner T, Vollmar B, Menger MD, Waldner H, Messmer K. Determinants of pancreatic microcirculation in acute pancreatitis in rats. J Surg Res 1996; 62:165.
38. Menger MD, Vollmar B. Role of microcirculation in transplantation. Microcirculation 2000; 7:291.
39. Li G, Chen S, Lou W, Lu E. Protective effects of ischemic preconditioning on donor lung in canine lung transplantation. Chest 1998; 113:1356.
40. Biberthaler P, Luchting B, Massberg S, et al. The influence of organ temperature on hepatic ischemia-reperfusion injury: a systematic analysis. Transplantation 2001; 72:1486.
41. Heijnen BH, van Veen SQ, Straatsburg IH, van Gulik TM. Pronounced effect of minor changes in body temperature on ischemia and reperfusion injury in rat liver. J Appl Physiol 2001; 91:265.
42. Wass CT, Lanier WL, Hofer RE, Scheithauer BW, Andrews AG. Temperature changes of > or = 1 degree C alter functional neurologic outcome and histopathology in a canine model of complete cerebral ischemia. Anesthesiology 1995; 83:325.
43. Dembinski A, Warzecha Z, Ceranowicz P, et al. Ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis. Eur J Pharmacol 2003; 473:207.
44. Kirsch M, Farhat F, Garnier JP, Loisance D. Acute brain death abolishes the cardioprotective effects of ischemic preconditioning in the rabbit. Transplantation 2000; 69:2013.
45. Farhat F, Loisance D, Garnier JP, Kirsch M. Norepinephrine release after acute brain death abolishes the cardioprotective effects of ischemic preconditioning in rabbit. Eur J Cardiothorac Surg 2001; 19:313.