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• *BASIC RESEARCH* •

Ischemic preconditioning inhibits development of edematous cerulein-induced pancreatitis: Involvement of cyclooxygenases and heat shock protein 70

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Abstract

AIM: To determine whether ischemic preconditioning (IP) affects the development of edematous cerulein-induced pancreatitis and to assess the role of cyclooxygenase-1 (COX-1), COX-2, and heat shock protein 70 (HSP 70) in this process.

METHODS: In male Wistar rats, IP was performed by clamping of celiac artery (twice for 5 min at 5-min intervals). Thirty minutes after IP or sham operation, acute pancreatitis was induced by cerulein. Activity of COX-1 or COX-2 was inhibited by resveratrol or rofecoxib, respectively (10 mg/kg).

RESULTS: IP significantly reduced pancreatic damage in cerulein-induced pancreatitis as demonstrated by the improvement of pancreas histology, reduction in serum lipase and poly-C ribonuclease activity, and serum concentration of pro-inflammatory interleukin (IL)-1β. Also, IP attenuated the pancreatitis-evoked fall in pancreatic blood flow and pancreatic DNA synthesis. Serum level of anti-inflammatory IL-10 was not affected by IP. Cerulein-induced pancreatitis and IP increased the content of HSP 70 in the pancreas. Maximal increase in HSP 70 was observed when IP was combined with cerulein-induced pancreatitis. Inhibition of COXs, especially COX-2, reduced the protective effect of IP in edematous pancreatitis.

CONCLUSION: Our results indicate that IP reduces pancreatic damage in cerulein-induced pancreatitis and this effect, at least in part, depends on the activity of COXs and pancreatic production of HSP 70.

Key words: Acute pancreatitis; Ischemic preconditioning; Cyclooxygenase-2; Interleukin-1β; Heat shock protein-70

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INTRODUCTION

Various organs including the heart[1], brain[2], kidney[3], liver[4], skeletal muscle[5], and stomach[6] respond to brief exposure to ischemia with an increase in resistance to severe ischemia, and this phenomenon is called ischemic preconditioning (IP). Also, the protective effect of IP has been found in the pancreas against ischemia/reperfusion-induced pancreatitis[7]. However, no study so far has been undertaken to determine whether IP is also able to prevent the acute pancreatic damage induced by other primary non-vascular factors.

Cyclooxygenase (COX), the key enzyme for prostaglandin synthesis, exists in two isoforms as COX-1 and COX-2[8]. COX-1 is constitutively expressed in most tissues and has been suggested to mediate the synthesis of prostaglandins required for physiological functions and maintenance of organ integrity. COX-2 is undetectable in most tissues in normal condition, but is highly inducible by cytokines, mitogens, and endotoxins, and is responsible for an increased production of prostaglandins during inflammation[8]. However, it was reported that both COXs contribute to gastric mucosal defense[9]. Inhibition of COXs activity by nonselective nonsteroidal anti-inflammatory drugs leads to induction of gastric ulcers and delays the healing of gastric mucosa[10,11], while the selective inhibition of COX-2 delays gastric ulcer healing[12]. The role of COX-2 in pancreatic pathology is unclear. Studies performed by Song *et al*.[13], and Ethridge *et al*.[14], with mice have shown that pharmacological inhibition of COX-2 or

COX-2 gene disruption reduces the severity of pancreatitis and pancreatitis-associated lung injury. On the other hand, our own study has shown that inhibition of COX-2 abolishes the protective effect of hepatocyte growth factor (HGF) against cerulein-induced pancreatitis[15].

Heat shock proteins (HSPs) are cytoprotective molecules that help to maintain the metabolic and structural integrity of cells. HSPs are induced by a variety of stresses, including heat, free radicals, and toxins[16]. In the pancreas, HSPs have been shown to provide the protection against cerulein-[17,18] and arginine-induced[19] acute pancreatitis. However, it is not investigated whether IP interacts with HSPs and what is the biological consequence of this potential interaction in the development of cerulein-induced pancreatitis.

The present study was to determine the effect of IP on the development of acute cerulein-induced pancreatitis, to evaluate the role of COX-1 and COX-2 in pancreatic IP, and to assess the effect of IP on the pancreatic synthesis of HSP 70.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 180-220 g and following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University. Rats were fasted for 18 h before final experiment, but they had free access to drinking water. Animals were housed in cages with wire mesh bottoms at normal room temperature in 12-h light–dark cycle.

Experiments were carried out in the following experimental groups (10 animals in each group); (1) sham-operated control group; (2) IP group; (3) sham-operated group treated with resveratrol (Cayman Chemicals, Ann Arbor, MI, USA, 10 mg/kg, intragastrically (i.g.) 1 h before sham operation); (4) sham-operated group treated with rofecoxib (Vioxx, Merck Sharp & Dohme Idea Inc., Glattbrugg, Switzerland, 10 mg/kg, i.g. 1 h before sham operation); (5) sham-operated group with cerulein-induced pancreatitis; (6) IP group with cerulein-induced pancreatitis; (7) sham-operated group with cerulein-induced pancreatitis and treated with resveratrol (10 mg/kg, i.g. 1 h before sham operation); (8) cerulein-induced pancreatitis group treated with rofecoxib (10 mg/kg, i.g. 1 h before sham operation); (9) IP group with cerulein-induced pancreatitis and treated with resveratrol (10 mg/kg, i.g. 1 h before IP); (10) IP group with cerulein-induced pancreatitis and treated with rofecoxib (10 mg/kg, i.g. 1 h before IP).

IP of the pancreas was performed under ketamine anesthesia (50 mg/kg i.p., Bioketan, Biowet, Gorzów Wlkp., Poland). After longitudinal laparotomy, the celiac artery was clamped twice for 5 min at 5-min intervals. In sham-operated rats, longitudinal laparotomy and mobilization of the pancreas without the clamping of any artery was performed.

Thirty minutes after IP or sham operation, acute pancreatitis was induced by cerulein (Takus, Pharmacia & Upjohn GmbH, Erlangen, Germany) i.p. five times at 1-h intervals at a dose 10 μg/kg per injection. Animals without induction of acute pancreatitis were treated with 0.9% NaCl i.p. at the same time as cerulein.

Determination of pancreatic blood flow

After the last injection of cerulein or saline, animals were anesthetized with ketamine and the abdomen was opened. The pancreas was exposed for the measurement of pancreatic blood flow by a laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously[20]. Pancreatic blood flow was measured in five different portions of the pancreas. The area of laser emission of the probe was about 1 mm^2, while the depth of measurement reached about 3 mm. Data were presented as percent change from control value obtained in sham-operated rats injected with saline.

Determination of serum lipase activity and serum interleukin-1β and interleukin-10 concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of serum lipase activity and serum interleukin-1β (IL-1β) and IL-10 concentration. Serum lipase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) using Lipa DT slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA). Serum lipase activity was expressed as units per liter. Serum IL-1β and IL-10 concentrations were measured in duplicate, using appropriate BioSource Cytoscreen rat kits based on a solid phase sandwich ELISA (BioSource International, Camarillo, CA, USA). Concentration of IL was determined from standard curves for recombinant IL-1β or IL-10, respectively. Serum IL-1β or IL-10 concentration was expressed as picogram per milliliter.

Determination of serum poly-C specific ribonuclease activity

Poly-C specific ribonuclease activity was determined using Warsaw and Lee's procedure[21], employing polycytydylic acid (poly-C) as a ribonuclease substrate, as described previously in detail[22]. Poly-C specific ribonuclease activity was expressed in units per liter.

Protein extraction and analysis of pancreatic HSP 70 expression by Western blot analysis

Shock-frozen tissue from rat pancreas was homogenized in a lysis buffer (100 mmol/L Tris-HCl, pH 7.4, 15% glycerol, 2 mmol/L EDTA, 2% sodium dodecyl sulfate (SDS), 100 mmol/L D,L-dithiothreitol) by the addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mmol/L phenylmethylsulfonyl fluoride. Insoluble materials were removed by centrifugation at 12 000 *g* for 15 min. Approximately 50 μg of the total protein extract was loaded on SDS-polyacrylamide gels and run 40 mA, followed by transfer on nitrocellulose membrane (Protran, Schleicher & Schuell, Germany) by electroblotting. Bovine serum albumin (30 g/L, Sigma-Aldrich, Germany) in Tris buffered saline (TBS)-Tween-20 buffer (137 mmol NaCl, 20 mmol Tris-HCl, pH 7.4, 0.1% Tween-20) was used to block filters for at least 1 h at room temperature. Specific primary antibody against HSP 70 (mouse monoclonal, 1:200 dilution; Stressgen Biotechnologies Corp., Canada) or β-actin (mouse monoclonal, dilution 1:5 000; Sigma-Aldrich, Germany) was added to the membrane, followed by an anti-mouse-IgG or anti-mouse IgG horseradish

peroxidase-conjugated secondary antibody (dilution 1:20 000; Promega, WI, USA) dissolved in 1% non-fat milk in TBS-Tween-20 buffer. Incubation of primary antibody was followed by washing thrice with TBS-Tween-20 buffer for 10 min. Incubation of the secondary antibody was followed by five washes for 10 min. Immunocomplexes were detected by the SuperSignal West Pico chemiluminescent kit (Pierce, USA). Thereafter, the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparison between different treatment groups was made by determining the HSP 70/β-actin ratio of the immunoreactive area by densitometry.

Determination of pancreatic DNA synthesis

The rate of DNA synthesis in samples of pancreatic tissue was determined as described previously[23]. Briefly, the minced pancreatic tissue was incubated at 37 ¡æfor 45 min in 2 mL of medium containing 8 μCi/mL of [^{3}H]thymidine ([6-^{3}H] thymidine, 20-30 Ci/mmol; Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic). The incorporation of [^{3}H]thymidine into DNA was measured by counting DNA containing solution in a liquid scintillation system. DNA synthesis was expressed as [^{3}H]thymidine disintegrations per minute per microgram DNA (dpm/μg DNA).

Histological examination

Samples of pancreatic tissue for histological examination were fixed in 40 g/L formaldehyde, embedded in paraffin and sections were sliced and stained with hematoxylin and eosin. Slides were examined by two experienced pathologists without the knowledge of the treatment given (four slides per animal). The histological grading of edema was made using our scale ranging from 0 to 3: 0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = severe interlobular and intralobular edema. Hemorrhage was graded: 0 = absent, 1 = 1-2 foci per slide, 2 = 3 to 5 foci per slide, 3 = more than 5 foci per slide. Leukocyte infiltration was graded: 0 = absent, 1 = scare perivascular infiltration, 2 = moderate perivascular and scare diffuse infiltration, 3 = abundant diffuse infiltration. Acinar necrosis was graded: 0 = absent, 1 = less than 15% of cells involved, 2 = 15–35% of cells involved, 3 = more than 35% of cells involved. Grading of vacuolization was based on the percentage of cells involved: 0 = absent, 1 = less than 25%, 2 = 25-50% and 3 = more than 50%.

Statistical analysis

Results were expressed as mean±SE. Statistical analysis was carried out by one-way analysis of variance followed by Tukey's multiple comparison test using GraphPadPrism (GraphPad Software, San Diego, CA, USA). $P<0.05$ was considered statistically significant.

RESULTS

Morphological features of pancreatic tissue

The pancreas of saline-treated sham-operated rats showed no tissue alteration macroscopically and at light microscopic level (Table 1). Exposure to IP combined with treatment with saline caused the mild interlobular edema and minimal vacuolization of acinar cells in cases 4 and 5, respectively. Rest of the animals exposed to IP and treated with saline did not show any histological alterations. Administration of the COX-1 inhibitor - resveratrol - in saline-treated animals did not affect pancreatic tissue morphology. Treatment with combination of the COX-2 inhibitor - rofecoxib - plus saline had no effect on pancreatic histology or caused mild interlobular edema. Administration of cerulein caused acute edematous pancreatitis in all rats that were tested (Table 1). The pancreas was grossly swollen and enlarged with a visible collection of edematous fluid. At light microscopic level, moderate or severe interlobular and intralobular edema was accompanied with moderate perivascular and scare diffuse inflammatory leukocyte infiltration. Vacuolization was observed in 25-50% of acinar cells. Additionally, in few cases, one or two foci of hemorrhages per slide were found. IP applied prior to cerulein-induced pancreatitis reduced histological signs of pancreatic damage such as pancreatic edema, inflammatory infiltration, and vacuolization of acinar cells. Also, administration of resveratrol or rofecoxib in combination with cerulein slightly reduced the cerulein-evoked pancreatic damage. In animals with IP applied prior to cerulein, inhibition of COXs abolished the beneficial effect of IP. This effect was especially pronounced after administration of rofecoxib and pancreatic damage was similar to that in animals treated with cerulein alone (Table 1).

Effect of ischemic preconditioning, resveratrol, and rofecoxib on serum lipase activity in rats without or with cerulein-induced pancreatitis

In sham-operated saline-treated rats, serum lipase activity reached 61.5±3.5 U/L (Figure 1A). IP, applied without

Table 1 Morphological features of pancreatic tissue of animals exposed to IP or sham-operated and treated with saline, resveratrol, rofecoxib or cerulein alone or in combination

	Edema	Inflammatory infiltration	Vacuolization	Hemorrhages	Necrosis
Sham-operation+saline (control)	0	0	0	0	0
IP+saline	0/1	0	0/1	0	0
Resveratrol+sham operation+saline	0	0	0	0	0
Rofecoxib+sham operation+saline	0/1	0	0	0	0
Sham-operation+cerulein	2/3	2	2	0/1	0
IP+cerulein	2	1	1	0	0
Resveratrol+sham operation+cerulein	2	1	2	0	0
Rofecoxib+sham operation+cerulein	2	1	2	0/1	0
Resveratrol+IP+cerulein	2	1/2	2	0	0
Rofecoxib+IP+cerulein	2/3	2	2	0/1	0

Numbers represent the predominant histological grading in each group.

induction of acute pancreatitis, did not affect serum lipase activity. Also, administration of the COX-1 inhibitor - resveratrol - or the COX-2 inhibitor - rofecoxib - had no effect on serum lipase activity in rats treated with saline. Administration of cerulein caused more than sevenfold increase in serum lipase activity, when compared to that of saline-treated control rats. IP markedly reduced the cerulein-evoked increase in serum lipase activity by 37% ($P<0.001$). Also, administration of resveratrol or rofecoxib reduced serum lipase activity in animals with cerulein-induced pancreatitis ($P<0.01$ or $P<0.05$, respectively). The effect of these blockers of COX on serum lipase activity was weaker than the effect of IP, but was still statistically significant. In contrast, administration of resveratrol or rofecoxib in combination with IP significantly abolished the IP-evoked reduction in serum lipase activity in animals with cerulein-induced pancreatitis ($P<0.001$). However, serum lipase activity in these animals was still lower than that in animals with cerulein-induced pancreatitis and treated with resveratrol or rofecoxib alone (Figure 1A).

Effect of ischemic preconditioning, resveratrol, and rofecoxib on serum concentration of IL-1β and IL-10 in rats without or with cerulein-induced pancreatitis

In control sham-operated rats treated with saline, the serum IL-1β concentration was 69.9±3.1 pg/mL (Figure 1B). IP or treatment with resveratrol or rofecoxib alone had no effect on serum IL-1β in rats without induction of acute pancreatitis. In sham-operated rats, cerulein caused more than threefold increase in serum IL-1β concentration and this increase was significantly diminished by IP ($P<0.001$). Lower but still significant reduction of serum IL-1β was also observed when administration of cerulein was combined with pretreatment with resveratrol ($P<0.05$). Administration of rofecoxib alone tended to reduce the cerulein-evoked increase in serum IL-1β concentration, but this effect was not statistically significant. Pretreatment with resveratrol or rofecoxib in animals with IP significantly reversed the

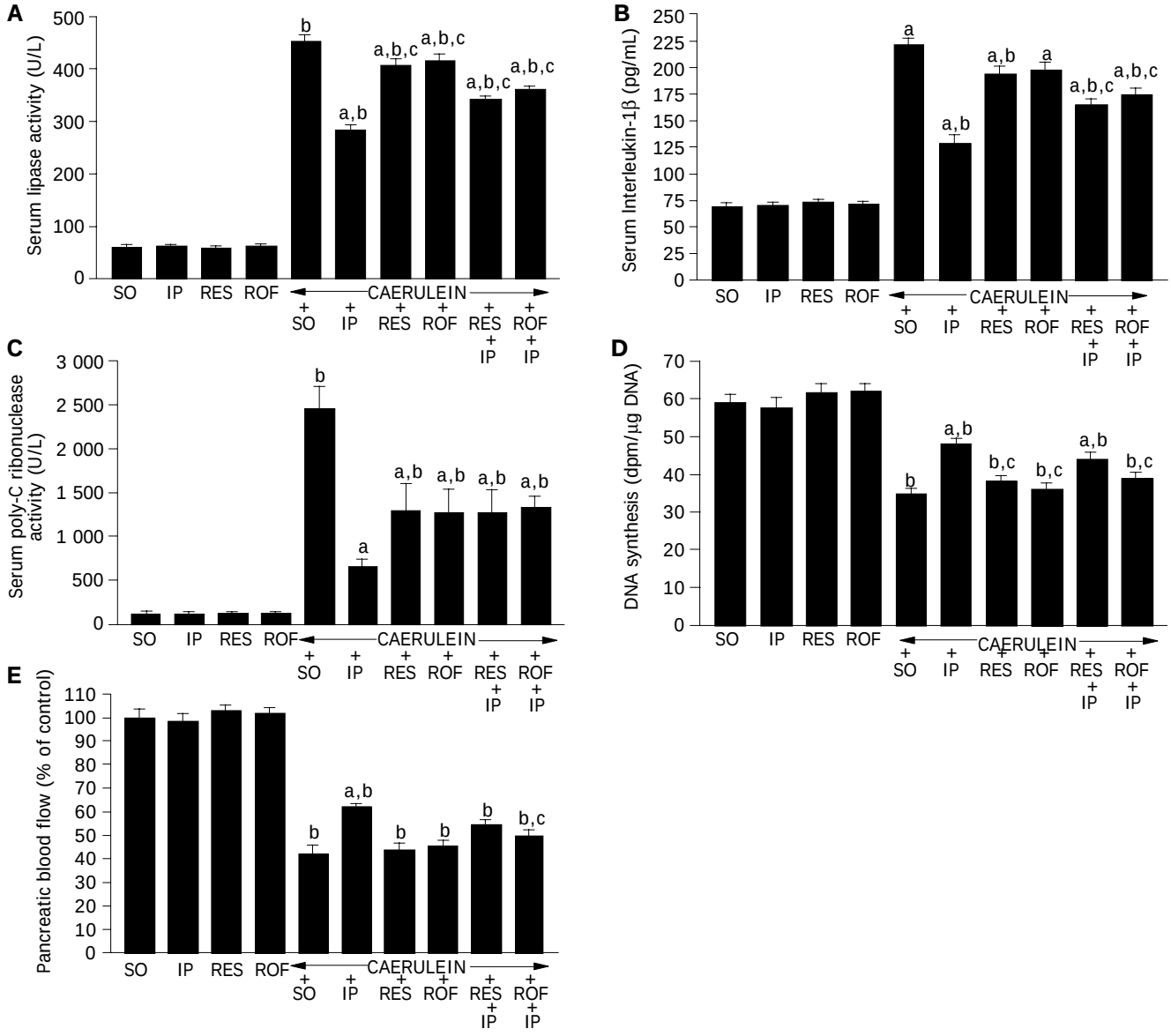

Figure 1 Effect of sham operation (SO), IP, resveratrol (RES) or rofecoxib (ROF) applied alone or in combination on serum lipase activity (A), IL-1β (B), poly-C ribonuclease (C), pancreatic DNA synthesis (D), and pancreatic blood flow (E) in rats with or without cerulein-induced pancreatitis. [a]$P<0.05$ *vs* sham-operated rats treated with cerulein alone, [b]$P<0.001$ *vs* sham-operated saline-treated control (SO), [c]$P<0.05$ *vs* rats exposed to IP and treated with cerulein.

IP-evoked decrease in serum IL-1β concentration in animals with cerulein-induced pancreatitis (P<0.001, Figure 1B).

In control sham-operated rats treated with saline, the serum IL-10 level reached 73.0±5.0 pg/mL (data not shown). Neither IP nor resveratrol or rofecoxib or cerulein applied alone or in their combination significantly affected the serum IL-10 concentration.

Effect of ischemic preconditioning, resveratrol, and rofecoxib on serum activity of poly-C ribonuclease in rats without or with cerulein-induced pancreatitis

In saline-treated sham-operated control rats, serum poly-C ribonuclease activity reached 125±25 U/L (Figure 1C). IP or administration of resveratrol or rofecoxib did not affect serum poly-C ribonuclease activity in animals without induction of acute pancreatitis. Cerulein-induced pancreatitis caused nearly 20-fold increase in serum poly-C ribonuclease activity and this effect was strongly and significantly inhibited by IP (P<0.001). Also administration of resveratrol or rofecoxib reduced the cerulein-induced increase in serum activity of poly-C ribonuclease, but the effect of these blockers of COX was weaker than that of IP (P<0.001). Combination of resveratrol or rofecoxib plus IP decreased the serum poly-C ribonuclease activity in cerulein-treated rats to the similar values as administration of resveratrol or rofecoxib in sham-operated rats with cerulein-induced pancreatitis (Figure 1C).

Effect of ischemic preconditioning, resveratrol, and rofecoxib on pancreatic DNA synthesis in rats without or with cerulein-induced pancreatitis

In saline-treated sham-operated control rats, pancreatic DNA synthesis reached 59.0±2.3 dpm/μg DNA (Figure 1D). IP as well as administration of resveratrol or rofecoxib did not affect pancreatic DNA synthesis in animals without induction of acute pancreatitis. In animals with cerulein-induced pancreatitis, pancreatic DNA synthesis was significantly reduced, reaching 34.7±1.6 dpm/μg DNA (P<0.001). IP significantly attenuated the pancreatitis-related reduction in pancreatic DNA synthesis (P<0.001). Resveratrol or rofecoxib given alone did not affect pancreatic DNA synthesis in animals treated with cerulein. Pretreatment with resveratrol tended to reduce the pancreatic DNA synthesis in animals treated with a combination of IP plus cerulein, but this effect was not statistically significant. In contrast, pretreatment with rofecoxib significantly reduced the pancreatic DNA synthesis in animals treated with IP plus cerulein (P<0.05).

Effect of ischemic preconditioning, resveratrol, and rofecoxib on pancreatic blood flow in rats without or with cerulein-induced pancreatitis

IP or treatment with resveratrol or rofecoxib did not affect pancreatic blood flow in rats without administration of cerulein (Figure 1E). Administration of cerulein for 5 h significantly reduced pancreatic blood flow by 58% when compared to that of saline-treated sham-operated control rats (P<0.001). Exposure to IP significantly reversed the cerulein-induced fall in pancreatic blood flow (P<0.001), whereas resveratrol or rofecoxib given alone had no effect on pancreatic blood flow in animals with cerulein-induced pancreatitis. Pretreatment with rofecoxib significantly abolished the IP-induced improvement of pancreatic blood flow in animals with pancreatitis (P<0.05).

Effect of ischemic preconditioning and cerulein-induced pancreatitis on synthesis of HSP 70 detected by Western blot

Figure 2 shows the effect of IP and cerulein-induced pancreatitis on the production of HSP 70 in the pancreatic tissue. In sham-operated control animals with an intact pancreas, the synthesis of HSP 70 was weak and the ratio of HSP 70 over β-actin reached a value of 0.11±0.01. IP or induction of acute pancreatitis by cerulein significantly increased the ratio of HSP 70 over β-actin in pancreatic tissue reaching 0.31±0.01 and 0.74±0.02, respectively (P<0.05 and P<0.001, respectively). The highest ratio of HSP 70 to β-actin in pancreatic tissue was observed in animals treated with combination of IP plus cerulein.

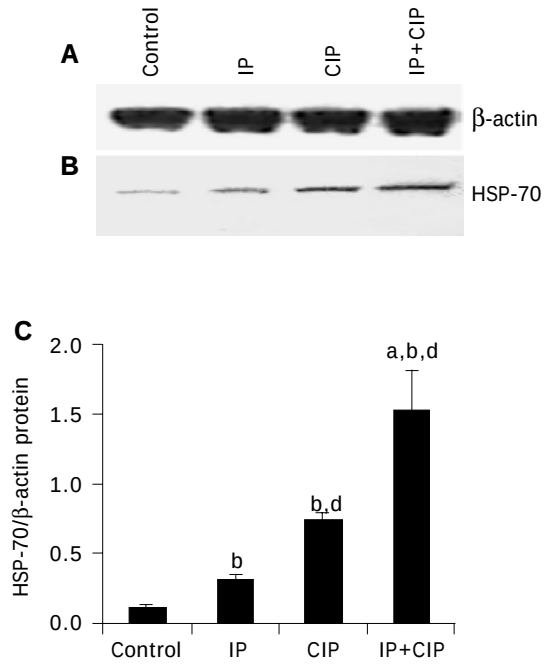

Figure 2 Representative Western blot analysis of β-actin protein (A) and HSP 70 (B), and the ratio of HSP 70 over β-actin protein (C) in pancreatic tissue of sham-operated control rats (lane 1), rats exposed to IP (lane 2), sham-operated rats with cerulein-induced pancreatitis (CIP, lane 3), and rats with IP applied prior to cerulein-induced pancreatitis (IP+CIP, lane 4). [b]P<0.001 *vs* sham-operated rats treated with cerulein alone (CIP), [a]P<0.05 *vs* sham-operated saline-treated control, [d]P<0.001 *vs* rats exposed to IP without induction of acute pancreatitis (CIP).

DISCUSSION

Our present study showed for the first time that: (1) IP could reduce the pancreatic damage in cerulein-induced acute pancreatitis; (2) IP and cerulein-induced pancreatitis could increase synthesis of HSP 70 in the pancreas and combination of these two factors could stimulate the production of HSP 70 to the highest extent; (3) inhibition of COXs, especially COX-2, could reduce the pancreatoprotective effect of IP against cerulein-induced pancreatitis.

Protective effect of IP against pancreatic damage evoked by cerulein was manifested by improvement of the biochemical and histological parameters. Exposure to IP prior to induction of acute pancreatitis significantly reduced the cerulein-evoked increase in serum lipase and poly-C ribonuclease activity, and serum concentration of pro-inflammatory IL-1β. A close relationship was found between the IP-evoked decrease in biochemical signs of pancreatitis and the improvement of pancreatic DNA synthesis, and pancreatic blood flow, as well as the reduction in histological score of pancreatic damage. Morphological features showed a decrease in pancreatic edema, vacuolization of acinar cells, and leukocyte infiltration of pancreatic tissue.

The increase in serum lipase activity is a well known manifestation of acute pancreatitis with high sensitivity and specificity[24]. Pancreatic-type poly-C specific ribonuclease is one of the few direct markers of severe pancreatic injury and pancreatic necrosis[21,25]. In our present study, acute pancreatitis was evoked by stimulation of pancreatitis by overdose of cerulein. This procedure leads to the development of mild edematous acute pancreatitis without pancreatic necrosis[26,27]. However, also in this case, we observed increase in serum activity of poly-C specific ribonuclease, suggesting that serum activity of pancreatic-type poly-C ribonuclease is not specific for severe pancreatic injury and pancreatic necrosis, and that serum activity of this enzyme increases in all types of acute pancreatitis, whereas severity of acute pancreatitis affects only the degree of poly-C ribonuclease activity in the serum. This discrepancy between our data and reports of Warshaw and Lee[21] or Naskalski *et al.*[25], may also be a result of species differences. On the other hand, our finding that IP applied before induction of acute pancreatitis reduced serum poly-C ribonuclease activity provides an additional support for the protective effect of IP on the pancreas in cerulein-induced pancreatitis.

In our present study, IP applied alone did not significantly affect pancreatic DNA synthesis. Induction of acute pancreatitis by cerulein caused a reduction in the pancreatic DNA synthesis, which is in agreement with previous observations[28,29] and may be considered as an index of pancreatic damage. In rats with induction of pancreatitis, IP attenuated the pancreatitis-evoked fall in pancreatic DNA synthesis. This observation brings the additional support for the concept that IP enhances resistance of the pancreas against tissue damage.

Our present observation that IP reduced pancreatic damage in cerulein-induced pancreatitis confirms and extends our previous finding that exposure to IP could protect the pancreas against damage evoked by severe ischemia followed by reperfusion[7]. These data suggest that IP, causing a mild damage, enhances resistance against pancreatic damage independently to the etiology of acute pancreatitis. This hypothesis is also supported by the observation that exposure to other mild damaging factors such as bacterial lipopolysaccharides[30], grapefruit-seed extract[22], or low doses of capsaicin[31] exhibits protective effect against acute pancreatitis.

The role of COX-2 in acute pancreatitis is unclear. Previous experimental studies on mice[13,14] have shown that pharmacological inhibition of COX-2 or COX-2 gene disruption ameliorates the severity of pancreatitis and the pancreatitis-associated lung injury. In contrast, in the rat model of pancreatitis, Foitzik *et al.*[32], have found some beneficial systemic effects of COX-2 inhibition on acute pancreatitis, such as an improvement of renal and respiratory function, but they have not observed any significant effect of COX-2 inhibition on histological score of pancreatic damage or plasma level of trypsinogen activation peptides. In our present study, blockade of COX-1 or COX-2 significantly reduced serum lipase and serum poly-C ribonuclease activity, as well as decreased pancreatic edema and inflammatory infiltration in morphological features in animals with cerulein-induced pancreatitis. The new and important finding of the present study is that inhibition of COXs, especially COX-2, reduces the protective effect of IP against pancreatic damage evoked by cerulein. This observation indicates that activity of COX-2 is necessary for the beneficial effect of IP. The involvement of COX-2 in IP effects on the pancreas is similar to that observed after treatment with HGF. Serum concentration of HGF is increased in patients with acute pancreatitis, and the HGF level reflects the clinical severity of pancreatitis and organ dysfunction[33,34]. Also experimental studies have shown the increase in plasma HGF level and tissue HGF overexpression in acute pancreatitis[35,36]. On the other hand, administration of antibodies that neutralize HGF aggravates the organ dysfunction and increases apoptosis in the course of acute pancreatitis[35], whereas treatment with HGF reduces pancreatic damage in cerulein-induced pancreatitis[37]. These data indicate that HGF is not a pro-inflammatory factor, but a result of pancreatic damage in acute pancreatitis and the elevation of HGF level plays a role of self-defense mechanism, limiting the intensity of inflammatory process. It was reported that inhibition of COX-2 abolishes the protective effect of HGF against acute pancreatitis[15]. Our present study showed that inhibition of COXs, especially COX-2, reduced the protective effect of IP against damage evoked by cerulein. These findings taken together suggest that pancreatoprotective effects evoked by IP and HGF involve similar but not strictly the same mechanisms. In contrast to the administration of HGF[37], exposure to IP did not increase the serum level of anti-inflammatory IL-10.

Another finding of our present study is the observation that cerulein-induced pancreatitis and IP increased pancreatic synthesis of HSP 70 and these two factors applied together caused maximal induction of HSP 70 synthesis in the pancreas. HSPs are highly conserved cytoprotective proteins that are induced by a variety of stresses including hyper- and hypothermia, toxin, heavy metals, and free radicals[16]. Previous studies demonstrated that both thermal and non-thermal stresses protect against cerulein-induced pancreatitis and prevent trypsinogen activation in the pancreas and this effect is mediated by HSP 70[38]. HSPs have also been shown to provide the protection against arginine-[19] and taurocholate-induced[39] pancreatitis. The potential protective mechanisms of HSPs on pancreas include stabilization and refolding of damaged proteins, resistance of cells to apoptosis or necrosis, decrease in the level of pro-inflammatory cytokines, antioxidant effects and interference with intra-acinar zymogen activation[16,38,40]. Our present results are consistent with the

previous studies showing an increase of HSP 70 at the mRNA and protein level after induction of cerulein-induced pancreatitis[41]. The major finding is, however, the observation of the further increase in HSP 70 synthesis in rats with IP, which indicates that the protective effects of IP could be mediated via this HSP.

In conclusion, exposure to IP reduces pancreatic damage in cerulein-induced acute pancreatitis and this protective effect of IP, at least in part, depends on COXs activity and production of HSP 70.

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