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Z. WARZECHA[1], A. DEMBIŃSKI[1], P. CERANOWICZ[1], S.J. KONTUREK[1],
R. TOMASZEWSKA[2], J. STACHURA[2], P.C. KONTUREK[3]

IGF-1 STIMULATES PRODUCTION OF INTERLEUKIN-10 AND INHIBITS DEVELOPMENT OF CAERULEIN-INDUCED PANCREATITIS.

[1]Department of Physiology and [2]Department of Pathology, Jagiellonian University Medical School, Kraków, Poland
[3]Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

Background/Aim: Insulin-like growth factor-1 (IGF-1) and other growth factors overexpression was reported in acute pancreatitis. Previous studies have shown the protective effect of epidermal growth factor (EGF), Hepatocyte Growth Factor (HGF) and Fibroblast Growth Factor (FGF) in the course of experimental acute pancreatitis. The aim of our studies was to determine the effect of IGF-1 administration on the development of caerulein-induced pancreatitis. Methods: Acute pancreatitis was induced by infusion of caerulein (10 μg/kg/h) for 5 h. IGF-1 was administrated twice at the doses: 2, 10, 50, or 100 μg/kg s.c. Results: Administration of IGF-1 without induction of pancreatitis increased plasma interleukin-10 (IL-10). Infusion of caerulein led to development of acute edematous pancreatitis. Histological examination showed pancreatic edema, leukocyte infiltration and vacuolization of acinar cells. Also, acute pancreatitis led to an increase in plasma lipase and interleukin 1β (IL-1β) level, whereas pancreatic DNA synthesis and pancreatic blood flow were decreased. Treatment with IGF-1, during induction of pancreatitis, increased plasma IL-10 and attenuated the pancreatic damage, what was manifested by histological improvement of pancreatic integrity, the partial reversion of the drop in pancreatic DNA synthesis and pancreatic blood flow, and the reduction in pancreatitis-evoked increase in plasma amylase, lipase and IL-1β level. Protective effect of IGF-1 administration was dose-dependent. Similar strong protective effect was observed after IGF-1 at the dose 2 x 50 and 2 x 100 μg/kg. Conclusions: (1) Administration of IGF-1 attenuates pancreatic damage in caerulein-induced pancreatitis; (2) This effect is related, at least in part, to the increase in IL-10 production, the reduction in liberation of IL-1β and the improvement of pancreatic blood flow.

Key Words: *pancreatitis; IGF-1 (insulin-like growth factor-1); interleukin-10, interleukin-1β; pancreatic blood flow*

INTRODUCTION

Insulin-like growth factor-1 (IGF-1), also called somatomedin C is a single chain polypeptide of 70 amino acid residues cross-linked by three disulfide bridges (1). During fetal development, IGF-1 plays an important role in tissue development (2, 3). It is expressed primary by mesenchymal-derived cells and probably regulates proliferation of neighboring cells (2, 3). After delivery, the major source of circulating (endocrine) IGF-1 is the liver, but also most of extrahepatic tissues synthesize IGF-1, where it acts as paracrine regulator (4, 5). IGF-1 affects the growth and differentiation of the variety of tissues. It is mitogenic for wide range of cells including fibroblasts, osteoblasts, smooth muscle cells, fetal brain cells, neuroglial cells and erythroid progenitor cells (6).

There is a growing evidence that IGF-1 plays an important role in tissue regeneration. After injury, IGF-1 mRNA is overexpressed in a variety tissues, including bone (7), muscle (7, 8), kidney (9, 10) and liver (10). Administration of exogenous IGF-1 has been shown to promote end-to-side nerve repair (11), healing of gastric ulcer (12) and wound healing of skin (13) and muscle (14). Other effect of IGF-1 is a reduction of insulin resistance in diabetics (15, 16) and a reversion of catabolism in severe illness and injury (17).

In the pancreas, IGF-1 is described as an important regulator of pancreatic development (18). In cell culture, IGF-1 was shown to induce proliferation of pancreatic acinar cells (19) and β-cells (20). IGF-1 mRNA is overexpressed in the pancreas after subtotal pancreatectomy (18, 21) and after acute pancreatitis (18, 22) suggesting involvement of endogenous IGF-1 in pancreatic regeneration.

IGF-1 belongs to the family of growth factors signaling via receptor-tyrosine-kinase. Other members of this family, such as epidermal growth factor (EGF) (23, 24), basic fibroblast growth factor (bFGF) (25) and hepatocyte growth factor (HGF) (26) were shown to attenuate pancreatic damage in the course of acute experimental pancreatitis and accelerate pancreatic recovery. These data suggest that treatment with IGF-1 may exhibit beneficial effect on the pancreas in the course of acute pancreatitis. The aim of the present study was to determine the effect of IGF-1 administration on the development of caerulein-induced pancreatitis.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 160-180 g and were conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University. Animals were housed in cages with wire mesh bottoms, with normal room temperature and a 12-h light-dark cycle.

Acute pancreatitis was induced by caerulein infusion in rats kept in individual cages. Caerulein (Takus, Pharmacia & Upjohn GmbH, Erlangen, Germany) was diluted in saline and infused s.c. for 5 h at the dose 10 μg/kg/h and at a volume 1.0 ml/h. Studies were carried out on the following experimental groups (ten animals in each group); (1) animals infused with 0.9% NaCl s.c. for 5 h (control); (2) animals infused with 0.9% NaCl s.c. for 5 h and treated twice with interleukin-10 (IL-10) (30 min before 0.9% NaCl infusion, and 3 h later) at the dose 8 μg/kg/injection s.c.; (3-6) animals infused with 0.9% NaCl s.c. for 5 h and treated twice s.c. with IGF-1 (30 min before 0.9% NaCl infusion, and 3 h later) at the doses: 2, 10, 50 or 100 μg/kg, respectively; (7) animals with caerulein induced pancreatitis; (8) animals with caerulein induced pancreatitis and treated twice with IL-10 (30 min before caerulein infusion, and 3 h later) at the dose 8 μg/kg/injection s.c.; (9-12) animals with caerulein induced pancreatitis and treated twice s.c. with IGF-1 (30 min before caerulein infusion, and 3 h later) at the doses: 2, 10, 50 or 100 μg/kg, respectively.

Groups treated with IL-10 were added to compare the protective effect of IGF-1 and IL-10 administration. We used IL-10 at the dose 8 μg/kg because this dose has been shown to exhibit protective effect against development of acute edematous pancreatitis in the previous study (27). Human recombinant IL-10 and human recombinant IGF-1 were purchased from Sigma (Saint Louis, Missouri, USA).

Determination of pancreatic blood flow

Following the infusion of saline or caerulein, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened. Pancreata were exposed for the measurement of the blood flow by laser Doppler flowmeter, using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (28). The pancreatic blood flow was presented as percent change from control value obtained in rats infused with saline.

Determination of plasma lipase activity, and plasma IL-1β and IL-10 concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of plasma lipase activity, and plasma IL-1β and IL-10. Plasma 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). The values of plasma lipase activity were expressed as units/liter (U/L). Plasma IL-1β, and IL-10 were measured in duplicate, using the BioSource Cytoscreen rat IL-1β and IL-10 kit based on a solid phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, California, USA). Concentrations ware expressed as pg/ml.

Determination of pancreatic DNA synthesis

After the blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, the duodenum, and the spleen. Fat and excess tissue were trimmed away. Samples of pancreatic tissue were taken for study of DNA synthesis and morphological examination. The rate of DNA synthesis in the portion of minced pancreatic tissue was determined by incubating the tissue at 37°C for 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 reaction was stopped with 0.4 N perchloric acid containing carrier thymidine (5 mM). Tissue samples were centrifuged and the precipitate washed twice in cold 0.2 N perchloric acid and recentrifuged. RNA was hydrolyzed in 0.3 M KOH incubated for 90 min at 37°C. DNA

and protein were reprecipitated with 10% perchloric acid. After standing for 10 min on ice, the tubes were centrifuged and the supernatant was discarded. DNA in the residual pellets was solubilized in 10 % perchloric acid by heating at 70°C for 20 min. Denaturated protein was removed by centrifugation for 20 min. Using calf thymus as a standard, the DNA concentration was determined by Giles and Myers procedure (29). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant 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 excised from the body portion for morphological examination were fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxilin and eosin. Two experienced pathologists without the knowledge of the treatment given examined the slides histologically. The histological grading of edema was made using a scale raging from 0 to 3 (0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema). Leukocytic infiltration was also graded from 0 to 3 (0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration, 3 = abundant diffuse infiltration) Grading of vacuolization was based on the appropriate percentage of acinar cells involved: 0 = absent, 1 = less than 25%, 2 = 25-50% and 3 = more than 50% of acinar cells.

Statistical analysis

Comparison of the differences between the mean values of various groups of experiments was made by analysis of variance and the Student's T test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (± S.E.M.).

RESULTS

Histological findings

Pancreata of saline-infused animals showed macroscopically and at light microscopic level no tissue alteration (*Table 1*). Also treatment with IL-10 or any dose of IGF-1 in saline-infused animals did not affect morphology of pancreatic tissue (*Table 1*). Infusion with caerulein caused acute edematous pancreatitis in all rat tested (*Table 1*). Pancreas was grossly swollen and enlarged with a visible collection of edematous fluid. At light microscopic level (*Fig. 1*), prominent interlobular and severe or moderate intralobular edema (grade 3 and 2) was accompanied with moderate perivascular and scarce diffuse inflammatory leukocyte infiltration. Vacuolization was observed in 25-50% of acinar cells.

Treatment with IL-10, before and during infusion of caerulein infusion, strongly reduced pancreatic damage (*Table 1*). Pancreatic edema was limited to interlobular space, inflammatory leukocyte infiltration was scare and perivascular. Vacuolization was seen in less than 25% of acinar cells.

Treatment with IGF-1, during induction of pancreatitis, attenuated the pancreatic tissue damage and this protective effect was dose-dependent (*Table 1*).

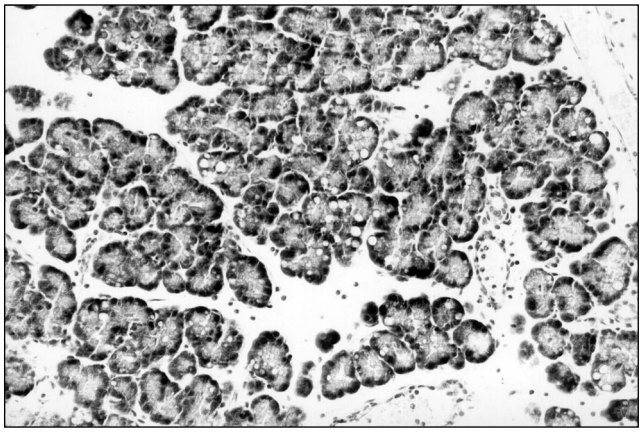

Fig.1. Histological section of pancreas from rats with caerulein-induced pancreatitis. Hematoxylin-eosine stain, magnification × 165.

Administration of IGF-1 at the lowest dose: 2 μg/kg slightly reduced a caerulein-induced leukocyte infiltration and pancreatic edema but was without marked effect on vacuolization of acinar cells. IGF-1 at the dose: 10 μg/kg caused an additional decrease in pancreatic edema and reduced acinar cells vacuolization. Treatment with IGF-1, at the doses 50 and 100 μg/kg, before and during caerulein infusion caused similar and strong beneficial effect on pancreatic histology (*Fig. 2*). In this group of animals, pancreatic edema was limited to interlobular space, inflammatory leukocyte infiltration was scare and perivascular. Vacuolization involved less than 25% of acinar cells.

Pancreatic blood flow

Treatment with IL-10 or any doses of IGF-1 was without any effect on pancreatic blood flow in animals infused with saline (*Fig. 3*). Infusion of caerulein for 5 h reduced pancreatic blood flow by 56% when compared to saline-infused control group. Treatment with IL-10 caused a strong reversion of

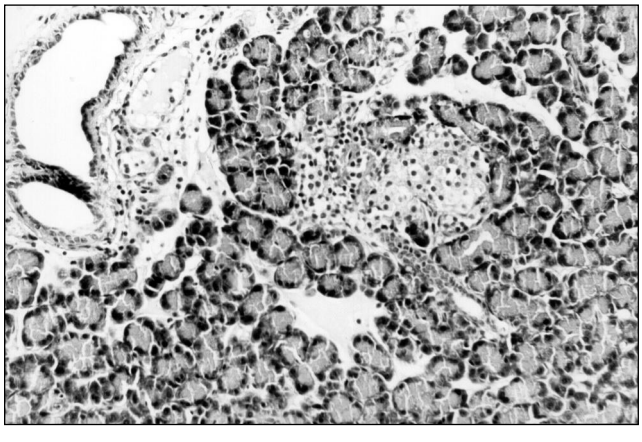

Fig. 2. Typical histological appearance of pancreas obtained from rats with caerulein-induced pancreatitis and pretreated with IGF-1 at the dose 50 μg/kg. Hematoxylin-eosine stain, magnification × 165.

Table 1. Morphological features of pancreatic tissue in rats treated with saline (control), IL-10 or IGF-1 (2 x 2, 10, 50 or 100 μg/kg) given in combination with saline or caerulein infusion.

	EDEMA (0-3)	INFILTRATION (0-3)	VACUOLIZATION (0-3)
saline (control)	0	0	0
IL-10	0	0	0
IGF 2μg/kg + saline	0	0	0
IGF 10μg/kg + saline	0	0	0
IGF 50μg/kg + saline	0	0	0
IGF 100μg/kg + saline	0	0	0
caerulein	2/3	2	2
IL-10 + caerulein	1	1	1
IGF 2μg/kg + caerulein	2	1/2	2
IGF 10μg/kg + caerulein	1/2	1/2	1/2
IGF 50μg/kg + caerulein	1	1	1
IGF 100μg/kg + caerulein	1	1	1

Numbers represent the predominant histological grading in each group.

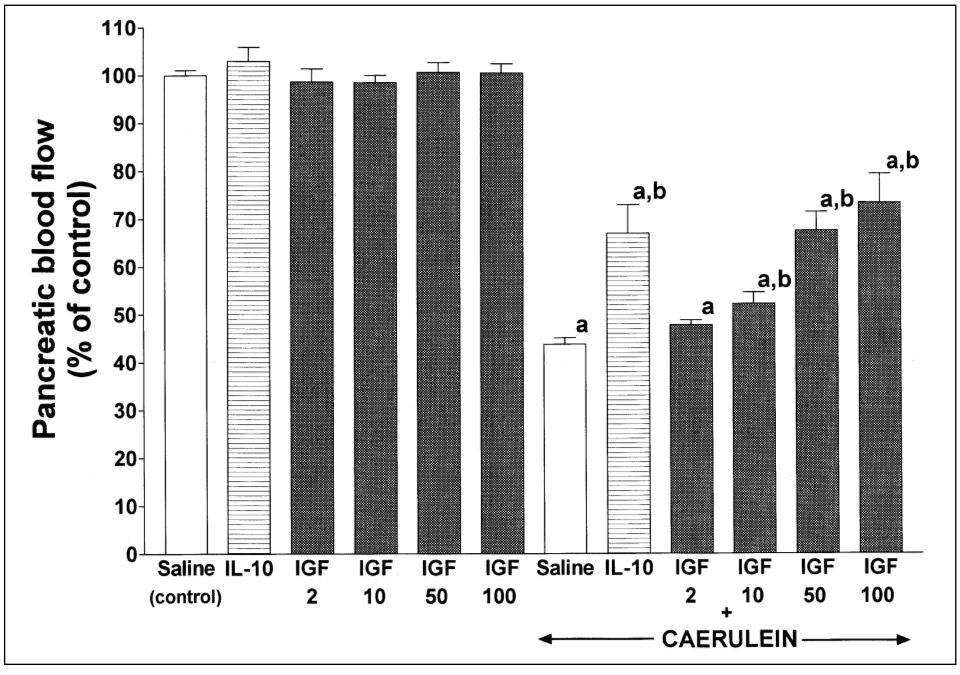

Fig. 3. Pancreatic blood flow in rats with or without caerulein-induced pancreatitis treated with saline (control), IL-10 or IGF (given twice at the dose 2, 10, 50 or 100 μg/kg). Mean ± S.E.M. N=10 in each group of animals. [a]P<0.05 compared with control, [b]P<0.05 compared with caerulein given alone.

caerulein-induced reduction in pancreatic blood flow. Administration of IGF-1 at the dose 2 μg/kg did not significantly affect the pancreatic blood flow in rats infused with caerulein. Treatment with IGF-1 at the higher doses led to a marked and dose dependent reversion of caerulein-induced fall of pancreatic blood flow. IGF-1 at the dose 50 and 100 μg/kg caused a similar and strong effect on pancreatic blood flow in animals with caerulein infusion.

Biochemical parameters

In saline-infused control rats, pancreatic DNA synthesis reached 58.9 ± 2.1 dpm/μg DNA (*Fig. 4*). Treatment with IGF-1 at any dose did not affect significantly pancreatic DNA synthesis in animals infused with saline. Also administration of IL-10 was without effect on pancreatic DNA synthesis in animals infused with saline. In animals with caerulein-induced pancreatitis, pancreatic DNA synthesis was reduced by 44%. In this group of animals, treatment with IL-10 reversed a caerulein-induced fall in pancreatic DNA synthesis. Administration of IGF-1 at the doses 10, 50 and 100 μg/kg significantly attenuated the reduction in DNA synthesis in rats with caerulein

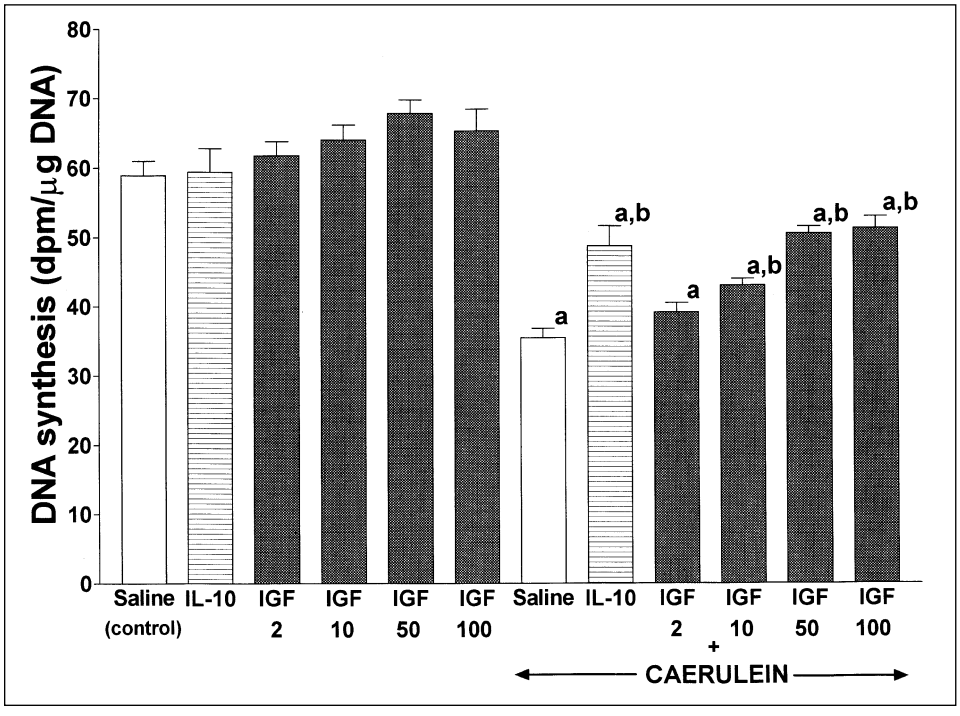

Fig. 4. Effect of IL-10 (8 μg/kg) or IGF-1 administration (given twice at the dose 2, 10, 50 or 100 μg/kg) on pancreatic DNA synthesis in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. [a]P<0.05 compared with control, [b]P<0.05 compared with caerulein given alone.

infusion. IGF-1 given at the dose 2 μg/kg was without significant effect on caerulein-evoked fall in pancreatic DNA synthesis.

Plasma lipase activity (*Fig. 5*) in control saline infused rats reached 58.7 ± 10.2 U/L. Administration of IL-10 or IGF-1 did not significantly affect plasma lipase activity in rats infused with saline. Infusion with caerulein for 5 h, causing acute pancreatitis, increased plasma lipase activity to 453.5 ± 17.1 U/L. Administration of IL-10 strongly reduced a plasma lipase activity by 42% in animals infused with caerulein. Treatment with IGF-1, at the dose 2, 10, 50 and 100 μg/kg, markedly reduced caerulein-evoked increase in plasma lipase activity by 17, 27, 41 and 46%, respectively.

In control rats infused with saline, plasma IL-1β concentration was 76.8 ± 4.5 pg/mL (*Fig. 6*). Treatment with IL-10 or IGF-1 was without a significant effect on plasma IL-1β level in saline-infused rats, except for the highest dose of IGF-1 (100 μg/kg). Caerulein caused an increase in plasma IL-1β concentration to value 252.6 ± 7.0 pg/mL and this increase was diminished by treatment with IL-10 or IGF-1. IGF-1 administrated at the dose 10, 50 or 100 μg/kg led to a significant and dose-dependent reduction in caerulein-evoked increase in plasma IL-1β concentration.

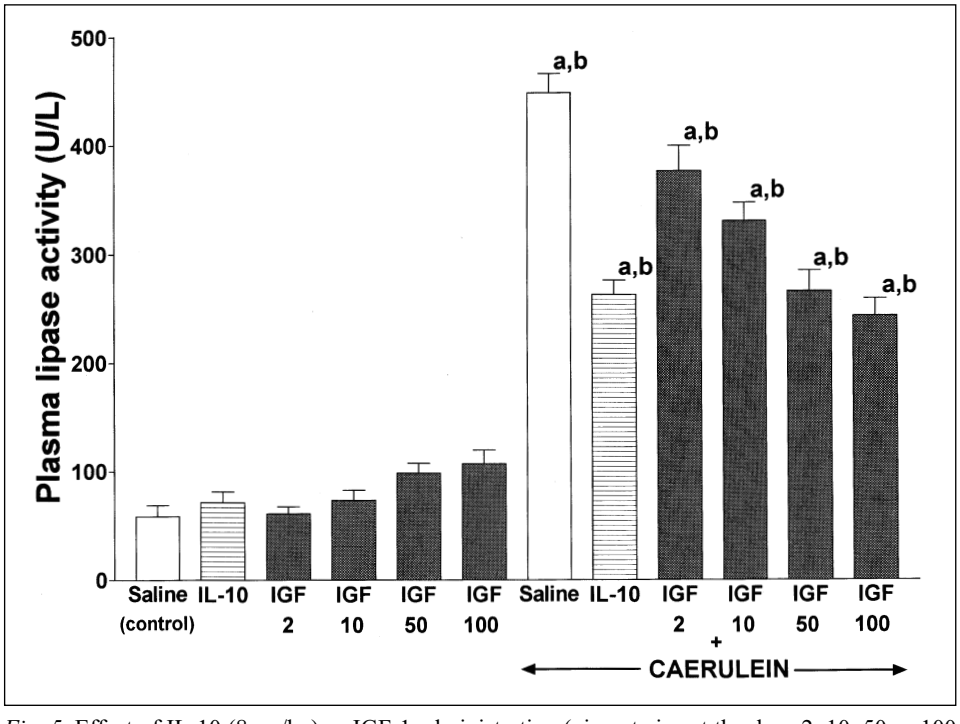

Fig. 5. Effect of IL-10 (8 μg/kg) or IGF-1 administration (given twice at the dose 2, 10, 50 or 100 μg/kg) on plasma lipase activity in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. [a]P<0.05 compared with control, [b]P<0.05 compared with caerulein given alone.

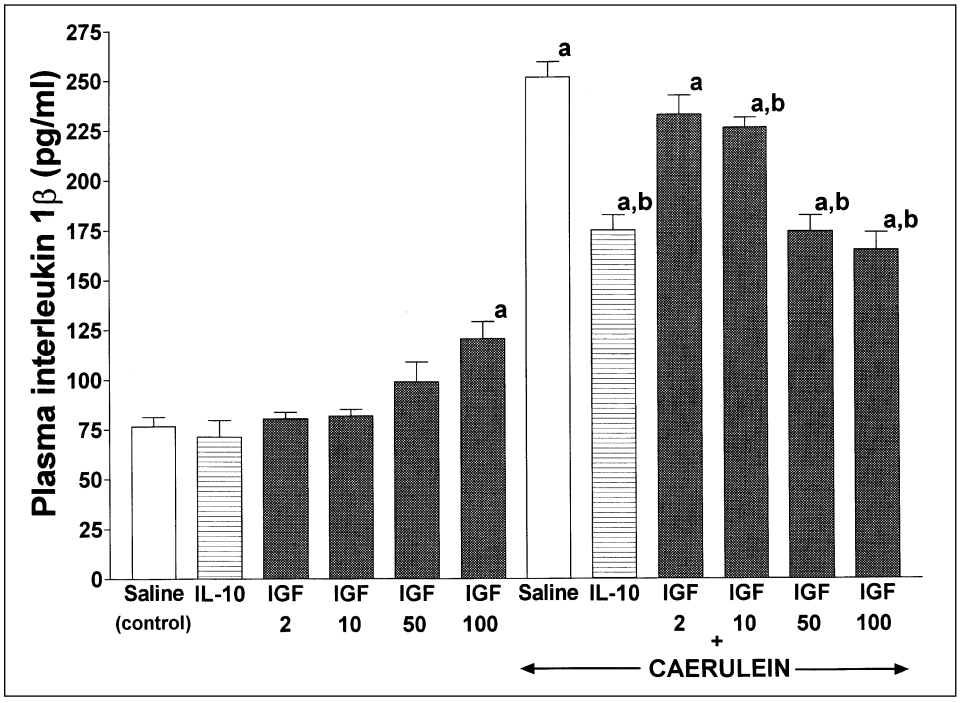

Fig. 6. Effect of IL-10 (8 μg/kg) or IGF-1 administration (given twice at the dose 2, 10, 50 or 100 μg/kg) on plasma interleukin-1β concentration in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. [a]P<0.05 compared with control, [b]P<0.05 compared with caerulein given alone.

IGF-1 given at the lowest dose 2 μg/kg was without significant effect on plasma IL-1β concentration in animals with caerulein-induced pancreatitis.

In control rats, plasma IL-10 reached a value 72.2 ± 5.8 pg/ml (*Fig. 7*). Infusion of caerulein for 5 h did not significantly affect the plasma IL-10 concentration. Treatment with IGF-1 at the higher doses (50 and 100 μg/kg) caused a significant increase in plasma concentration of IL-10 in rats infused with saline, as well as, with caerulein. In rats without caerulein-induced pancreatitis this effect was significantly more pronounced. In animals treated with human recombinant IL-10, plasma rat IL-10 concentration was not tested.

DISCUSSION

Our study has shown for the first time that administration of IGF-1 reduces the severity of caerulein-induced pancreatitis. The beneficial effect of treatment with IGF-1 was manifested by a reduction in plasma lipase activity, a decrease in plasma concentration of pro-inflammatory IL-1β, and a prevention of pancreatic

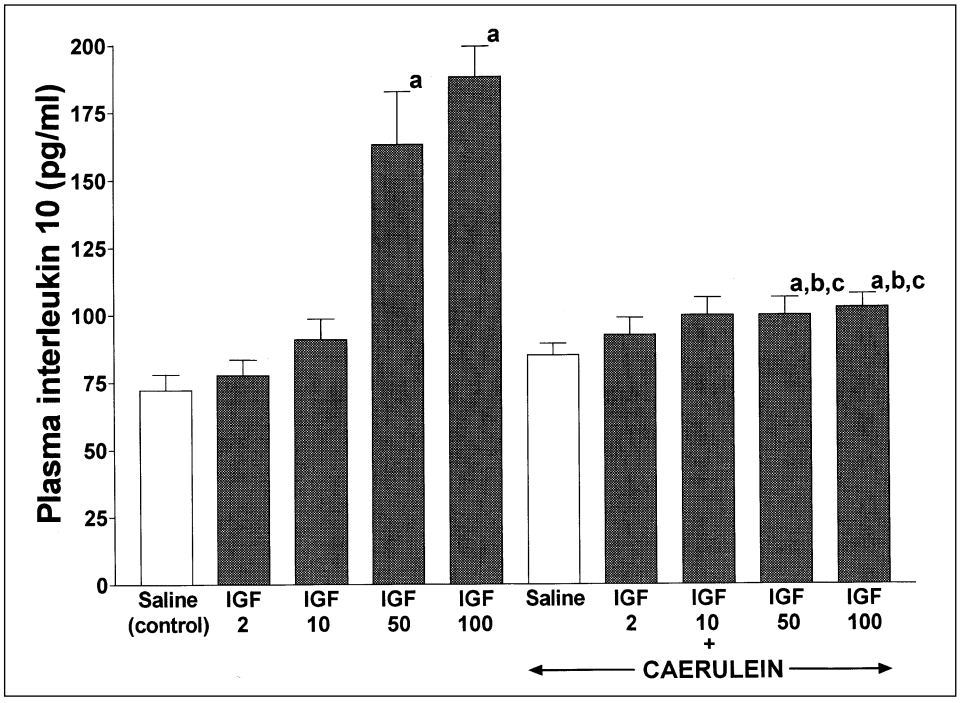

Fig. 7. Effect of IGF-1 administration (given twice at the dose 2, 10, 50 or 100 μg/kg) on plasma interleukin-10 concentration in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. [a]P<0.05 compared with control, [b]P<0.05 compared with caerulein given alone. [c]P<0.05 compared with IGF-1 at the same dose without caerulein infusion.

DNA synthesis reduction. There was found close relationship between evoked by IGF-1 the decrease in biochemical signs of pancreatitis and the improvement of pancreatic blood flow, as well as, the reduction in histological score of pancreatic damage. Morphological features have shown that IGF-1 administration decreases a pancreatic edema, acinar cells vacuolization of acinar cells and inhibits leukocyte infiltration of pancreatic tissue.

Acute pancreatitis is a pathological process dependent on autodigestion of pancreatic tissue and results from premature activation of inactive zymogens into active digestive enzymes but recent experimental data indicate that leukocyte infiltration and release of pro-inflammatory cytokines are responsible for local pancreatic injury and the development of systemic inflammatory response system (SIRS) and multiple organ failure (MOF) (30). Pro-inflammatory cytokines, such as IL-1β, interleukin-6 (IL-6) and TNF-α (Tumor Necrosis Factor) are produced within pancreas and subsequently within distant organs, which develop dysfunction during severe pancreatitis (31). Pro-inflammatory cytokine production correlates with disease severity (31) and among them IL-1β plays the most important role in the induction of systemic acute phase response and in the

release of other members of pro-inflammatory cytokine cascade (32). The role of IL-1β in the development of acute pancreatitis has been shown by Norman *et al.* (33). They have shown that blockade of IL-1β prevents the rise in serum IL-6 and TNF-α level, and protects against pancreatic damage in the course of experimental acute pancreatitis. These observations are in agreement with our present data and partly elucidate the mechanism of protective activity of IGF-1 administration on the pancreas. Treatment with IGF-1 has reduced the leukocyte infiltration of pancreatic tissue and the production of IL-1β, leading to the reduction of pancreatic damage.

The important finding of our present study is the observation that treatment with IGF-1 increases plasma IL-10 concentration. In contrast to IL-1β, IL-10 has been found to be a major anti-inflammatory cytokine. IL-10 reduces activation of macrophages and inhibits the production of reactive oxygen species (34) and pro-inflammatory cytokines (35). The study performed by Van Laethem *et al.* (36) has shown that administration of IL-10 before and during induction of acute pancreatitis decreases the severity of pancreatitis. The same effect has been observed in our present study. Administration of exogenous IL-10 in animals with caerulein infusion reduced pancreatic damage, plasma lipase activity, plasma IL-1β concentration and increased pancreatic blood flow and pancreatic DNA synthesis.

Previous study has shown that an increase in IL-10 level in the course of acute pancreatitis is a consequence of an increase in IL-1β and IL-10 plays a role of self-defense mechanism, limiting the intensity of inflammatory process (37). Contrary, a reduction in plasma pro-inflammatory IL-1β concentration leads to secondary reduction in plasma anti-inflammatory IL-10 in the course of this disease (38). In our present study, an increase in plasma interleukin-1β concentration was observed immediately after induction of pancreatits by caerulein as a result of leukocyte activation and tissue damage. In contrast to interleukin-1β, plasma interleukin-10 in animals with caerulein-induced pancreatitis was still low because a short time of inflammatory process. Treatment with IGF-1 reduced plasma IL-1β in animals infused with caerulein and increased plasma IL-10 concentration in animals infused with saline, as well as, in animals infused with caerulein. In animals without caerulein-induced pancreatitis, an increase in plasma IL-10 was significantly more pronounced. This observation indicates that IGF-1 stimulates a release of interleukin-10 independently to alteration of plasma IL-1β. Moreover, higher concentration of IL-10 in animals treated with IFG-1 without induction of pancreatitis than in animals with induction of pancreatitis suggests that this effect may be a result of indirect extrapancreatic mechanisms. IGF-1 is a member of hormonal axis: growth hormone (GH)-IGF-1-HGF. Postnatally, endogenous IGF-1 is produced mainly in the liver by hepatocytes and this organ is responsible for over 90% of circulating IGF-1 (39). Hepatic production of IGF-1 is GH-dependent. IGF-1 acts in endocrine and paracrine manner and in the liver, stimulates secretion of HGF

by hepatic stellate cells (40). On the other hand, HGF has been shown to stimulate a release of plasma IL-10 and attenuate the development of acute pancreatitis (26). These data suggest that protective effect of IGF-1 administration in the course of acute pancreatitis may be dependent on release of HGF.

Bacterial translocation from the gut has been demonstrated in numerous studies of major trauma such as burns (41), shock (42) or acute pancreatitis (43). Bacterial infection is a major cause of death in patients with acute necrotizing pancreatitis and approximately 75% of organisms that infect the pancreas belong to intestinal flora, including *Escherichia coli*, *Klebsiella* and other Gram-negative rods (44). Also, *Helicobacter pylori* infection aggravates acute pancreatitis (45). On the other hand, the study performed by Huang *et al.* (46) has shown that administration of IGF-1 reduces gut atrophy and bacterial translocation after severe burn injury. This observation suggests another possible mechanism of beneficial effect of IGF-1 administration in the course of acute pancreatitis. An additional support for this idea gives a study performed by Wang *et al.* (47). They have found that administration of GH reduces bacterial translocation throughout gut in the course of acute pancreatitis and causes overexpression of IGF-1 mRNA in the ileum.

Treatment with IGF-1 without induction of acute pancreatitis did not affect pancreatic DNA synthesis, probably due to the short time of IGF-1 administration (5.5 h). Induction of acute pancreatitis caused a reduction in the pancreatic DNA synthesis, what may be considered as an index of pancreatic damage. In rats with induction of pancreatitis, administration of IGF-1 attenuated the caerulein-induced fall in pancreatic DNA synthesis. This observation is an additional evidence of protective effect of IGF-1 administration on the pancreas.

Experimental and clinical studies have shown that pancreatic ischemia plays an important role in the development of acute pancreatitis. Pancreatic microvascular failure may be a primary reason of clinical (48-50) and experimental acute pancreatitis (37, 51), but an early disturbance of pancreatic circulation is observed in acute pancreatitis caused by other, primary non-vascular factors (51-53). It is well known that a disturbance of pancreatic microcirculation leads to the formation of thrombi in capillaries, activation of leukocytes, release of proteolytic enzymes, formation of oxygen-derived free radicals and pro-inflammatory cytokines (51). The severity of such experimental pancreatitis is closely correlated with tissue ischemia (54). An additional reduction in pancreatic circulation aggravates pancreatic damage in the course of acute pancreatitis (51, 55, 56), whereas a vasodilatation and an improvement of pancreatic blood flow have been found to reduce the development of acute pancreatitis (56-58). In our present study, pancreatic overstimulation with caerulein induced acute edematous pancreatitis and reduced the pancreatic blood flow. Treatment with IGF-1 caused a partial reversion of pancreatitis-induced fall of pancreatic blood flow. This observation suggests that the improvement of pancreatic microcirculation may contribute to the protective effect of IGF-1

administration. The mechanism of vascular effect of IGF-1 administration is not clear. IGF-1 given alone without induction of acute pancreatitis did not affect pancreatic blood flow. These data indicate that IGF-1 acts indirectly on pancreatic blood flow, probably by the limitation of caerulein-induced pancreatic edema and leukocyte activation.

In summary, our results provide the evidence that treatment with IGF-1 reduces development of acute pancreatitis. The improvement in pancreatic blood flow, the decrease in leukocyte activation, the limitation of pro-inflammatory IL-1β release, as well as, the increase in anti-inflammatory IL-10 are probably the major mechanisms involved in beneficial effect of IGF-1 on the pancreas.

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Authors address: Professor Artur Dembiński, MD, PhD; Department of Physiology, Jagiellonian University Medical School, 16 Grzegórzecka Street, 31-531 Kraków; Poland, tel.: +48-12-4211006, fax: +48-12-4211578
E-mail: mpdembin@cyf-kr.edu.pl