Acute pancreatitis leads to pancreatic damage followed by subsequent regeneration. The aim of our study was to evaluate the presence of growth factors in the course of spontaneous pancreatic regeneration after ischemia/reperfusion (I/R)-induced pancreatitis. Methods: In rats, I/R was evoked by clamping of splenic artery for 30 min followed by reperfusion. Rats were sacrificed 1, 5, 12 h or 1, 2, 3, 5, 7, 9 or 21 days after removal of vascular clips. Pancreatic blood flow (PBF), plasma lipase, interleukin-1β (IL-1β), interleukin-10, pancreatic cells proliferation and morphological signs of pancreatitis were determined. Pancreatic presence of fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), platelet-derived growth factor-A (PDGF-A) and transforming growth factor-β type II receptor (TGF-β RII) was detected by immunohistochemistry. Results: Exposure to I/R led to the development of acute necrotizing pancreatitis followed by regeneration. Morphological features showed maximal pancreatic damage between the 1st and 2nd day of reperfusion. It was correlated with a maximal increase in plasma lipase, and pro-inflammatory IL-1β concentration, as well as, a reduction in PBF and pancreatic DNA synthesis. I/R increased FGF-2 content in pancreatic acinar cells between the 12th and 24th h, and between 5th and 9th day of reperfusion. At the 2nd day the presence of FGF-2 in pancreatic acinar cells was reduced. After I/R PDGF-A appeared in pancreatic vessels from the 12th h to 5th day of reperfusion. PDGF-A was not observed in pancreatic acinar cells in the control or in I/R group. In pancreatic ducts, the presence of PDGF-A was reduced between the 1st and 3rd, and between 7th and 9th day of reperfusion. In acinar cells, VEGF content was increased after I/R at the time between the 1st and 24th h, and between 3rd and 7th day of reperfusion. At the 2nd day of reperfusion, VEGF was not detected in the pancreatic acinar cells. Moreover, VEGF was found in the inflammatory infiltration, in the tubular complexes between the 2nd and 5th day, and in granulation tissue at the 9th day of reperfusion. In pancreatic acinar cells, I/R caused an increase in TGF-β RII presence between the 5th and 24th h, and between 7th and 9th day of reperfusion. Between the 2nd
and 5th day of reperfusion the acinar presence of TGFβ RII was reduced. In the pancreatic ducts, the presence of TGFβ RII was increased after I/R from the 1st h to 9th day of observation. Four weeks after induction of acute pancreatitis, the pancreatic regeneration was completed and the presence of growth factors tested returned to control value. Conclusions: The presence of FGF, VEGF, PDGF-A and TGFβ RII is modified in the course of I/R-induced acute pancreatitis. Maximal content of FGF, VEGF and TGFβ RII has been observed in early stage of pancreatic regeneration suggesting the involvement these factors in pancreatic recovery.

**Key words:** acute pancreatitis, pancreatic regeneration, FGF-2, VEGF, PDGF-A, TGF-β RII.

**INTRODUCTION**

Acute pancreatitis leads to pancreatic damage followed by subsequent regeneration (1-3). The sequence of events during pancreatic damage and regeneration includes leukocyte infiltration and inflammation, transient activation and proliferation of fibroblasts, and deposition of extracellular matrix. Next step of regeneration involves proliferation of acinar cells and degradation of extracellular matrix (3, 4). Previous studies have shown that growth factors are involved in pancreatic development, growth and regeneration (5, 6). Growth factors are expressed in low levels even in normal pancreas (7). In experimental rat model of edematous acute pancreatitis, the pancreatic mRNA transcript is overexpressed for growth factors such as fibroblast growth factor-1 (FGF-1) (7, 8), fibroblast growth factor-2 (FGF-2) (7, 8), insulin-like growth factor-1 (IGF-1) (7-9), hepatocyte growth factor (HGF) (7, 8), transforming growth factor-α (TGF-α) (7, 8), transforming growth factor-β (TGF-β) (7, 10, 11) and epidermal growth factor (EGF) (10, 11). Acute experimental pancreatitis causes the increase in the expression of mRNA for HGF-receptor in the pancreas (7, 8).

Moreover, clinical studies, performed on pancreatic samples obtained from patients with acute necrotizing pancreatitis, have shown a marked increase in pancreatic expression of growth factors such as TGF-β (12, 13), connective tissue growth factor (CTGF) (13), FGF-1 and FGF-2 (14).

The influence of growth factors on the course of acute pancreatitis has been studied by administration of exogenous peptides. The most of these studies has been performed using caerulein-induced model of acute pancreatitis. Treatment with EGF (15-17), FGF-2 (18), HGF (19) or IGF-1 (20, 21) attenuated the pancreatic damage in the course of acute experimental pancreatitis and accelerated the pancreatic recovery. This observation is the evidence that growth factors may limit the pancreatic damage in the course of pancreatitis and stimulate pancreatic regeneration.

Vascular endothelial growth factor (VEGF) (22), and platelet-derived growth factor-A (PDGF-A) (23) have been shown to participate in wound and tissue repair, however the role of endogenous VEGF and PDGF-A in the course of acute
Pancreatitis is unknown. Pancreatic mRNA for FGF-2 and TGFβ has been found to be overexpressed in the course of acute pancreatitis, but it is not a proof of the presence of final product of translation - a protein. Also, it is of interest what is a target for TGFβ in the course of pancreatitis. The aim of our study was to evaluate the pancreatic presence and localization of PDGF-A, FGF-2, VEGF and TGFβ type II receptor in the course of ischemia/reperfusion-induced pancreatitis.

**MATERIALS AND METHODS**

*Animals and treatment*

Studies were performed on male Wistar rats weighing 200-250 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-hour light-dark cycle. Acute pancreatitis was induced by limitation of pancreatic blood flow in inferior splenic artery followed by reperfusion as described previously (3). Briefly, after fasting for 24 h with free access to water, rats were anesthetized with ketamine (50 mg/kg intraperitoneally, Bioketan, Biowet, Gorzów, Poland). After longitudinal laparotomy, ischemia in the splenic region of the pancreas was induced by clamping of inferior splenic artery using microvascular clips. Thirty min later, microvascular clips were removed to obtain pancreatic reperfusion and the abdominal cavity was closed by suture. In sham operated-control animals, longitudinal laparotomy and mobilization of pancreas without clamping any arteries was performed. Animals were anesthetized again and sacrificed after 1, 5, 12 h or 1, 2, 3, 5, 7, 9 or 21 days from the start of reperfusion.

*Determination of pancreatic blood flow*

At the time of experiment cessation animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdominal cavity was opened. The pancreas was exposed for the measurement of the blood flow in the pancreatic tissue by laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (24). The pancreatic blood flow was presented as percent change from control value obtained in sham-operated rats.

*Determination of plasma lipase activity, and plasma 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 plasma lipase activity, and plasma interleukin-1β, and interleukin-10 concentration. 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. Plasma interleukin-1β and interleukin-10 were measured in duplicate using the BioSource Cytoscreen rat interleukin-1β and interleukin-10 kits (BioSource International, Camarillo, California, USA) based on ELISA. Concentration of interleukin was 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 peripancreatic 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 [3H]thymidine ([6-3H]thymidine, 20-30 Ci/mmol; Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic), as described previously (25). The incorporation of [3H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [3H]thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

Histological examination of pancreatic damage

Samples of pancreatic tissue excised from the body portion for morphological examination were fixed for 24 h in buffered 10% formalin, embedded in paraffin and sections were stained with hematoxilin and eosin. The histological grading of edema was made using a scale ranging 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. Hemorrhagia was graded: 0 = no hemorrhagia, 1 = 1-2 hemorrhagic foci per slide, 2 = 3-5 hemorrhagic foci per slide, 3 = more than 5 hemorrhagic foci per slide. Necrosis was graded: 0 = no necrosis, 1 = less than 15% of pancreatic cells involved, 2 = 15-35% of cells involved, 3 = more than 35% of cells involved.

Immunohistochemistry

Unstained sections were deparaffinized and rehydrated with xylene and graded ethanol. After rinsing in distilled water, endogenous peroxidase was blocked by 3% hydrogen peroxide for 8 min. Afterwards sections were treated with sodium citrate buffer and then submitted to microwave antigen retrieval. The slides were incubated with primary antibodies (Santa Cruz Biotechnology Inc., USA) in a moist chamber for 1 h at room temperature. Rabbit polyclonal antibodies against FGF-2 (147: sc 79), PDGF-A (N-30) and TGF-β RII (L-21), and mouse monoclonal antibody against VEGF (C-1) were used at the dilution 1:50. Primary antibodies were detected using DAKO Envision™ + System Labeled Polymer HRP (DAKO, Glostrup, Denmark). Visualization was performed using AEC (3-amino-9-ethyl-carbazole) as a chromogen (AEC-Substrate Chromagen ready-to-use, DAKO, Glostrup, Denmark). Sections were counterstained with hematoxylin and mounted in glycergel. In each specimen staining intensity in pancreatic acinar cells, ductal epithelial cells, islet cells and stromal cells was recorded in a whole tissue fragment and graded as: 0 = negative; 1 = minimal immunostaining in some cells; 2 = weak and 3 = -strong immunostaining.

Statistical analysis

Statistical analysis was made by analysis of variance and Student’s T test for unpaired data. A difference with a p value of less than 0.05 was considered significant. Results are expressed as means (± S.E.M).
RESULTS

The pancreas of sham-operated control rats showed macroscopically and microscopically no tissue damage (Table 1). Weak FGF-2 immunoreactivity was found in pancreatic acinar cells, whereas strong FGF-2 immunostaining was detected in ductal epithelial cells and islets cells (Table 2). In pancreatic blood vessels, no FGF-2 immunoreactivity was observed. PDGF was not detected in pancreatic acinar cells, but weak immunostaining for PDGF was found in ductal and islet cells (Table 3). In some cases of control rats, PDGF was absent in pancreatic blood vessels; in other cases PDGF was minimally expressed in endothelial cells. VEGF was present only in few pancreatic acinar cells (Table 4). No immunostaining for VEGF was found in ductal epithelial cells, islet cells or blood vessels in the pancreas of control rats. Type II receptor for TGF-β was weakly expressed in pancreatic acinar cells (Table 5). In ductal epithelial cells and islets cells, immunostaining for TGF-β RII was minimal. In pancreatic blood vessels of control rats, type II receptor for TGF-β was not present.

Exposure to pancreatic ischemia followed by reperfusion led to the development of acute necrotizing pancreatitis in all rats tested (Table 1). After 1-h reperfusion, moderate inter- and intralobular edema was accompanied with scarce or moderate perivascular inflammatory leukocyte infiltration. In some cases, scarce diffuse inflammatory infiltration was also observed. Vacuolization or necrosis of acinar cells was not found. Hemorrhages were limited to 1 to 2 foci per slide.

Table 1. Morphological features of the pancreas in the course of ischemia/reperfusion-induced pancreatitis

<table>
<thead>
<tr>
<th>Time of reperfusion</th>
<th>edema</th>
<th>inflammatory infiltration</th>
<th>vacuolization</th>
<th>necrosis</th>
<th>hemorrhages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Time of reperfusion: 1 h</td>
<td>2</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5 h</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12 h</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1/2</td>
</tr>
<tr>
<td>1 day</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1/2</td>
</tr>
<tr>
<td>2 days</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3 days</td>
<td>1/2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>5 days</td>
<td>1/2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>7 days</td>
<td>1/2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>9 days</td>
<td>1/2</td>
<td>1/2</td>
<td>0</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>21 days</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers represent the predominant grading in each group
Morphological features of pancreatic damage, such as edema, vacuolization and necrosis reached maximal intensity after 1-day reperfusion (Table 1). Hemorrhages and leukocyte inflammatory infiltration were maximally expressed after 2-days reperfusion. From the 3rd day of reperfusion, histological signs of pancreatic damage tended to reduce. Pancreatic damage was followed by regeneration with temporary forming of features typical for chronic pancreatitis. Deposition of extracellular matrix, acinar cells loss, formation of tubular complexes and dilatation of pancreatic ducts were observed between the second and ninth day of reperfusion. Regeneration involved acinar cells and tubular complexes, as evidenced by mitotic figures observed in these structures. Twenty one days after induction of acute pancreatitis, the morphological features showed almost normal pancreatic tissue, except scarce slight perivascular leukocyte infiltration.

Histological findings were correlated with biochemical signs of acute pancreatitis. After 1-h reperfusion, plasma lipase activity (Fig. 1) and plasma
concentration of pro-inflammatory interleukin-1β (Table 6) were increased, whereas pancreatic blood flow (Fig. 2) and pancreatic DNA synthesis (Table 6) were reduced. Maximal increase in plasma lipase activity (Fig. 1) and plasma interleukin-1β concentration (Table 6) was observed after 2-days reperfusion. Plasma lipase activity and plasma interleukin-1β concentration returned to control value at the 21st day of reperfusion. Pancreatic blood flow (Fig. 2) and pancreatic DNA synthesis (Table 6) reached the lowest value after 2 days of reperfusion. Twenty one days after induction of acute pancreatitis pancreatic blood flow and pancreatic DNA synthesis returned to control value. Plasma concentration of interleukin-10 was unchanged 1 h after removal of vascular clips (Table 6). Plasma interleukin-10 concentration was significantly increased between the 1st and 7th day of reperfusion, reaching maximal value after 2-day reperfusion.

Table 3. Immunohistochemical localization of PDGF-A in pancreatic tissue in the course of ischemia/reperfusion-induced pancreatitis

<table>
<thead>
<tr>
<th>Time of reperfusion</th>
<th>pancreatic acinar cells</th>
<th>ductal epithelial cells</th>
<th>islet cells</th>
<th>blood vessels</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0/1</td>
<td>-</td>
</tr>
<tr>
<td>5 h</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0/1</td>
<td>-</td>
</tr>
<tr>
<td>12 h</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1/2</td>
<td>inflammatory infiltration cells</td>
</tr>
<tr>
<td>1 day</td>
<td>0</td>
<td>1/2</td>
<td>2</td>
<td>0</td>
<td>inflammatory infiltration cells</td>
</tr>
<tr>
<td>2 days</td>
<td>0</td>
<td>1/2</td>
<td>2</td>
<td>2</td>
<td>tubular complexes inflammatory infiltration cells</td>
</tr>
<tr>
<td>3 days</td>
<td>0</td>
<td>1/2</td>
<td>2</td>
<td>1/2</td>
<td>fibroblast or fibroblast-like cells</td>
</tr>
<tr>
<td>5 days</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1/2</td>
<td>fibroblast or fibroblast-like cells</td>
</tr>
<tr>
<td>7 days</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0/1</td>
<td>fibroblast or fibroblast-like cells</td>
</tr>
<tr>
<td>9 days</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0/1</td>
<td>fibroblast or fibroblast-like cells</td>
</tr>
<tr>
<td>21 days</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0/1</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers represent the predominant grading in each group
Ischemia followed by reperfusion increased FGF-2 content in pancreatic acinar cells between the 12th and 24th h, and between the 5th and 9th day of reperfusion (Table 2). After 2-days reperfusion the presence of FGF-2 in acinar cells was reduced. In ductal epithelial cells a moderate decrease in FGF-2 content was observed after 12-h and 3-days reperfusion. In islet cells, strong immunostaining for FGF-2 was observed during full time of observation, except a slight decrease in FGF-2 level after 12-h reperfusion. In contrast, weak immunostaining for FGF-2 in pancreatic vessels was found only after 2-days reperfusion. In remaining time FGF-2 was undetected in endothelial cells. Additionally, the presence of FGF-2 was temporary observed in fibroblasts or fibroblast-like cells and tubular complexes. In fibroblasts or fibroblast-like cells, immunostaining for FGF-2 was found between the 1st and 9th day of reperfusion. In tubular complexes, FGF-2 was present at the 2nd day of reperfusion (Table 2, Fig. 3).
Table 5. Immunohistochemical localization of TGFβ R II in pancreatic tissue in the course of ischemia/reperfusion-induced pancreatitis

<table>
<thead>
<tr>
<th></th>
<th>pancreatic acinar cells</th>
<th>ductal epithelial cells</th>
<th>islet cells</th>
<th>blood vessels</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of reperfusion: <strong>1 h</strong></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>5 h</td>
<td>2/3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>12 h</td>
<td>2/3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>1 day</td>
<td>2/3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>2 days</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>tubular complexes 2</td>
</tr>
<tr>
<td>3 days</td>
<td>1/2</td>
<td>1/2</td>
<td>0/1</td>
<td>0</td>
<td>fibroblast or fibroblast-like cells 1</td>
</tr>
<tr>
<td>5 days</td>
<td>1</td>
<td>1/2</td>
<td>0/1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>7 days</td>
<td>2/3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>9 days</td>
<td>2/3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>21 days</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers represent the predominant grading in each group

Fig. 1. Plasma lipase activity in the course of ischemia/reperfusion-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. *p<0.05 compared with control.
Fig. 2. Pancreatic blood flow in the course of ischemia/reperfusion-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. *p<0.05 compared with control.

Table 6. Plasma interleukin-1β and interleukin-10 concentration, and pancreatic DNA synthesis in the course of ischemia/reperfusion-induced pancreatitis

<table>
<thead>
<tr>
<th>Time of reperfusion</th>
<th>Plasma interleukin-1β concentration (pg/ml)</th>
<th>Plasma interleukin-10 concentration (pg/ml)</th>
<th>Pancreatic DNA synthesis (dpm/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (control)</td>
<td>49.5 ± 5.5</td>
<td>77.5 ± 6.5</td>
<td>56.6 ± 2.8</td>
</tr>
<tr>
<td>Time of reperfusion: 1 h</td>
<td>124.5 ± 7.5*</td>
<td>80.0 ± 7.0</td>
<td>41.7 ± 3.2*</td>
</tr>
<tr>
<td>5 h</td>
<td>135.5 ± 7.5*</td>
<td>83.5 ± 6.5</td>
<td>30.0 ± 2.2*</td>
</tr>
<tr>
<td>12 h</td>
<td>203.0 ± 8.0*</td>
<td>93.5 ± 6.5</td>
<td>17.4 ± 2.5*</td>
</tr>
<tr>
<td>1 day</td>
<td>229.5 ± 9.5*</td>
<td>304.5 ± 6.5*</td>
<td>15.5 ± 2.3*</td>
</tr>
<tr>
<td>2 days</td>
<td>216.0 ± 8.0*</td>
<td>554.0 ± 22.0*</td>
<td>17.5 ± 2.3*</td>
</tr>
<tr>
<td>3 days</td>
<td>175.5 ± 6.5*</td>
<td>344.0 ± 13.0*</td>
<td>24.0 ± 2.8*</td>
</tr>
<tr>
<td>5 days</td>
<td>163.0 ± 6.0*</td>
<td>226.0 ± 8.0*</td>
<td>30.3 ± 2.9*</td>
</tr>
<tr>
<td>7 days</td>
<td>129.0 ± 14.0*</td>
<td>114.5 ± 7.5*</td>
<td>32.5 ± 1.3*</td>
</tr>
<tr>
<td>9 days</td>
<td>106.0 ± 8.0*</td>
<td>94.0 ± 6.5</td>
<td>33.5 ± 1.6*</td>
</tr>
<tr>
<td>21 days</td>
<td>72.3 ± 9.0</td>
<td>82.0 ± 5.8</td>
<td>51.3 ± 2.5</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. N=10 in each group of animals. *p<0.05 compared with control.
PDGF-A was not observed in pancreatic acinar cells in the control rats or in rats with acute pancreatitis (Table 3). In pancreatic ducts, the presence of PDGF-A was reduced between the 1st and 3rd, and between the 7th and 9th day of reperfusion. Ischemia/reperfusion-induced pancreatitis did not affect PDGF-A immunostaining in pancreatic islet cells. In pancreatic blood vessels, the increase in PDGF-A content was observed after 12-h reperfusion, and between the 2nd and 5th day of reperfusion. PDGF-A was not detected in pancreatic vessels after 1-day reperfusion. Additionally, weak PDGF-A immunostaining was found in cells of inflammatory infiltration between the 12th h and 2nd day of reperfusion. Also, after 2-days reperfusion, weak immunostaining for PDGF-A was observed in tubular complexes (Fig. 4). Fibroblasts or fibroblast-like cells showed PDGF-A immunostaining between the 3rd and 9th day of reperfusion (Table 3).

Acinar VEGF content was slightly increased in animals with acute pancreatitis at the time between the 1st and 24th h, and between 3rd and 7th day of reperfusion (Table 4). At the 2nd day of reperfusion, VEGF was not detected in pancreatic acinar cells. Moreover, VEGF was found in ductal epithelial cells after 12-h reperfusion, and in islet cells between the 1st and 12th h of reperfusion. Minimal or weak VEGF immunostaining was found in inflammatory infiltration cells and in tubular complexes (Fig. 5) between the 2nd and 5th day of reperfusion. At the 7th day of reperfusion minimal or weak immunostaining for VEGF was found in
Fig. 4. Presence of PDGF-A in tubular complexes at the second day from the time of induction of acute pancreatitis.

Fig. 5. Presence of VEGF in tubular complexes at the second day from the time of induction of acute pancreatitis.
some fibroblasts or fibroblast-like cells. Additionally, weak immunostaining for VEGF was found in granulation tissue at the 9th day of reperfusion (Table 4).

Ischemia followed by reperfusion increased TGF-β RII immunostaining in pancreatic acinar cells between the 5th and 24th h (Fig. 6), and between 7th and 9th day of reperfusion. Between the 2nd and 5th day of reperfusion acinar content of TGF-β type II receptor was reduced. The presence of TGFβ RII in the pancreatic ducts was increased between the 1st and 24th h, and between 3rd and 9th day of reperfusion. In pancreatic islets, immunostaining for TGF-β RII was increased between the 1st h and 2nd day, and between 7th and 9th day of reperfusion. Between the 3rd and 5th day of reperfusion, the presence of TGF-β RII in pancreatic islets was reduced. TGF-β RII immunostaining was not observed in pancreatic blood vessels during full time of observation. Weak immunostaining for TGF-β type II receptor in tubular complexes was found after 2 days of reperfusion, minimal TGF-β RII immunostaining was temporary observed in some fibroblasts or fibroblast like cells (Table 5).

**Fig. 6.** The increase in the presence of TGFβ RII in pancreatic acini at the 12th h of reperfusion.

**DISCUSSION**

In our present study, exposure to ischemia followed by reperfusion has induced acute necrotizing pancreatitis. Morphological and biochemical examination showed maximal pancreatic damage between the 1st and 2nd day of
reperfusion and these findings were associated with maximal reduction of pancreatic blood flow. Since the 3rd day of reperfusion, we observed pancreatic regeneration. Histological and biochemical signs of pancreatic damage tended to reduce. During pancreatic repair, morphological features of pancreatic tissue showed the transient presence of fibroblasts or fibroblast like cells, deposition of extracellular matrix, formation of tubular complexes and dilatation of pancreatic ducts. Theses findings were observed between the second and ninth day of reperfusion and were followed by proliferation of acinar cells and degradation of extracellular matrix seen in morphological examination. Observation of mitotic figures in pancreatic cells was in agreement with a partial restoration of pancreatic DNA synthesis.

In normal pancreas, pancreatic stellate cells with the retinoid-storing phenotype are present in low number in periacinar regions (26, 27). After stimulation, pancreatic stellate cells change their phenotype to highly active myofibroblast-like cells and produce majority of extracellular matrix, including collagen type I, III and IV, fibronectin and proteoglican matrix (28). Activation of pancreatic stellate cells plays an essential role in the deposition of extracellular matrix in pancreatic regeneration after acute pancreatitis (29), but pancreatic stellate cells are also involved in the development of fibrosis in chronic pancreatitis (30). In our present study, we have found a temporary immunostaining for FGF-2, PDGF-A, VEGF and TGFβ RII in fibroblasts or fibroblast cells indicating the involvement of these factors in regulation of extracellular matrix deposition and pancreatic regeneration.

In general, TGF-β stimulates growth of cells of mesenchymal origin, but inhibits the growth of endothelial, hemopoetic and epithelial cells (31). TGF-β1 is overexpressed in acute pancreatitis (7, 10-13) and participates in the healing process. TGF-β1, as well as other members of TGFβ family mediate their activity by high affinity binding to the type II receptor (32). In our present study, we have studied the target cells for TGF-βs by immunohistochemical localization of TGF-β R II. We have found biphasic increase in the presence of TGF-β type II receptor in pancreatic acinar, ductal and islet cells and these results are in agreement with a biphasic increase in pancreatic expression of TGF-β1 mRNA in acute caerulein-induced pancreatitis, which was observed by Konturek et al (11) and Riesle et al (33). The first peak of the presence of TGF-β type II receptor in pancreatic acinar cells was observed between the 5th and 24th h of reperfusion, when the pancreatic DNA synthesis was progressively reduced. Between the 2nd and 5th day of reperfusion acinar content of TGF-β type II receptor was reduced, and at this time the partial restoration of pancreatic DNA synthesis was observed. The second peak of immunostaining for TGF-β RII in acinar cells was found between 7th and 9th day of reperfusion, and this effect was accompanied by lack of additional increase in pancreatic DNA synthesis. These data indicate the relationship between the increase in acinar immunostaining for TGF-β RII and inhibition of pancreatic acinar cell proliferation. This observation is supported by study
performed by Logsdon et al (34). They have found that TGF-β1 inhibits pancreatic acinar cell growth (34).

Study performed by Hahm et al (35) has shown that loss of TGF-β signaling contributes to autoimmune pancreatitis and increases susceptibility to caerulein-induced pancreatitis. This finding suggests that the first peak of the presence of TGF-β type II receptor in pancreatic acinar cells, observed in our present, study may be involved in preservation of the integrity of these cells.

TGF-β1 has been found to affect pancreatic stellate cells. TGF-β induces the changes in their phenotype from fat-storing cells to fibroblast-like cells (36) and stimulates these cells to the synthesis of pancreatic extracellular matrix (28, 36-37), metalloproteinases and tissue inhibitors of metalloproteinases (38). In our results, we have found immunostaining for TGF-β type II receptor in tubular complexes and fibroblasts or fibroblast like cells. These findings support the conception that TGF-β1 acting on pancreatic stellate cells or fibroblast may regulate the synthesis and degradation of extracellular matrix protein.

In our present study, immunostaining for TGF-β RII was increased between the 1st and 2nd day, and between 7th and 9th day of reperfusion in pancreatic islets. TGF-β1 has been found to be involved in the development of pancreatic islets (39). In the presence of a pan-specific TGF-β neutralizing antibody islets morphogenesis is abolished (39). These data and our results taken together indicate the influence of TGF-βs on pancreatic islet cells in the course of acute pancreatitis. It may depend on the regulation of the hormone release by islet cells and by this way on the regeneration of acinar cells or on pancreatic islets regeneration in the course of acute necrotizing pancreatitis.

Platelet-derived growth factor (PDGF) is a mitogen for mesoderm-derived cells and is released from platelets during clot formation at sites of vascular damage (40). It consists of two disulfide-bonded chains, A and B, and occurs as three isoforms, PDGF-AA, PDGF-AB, and PDGF-BB (41). PDGF stimulates the growth and migration of fibroblasts, endothelial cells and smooth muscle cells, and this polypeptide plays an important role in wound healing and tissue repair (23, 41-42). In acute pancreatitis, acinar cell injury causes aggregation of platelets in pancreatic capillaries and extravasation of mononuclear cells into the damaged tissue (43). These data are supported by our present observation. We have found that PDGF-A content in pancreatic blood vessels is increased after 12-h reperfusion, and between the 2nd and 5th day of reperfusion. Also, we have observed a presence of PDGF-A immunostaining in the cells of inflammatory infiltration between the 12th h and 2nd day of reperfusion. This last finding is in agreement with previous report that PDGF-A can be released, apart platelets, by mononuclear cells and activated macrophages (44).

Previous studies have shown that PDGF acts on pancreatic stellate cells stimulating their proliferation (28, 43). In our present study, we have found PDGF-A immunostaining in fibroblasts or fibroblast-like cells between the 3rd and 9th day of reperfusion. These data suggest that proliferation of fibroblast or
fibroblast-like cells occurs in this period of time in the course of ischemia/reperfusion-induced pancreatitis.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is secreted by different types of cells, including, pericytes, smooth muscle cells, macrophages and mast cells (45). VEGF stimulates the proliferation of endothelial cells and acts as a potent angiogenic factor (45). It is an important mediator of angiogenesis in physiological and pathological conditions. VEGF is involved in regulation of angiogenesis during organ development (45, 46), tumor growth (47, 48) and tissue repair (22, 49).

In our present study, pancreatic ischemia was a primary cause of acute pancreatitis and a decrease in pancreatic blood flow was associated with an appearance of immunostaining for VEGF in acinar and ductal epithelial cells, cells of pancreatic islets, as well as in tubular complexes, inflammatory infiltration cells and granulation tissue. This observation gives us three interesting pieces of information: [1] The increase in the presence of VEGF in pancreatic acinar and ductal cells suggests that VEGF is probably produced by these cells. This hypothesis is supported by study performed by Kuehn et al (47). They have found that VEGF is strongly expressed in ductal cells in chronic pancreatitis and in pancreatic cancer cells. [2] Our present finding confirms the observation that hypoxia is one of the most important stimulators of VEGF expression (50, 51). [3] The immunostaining for VEGF in granulation tissue at the ninth day of reperfusion suggests the involvement of endogenous VEGF in revascularization of pancreatic tissue during pancreatic repair in the course of acute pancreatitis.

FGF-2 has been shown to stimulate the proliferation of cells of mesenchymal, epithelial and neuroectodermal origin. It is involved in wound healing and tissue repair (52). In our present study, FGF-2 content in acinar cells was increased between the 12th and 24th h, and between the 5th and 9th day of reperfusion. At the 2nd day the presence of FGF-2 in the pancreatic acinar cells was reduced and this effect seems to be related to severity of pancreatic damage. These findings are consistent with the study performed by Ebert et al (53). They have found a loss of FGF-1 and FGF-2 immunoreactivity in regions with necrosis in human pancreatic tissues obtained from patient with acute pancreatitis. In contrast, in the regenerating areas, they have observed the increase in FGF-1, FGF-2 and FGF-receptor 1 immunostaining in exocrine-type cells.

FGF-2 in cooperation with VEGF exhibits a potent angiogenic activity (54). In our present study, we have observed a temporary immunostaining for FGF-2 in pancreatic blood vessels at the 2nd day of reperfusion. This finding indicates the involvement of FGF-2 in revascularization of pancreatic tissue during pancreatic regeneration in the course of ischemia/reperfusion-induced pancreatitis.

Our study has also shown the temporary presence of FGF-2 in fibroblasts or fibroblast-like cells. Immunostaining for FGF-2 in fibroblasts or fibroblast-like cells indicates the involvement of FGF-2 in extracellular matrix synthesis and this
observation is in harmony with the observation that FGF-2 stimulates the synthesis of extracellular matrix proteins by pancreatic stellate cells (36).

In our present study we have observed immunostaining for PDGF-A, VEGF, FGF-2 and TGF-β RII in tubular complexes. Previous study has shown that regeneration of pancreatic tissue after acute pancreatitis involves acinar cells and tubular complexes, as evidenced by mitotic figures observed in these structures (55). Cells of tubular complexes are supposed to originate from acinar cells by dedifferentiation and structures very similar to tubular complexes were observed in embryonic pancreas, what suggest that the cells forming tubular complexes may have recovered pluripotency, what is essential in pancreatic development and repair (55). Acinar (55), ductal (56) cells, as well as, endocrine islets cells (57, 58) can develop from cells of tubular complexes. These data and our observations indicate that PDGF-A, VEGF, FGF-2 and TGF-β are involved in regulation of tubular complexes formation, and by this in regulation of pancreatic cells restoration during pancreatic regeneration.

In conclusion, our data have shown that maximal alterations in the content of PDGF-A, FGF-2, VEGF and TGF-β RII occur in early stage of pancreatic regeneration. This observation suggests the involvement of these growth factors in pancreatic recovery after pancreatic damage evoked by ischemia/reperfusion-induced pancreatitis.

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