Leptin is involved in the regulation of food intake and previous studies have shown that leptin affects the inflammatory response in various tissues. The objective of this study was to examine the influence of leptin administration on the development and the course of acute ischemic pancreatitis. Acute pancreatitis was induced by limitation of pancreatic blood flow by clamping of inferior splenic artery for 30 min, followed by reperfusion. Leptin was administered three times daily at the dose 10 or 50 \( \mu \)g/kg. Animals were sacrificed 1, 3, 5, 10 and 21 days after removal of vascular clips. Administration of leptin reduced development of pancreatic damage and accelerated pancreatic regeneration what was manifested by the improvement of pancreatic histology, the decrease in serum lipase and amylase activity, and the reduction in serum interleukin-1\( \beta \) concentration. Also, treatment with leptin caused the increase in the pancreatic blood flow and pancreatic DNA synthesis. Leptin administration was without effect on serum interleukin-10 concentration. Leptin at the dose 50 \( \mu \)g/kg was more effective than 10 \( \mu \)g/kg. We conclude that leptin reduces the pancreatic damage in the course of ischemic pancreatitis and accelerates the pancreatic tissue repair. The beneficial effects of leptin appear to be dependent on the improvement of pancreatic blood flow, the increase in pancreatic cell growth, and the limitation of pro-inflammatory interleukin-1\( \beta \) release.

Key words: leptin, ischemic acute pancreatitis, pancreatic regeneration, interleukin-1\( \beta \).
INTRODUCTION

Leptin is a 16 kDa protein encoded by the obese \((ob)\) gene (1). Leptin is produced and secreted mainly by adipocytes (2) and plasma leptin level corresponds very closely to the amount of fat tissue (3). Leptin reaches the hypothalamus via a blood to induce satiety by suppression of neuropeptide Y release (4). Reducing the food intake, leptin plays a crucial role in the homeostasis of body weight and energy expenditure (5, 6). In \(ob(-)/ob(-)\) mice, which have leptin deficiency, the obesity is observed. Administration of recombinant leptin to these mice leads to a marked reduction in their body weight and adipose tissue (7).

During the last years, the participation of leptin in acute phase response to inflammation has been proposed (8-10). Cytokines and acute inflammation raise plasma level (9) and the role of leptin in the inflammatory response is further strengthened by the observation that in interleukin-1\(\beta\) (IL-1\(\beta\)) deficient mice the increase in leptin production is absent after an inflammatory stimulus (11).

Besides fat tissue and hypothalamus, the presence of leptin and expression of its receptors was detected in the others tissues like the stomach (12), liver (13) or pancreas (14, 15). The stomach is the second, besides fat tissue, source of leptin in the circulation. Feeding (12), cholecystokinin (12) or gastrin (16) greatly increase the concentration of leptin in the plasma and this effect is associated with a fall of leptin content in the gastric epithelium. Leptin from the stomach together with leptin from the fat tissue cooperate in the regulation of food intake. Exogenous and endogenous leptin (released by cholecystokinin, gastrin or meal) exerts a potent gastroprotective activity. Leptin inhibits the gastric ulcer development in various experimental models (14, 17) and it also promotes the healing of chronic gastric ulcer (18). This gastroprotective action of leptin seems to be dependent, at least in part, on vagal activity, intact capsaicin-sensitive sensory nerves and increased gastric blood flow (17). Also, the gastroprotective effects of leptin involve local generation of nitric oxide (NO) (17) and stimulation of transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) production (18).

Acute pancreatitis is an inflammatory disease associated with autodigestion of the pancreas due to intrapancreatic activation and release of digestive enzymes, but there is increasing evidence that pancreatic ischemia plays an important role in this disease. Pancreatic microvascular perfusion failure may be a primary reason of clinical (19-21) and experimental acute pancreatitis (22-23), but the early disturbance of pancreatic circulation is observed in acute pancreatitis caused by other, primary non-vascular factors (23-25). Reduction in the pancreatic circulation aggravates pancreatic damage in the course of acute pancreatitis (23, 26, 27). 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 (23).
Recent reports have shown that leptin administration inhibits the development of acute edematous caerulein-induced pancreatitis (14, 15). However, there is a lack of information about the influence of leptin administration on the development of acute pancreatitis caused by primary vascular factor. Therefore, the aim of present study was to assess the effect of leptin administration on the course of acute ischemic pancreatitis and on the post-inflammatory pancreatic regeneration.

**MATERIAL AND METHODS**

**Animals and treatment**

Studies were performed on male Wistar rats weighing 200-220 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. 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 for 30 min using microvascular clips. In sham operated-control animals longitudinal laparotomy and mobilization of pancreas without clamping any arteries was performed. After 30 min ischemia microvascular clips were removed for reperfusion and the abdominal cavity was closed. After operation, animals were treated with saline or leptin (mouse recombinant leptin, Sigma, Saint Louis, Missouri, USA) given at the dose 10 or 50 \( \mu \text{g/kg} \) (three s.c. injection daily, first injection 60 min after release of the microvascular clamp). Animals were anesthetized again and sacrificed at the time 1, 3, 5, 10 and 21 days after induction of acute pancreatitis by ischemia (n = 8-10 rats in each experimental group and at the each time of observation).

**Determination of pancreatic blood flow**

At the time of experiment cessation animals were anesthetized with ketamine 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 (28). The pancreatic blood flow was presented as percent change from control value obtained in sham-operated rats.

**Determination of serum amylase and lipase activity, and serum IL-\( \beta \) and IL-10 concentration**

Immediately after measurement of pancreatic blood flow the abdominal aorta was exposed and blood was taken for serum amylase, lipase, IL-1\( \beta \) and IL-10 determination. Serum amylase activity was determined by an enzymatic method (Amylase reagent set (kinetic), Alpha Diagnostic sp. z o.o., Warszawa, Poland). 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 Diagnosttic, Inc., Rochester, NY, USA). The values of serum amylase and lipase activity were expressed as units/liter. Serum IL-1\( \beta \) and IL-10 were measured in duplicate using the BioSource Cytoscreen rat IL-1\( \beta \) and IL-10 kit based on a solid phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, California, USA). Concentrations were expressed as pg/ml.
Determination of pancreatic DNA synthesis

After blood withdrawal the pancreas was carefully dissected from its attachment to the stomach, the duodenum and the spleen. Fat and peripancreatic tissue were trimmed away. The pancreas was rinsed with saline and blotted on paper. 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). The reaction was stopped with 0.4 M perchloric acid containing carrier thymidine (5 mM). Tissue samples were centrifuged and the precipitate washed twice in cold 0.2 M 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 [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

Samples of pancreatic tissue were excised, fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxylin and eosin. The slides were examined histologically by two experienced pathologists without knowledge of treatment given. The histological grading of edema was made using own 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.

Leukocytic infiltration was graded from 0 (absent) to 3 for maximal alterations (diffuse infiltration in the entire pancreatic gland).

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%.

Findings of acinar necrosis were graded: 0 = absent, 1 = less than 15% of cells involved, 2 = form 15 to 35% of cells involved, 3 = more than 35% of cells involved.

Grading of hemorrhages: 0 = absent, 1 = from 1 to 2 foci per slide, 2 = from 3 to 5 foci per slide, 3 = more than 5 foci per slide.

Statistical analysis

The differences between mean values from various groups of experiments were compared by variance analysis and 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

Morphological features

The pancreas of sham-operated animals showed macroscopically no tissue alteration and at light microscopic level minimal edema and no inflammation (Table 1). In contrast, pancreatic ischemia followed by reperfusion produced acute necrotizing pancreatitis in all tested rats.
One day after the initiation of reperfusion prominent edema was accompanied with abundant and diffuse inflammatory infiltration. There were more than 5 foci of hemorrhage in each slide. Vacuolization of acinar cells involved from less than 25 to 50% of cells. Acinar cells necrosis was seen in less than 15% of cells. There was also abundant inflammatory infiltrate in the peripancreatic fatty tissue and foci of enzymatic necrosis. After leptin administration, the histological signs of inflammation were less prominent. Pancreatic edema, hemorrhages, necrosis and leukocytic infiltrates were reduced. Treatment with leptin for one day was without effect on acinar cells vacuolization. Effects of leptin at the dose 50 µg/kg were more prominent than leptin at the dose 10 µg/kg.

After 3 days of reperfusion, in all cases treated with saline abundant inflammatory infiltrate was present. Edema was mostly interlobular and 1 to 2 foci of hemorrhage were seen. There were also small foci of acinar cells necrosis. Vacuolization involved less than 25% of acinar cells. After leptin administration at the dose 50 µg/kg, neither acinar cell necrosis nor hemorrhages were observed. The leukocytic infiltrate was scare perivascular or moderate perivascular and scare diffuse. Pancreatic edema was absent or slight interlobular. Vacuolization

Table 1. Morphological features of pancreatic damage in the course of ischemia/reperfusion-induced pancreatitis in the rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day).

<table>
<thead>
<tr>
<th>Time of reperfusion after ischemia</th>
<th>EDEMA (0-3)</th>
<th>HEMORRHAGES (0-3)</th>
<th>INFILTRATION (0-3)</th>
<th>VACUOLIZATION (0-3)</th>
<th>NECROSIS (0-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9 % NaCl</td>
<td>3</td>
<td>3</td>
<td>2/3</td>
<td>1/2</td>
<td>1</td>
</tr>
<tr>
<td>Leptin 10</td>
<td>2/3</td>
<td>3</td>
<td>2/3</td>
<td>1/2</td>
<td>0/1</td>
</tr>
<tr>
<td>Leptin 50</td>
<td>2</td>
<td>2/3</td>
<td>2</td>
<td>1/2</td>
<td>0/1</td>
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<tr>
<td>3 days</td>
<td></td>
<td></td>
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<tr>
<td>0.9 % NaCl</td>
<td>1/2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leptin 10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0/1</td>
<td>0/1</td>
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<tr>
<td>Leptin 50</td>
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<td>0</td>
<td>1/2</td>
<td>0/1</td>
<td>0</td>
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<td></td>
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</tr>
<tr>
<td>0.9 % NaCl</td>
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<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leptin 10</td>
<td>0</td>
<td>0/1</td>
<td>1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Leptin 50</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0/1</td>
<td>0/1</td>
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<tr>
<td>10 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9 % NaCl</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leptin 10</td>
<td>0/1</td>
<td>0/1</td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
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<td>21 days</td>
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<td></td>
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</tr>
<tr>
<td>0.9 % NaCl</td>
<td>0/1</td>
<td>0/1</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>Leptin 10</td>
<td>0</td>
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</table>

Numbers represent the predominant histological grading in each group.
involved from 0 to 25% of acinar cells. Beneficial effect of leptin administration at the dose 10 µg/kg was less pronounced.

After 5 days of reperfusion, in all cases moderate inflammatory infiltrate was present. Pancreatic tissue edema was slight and vacuolization affected less than 25% of cells. There were only small foci of acinar cells necrosis and from 1 to 2 foci of hemorrhage per slide. Leptin administration reduced all signs of pancreatic damage.

After 10 days of reperfusion, pancreatic edema and inflammatory infiltrate were moderate and vacuolization involved less than 25% of acinar cells. Additionally, there were also encountered small foci of hemorrhage and acinar necrosis. In group treated with leptin at the dose 10 µg/kg, only slight edema in some cases and scarce perivascular inflammatory infiltrate were seen. In animals treated with leptin at the dose 50 µg/kg, morphological features showed almost complete regeneration, only scarce perivascular inflammatory infiltrate was seen in some cases.

Twenty one days after the initiation of reperfusion, the pancreatic parenchyma appeared nearly normal except of scarce perivascular leukocytic infiltration and in some cases minimal edema and single foci of hemorrhage. In animals treated with leptin at the dose 10 µg/kg, almost full regeneration was observed. Only in two cases scarce perivascular leukocytic infiltration was found. In animals treated with leptin at the dose 50 µg/kg, normal pancreatic parenchyma was present in all cases tested.

**Pancreatic blood flow**

As shown in Fig. 1, one day after removal of microvascular clips, the pancreatic blood flow was reduced by 71%. From the 3rd day after the induction of ischemia, the pancreatic blood flow increased, reaching 95% of the control value 21 days after the induction of ischemia. The administration of leptin reduced a pancreatitis-induced fall of pancreatic blood flow. For leptin at the dose 10 µg/kg this effect was statistically significant 3 days after induction of pancreatic ischemia. Leptin at the dose 50 µg/kg caused a marked increase in pancreatic blood flow 1 and 3 days following reperfusion. Additionally, three days after induction of pancreatitis, the effect of leptin administration at the dose 50 µg/kg on the pancreatic blood flow was markedly larger than effect of leptin at the dose 10 µg/kg.

**Biochemical examination**

Pancreatic DNA synthesis in control rats reached 57.5 ± 1.1 dpm/µg DNA. Ischemia followed by reperfusion caused a decrease in pancreatic DNA synthesis (Fig. 2) and the lowest value of DNA synthesis (16.9 ± 0.6 dpm/µg DNA) was observed after 1 day of reperfusion. It was followed by a partial restoration of pancreatic DNA synthesis starting from the third day of reperfusion, but 21 days
Fig. 1. Pancreatic blood flow in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. \(^{a}P<0.05\) compared to control, \(^{b}P<0.05\) compared to saline treated rats at the same time of observation, \(^{c}P<0.05\) compared to rats treated with leptin at the dose 10 µg/kg at the same time of observation.

Fig. 2. Pancreatic DNA synthesis in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. \(^{a}P<0.05\) compared to control, \(^{b}P<0.05\) compared to saline treated rats at the same time of observation, \(^{c}P<0.05\) compared to rats treated with leptin at the dose 10 µg/kg at the same time of observation.
after the induction of ischemia, pancreatic DNA synthesis was still depressed. Leptin administration partly reversed pancreatitis-induced fall of pancreatic DNA synthesis. This effect was statistically significant after 1, 3 and 5 days of reperfusion for leptin at the dose 50 µg/kg. Leptin at the dose 10 g/kg significantly increased pancreatic DNA synthesis after 1 day of reperfusion. Statistically significant difference between effects of leptin at the dose 50 µg/kg and 10 µg/kg on pancreatic DNA synthesis was observed after 3 and 5 days of reperfusion.

Serum amylase (Fig. 3) and lipase (Fig. 4) activity in control rats reached 1226 ± 24 U/L and 52 ± 3 U/L, respectively. After the induction of acute pancreatitis by ischemia with reperfusion, serum amylase and lipase activity were elevated. Serum amylase reached the maximal value after 1 day of reperfusion (3167 ± 114 U/L). Serum lipase reached the maximal value after 3 days of reperfusion (209 ± 9 U/L). Ten days after induction of pancreatitis, serum amylase and lipase values were close to the control values. The leptin administration resulted in the decrease in serum amylase and lipase activity. Leptin at the dose 50 µg/kg significantly reduced serum amylase and lipase activity after 1 and 3 days of reperfusion. Leptin at the dose 10 µg/kg was less effective. This dose of leptin markedly reduced serum amylase and lipase activity only after 3 days of reperfusion.

In the control rats, serum IL-1β concentration was 73 ± 2 pg/ml (Fig. 5). Approximately a three-fold increase in serum IL-1β concentration levels was

![Graph showing serum amylase activity](image_url)

**Fig. 3.** Serum amylase in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. *P<0.05 compared to control, †P<0.05 compared to saline treated rats at the same time of observation.
Fig. 4. Serum lipase in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. *P<0.05 compared to control, \( ^{b}P<0.05 \) compared to saline treated rats at the same time of observation, \( ^{c}P<0.05 \) compared to rats treated with leptin at the dose 10 µg/kg at the same time of observation.

Fig. 5. Serum interleukin-1β in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. *P<0.05 compared to control, \( ^{b}P<0.05 \) compared to saline treated rats at the same time of observation, \( ^{c}P<0.05 \) compared to rats treated with leptin at the dose 10 µg/kg at the same time of observation.
observed one day after the acute pancreatitis induction. From the third day of reperfusion, serum IL-1β concentration tended to decrease but remained elevated above the control value till the 21st day of reperfusion. Following leptin administration, serum IL-1β levels were partly reduced. This effect was statistically significant 1 day after reperfusion for leptin at the dose 10 µg/kg and 1 and 3 days after reperfusion for leptin at the dose 50 µg/kg.

Ischemia/reperfusion-induced pancreatitis caused an increase in serum IL-10 concentration (Fig. 6). The maximal value of plasma IL-10 concentration was observed after one day of reperfusion (402 ± 14 pg/ml versus 48 ± 3 pg/ml) and the normalization of plasma IL-10 concentration was found after 21 days of reperfusion. Leptin administration at both doses was without effect on serum IL-10 concentration.

![Fig. 6. Serum interleukin-10 in the course of ischemia/reperfusion-induced pancreatitis in rats treated with saline (control) or leptin (10 or 50 µg/kg three times a day). Mean ± SEM. Number in circle indicates the number of rats used in each experimental group. *P<0.05 compared to control.](image)

**DISCUSSION**

Previous studies have shown the presence of leptin and leptin receptor within gastric mucosa (12, 30). Leptin participates in the maintenance of gastric mucosa integrity. Administration of leptin reduces gastric mucosa damage in various experimental models of gastric ulcer (14, 17) and promotes healing of chronic gastric ulceration (18). The presence of leptin receptor (31, 32) and expression of leptin mRNA (14) and leptin receptor mRNA has also been found in the pancreas, mainly in pancreatic islets (31, 32). Moreover, recent studies have shown that
administration of leptin inhibits development of acute edematous pancreatitis evoked by caerulein (14, 15).

Our present study demonstrated for the first time that administration of leptin reduces the severity of ischemia/reperfusion-induced pancreatitis and accelerates pancreatic repair. The beneficial effect of treatment with leptin was manifested by a reduction in serum amylase and lipase activity, a decrease in serum concentration of pro-inflammatory IL-1β, and an increase in pancreatic DNA synthesis. There was found close relationship between evoked by leptin a decrease in biochemical signs of pancreatitis and the improvement of pancreatic blood flow, as well as, a reduction in histological score of pancreatic damage. Morphological features have shown that leptin administration decreases pancreatic tissue edema, acinar cells necrosis and vacuolization of acinar cells. Also, treatment with leptin inhibits formation of foci of hemorrhage and reduces leukocyte infiltration.

Inflammatory infiltration plays an important role in development of pancreatic damage in the course of acute pancreatitis. Leukocytes adhere to the vascular endothelium of veins forming plagues and contribute to the injury by reducing blood flow via occlusion of microvessels (33). Moreover, leukocytes infiltrate pancreatic tissue and cause a release of pro-inflammatory cytokines such as IL-1β, interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) within the pancreas and systematically (34). IL-1β is a mediator in the disease and in the production of systemic acute phase responses (35). IL-1 plays a crucial role in the release of other members of the pro-inflammatory cytokine cascade (35). The study performed by Norman and co-workers (36) has 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 pancreatic protection after leptin administration. Treatment with leptin has reduced the leukocyte infiltration of pancreatic tissue and the production of IL-1β leading to the reduction of pancreatic damage. Similar effect of leptin administration has been also observed in other experimental model of acute pancreatitis. In acute edematous pancreatitis evoked by caerulein, treatment with leptin caused a reduction in leukocyte infiltration and a decrease in the release of pro-inflammatory cytokine: TNF-α (37).

In contrast to IL-1β, IL-10 has been found to be a major anti-inflammatory cytokine. It reduces activation of macrophages and inhibits the production of reactive oxygen species (38) and pro-inflammatory cytokines (39). The study performed by Van Laethem and co-workers (40) has shown that administration of IL-10 before and during induction of acute pancreatitis decreases the severity of pancreatitis, mainly by inhibiting the development of acinar cell necrosis. In our present study, the development of acute ischemic pancreatitis led to an increase in serum IL-10 concentration. Maximal serum concentration of IL-10 was observed after one day of reperfusion. Treatment with leptin has not affected
markedly serum IL-10 concentration in the course of acute pancreatitis, but this result seems to indicate some stimulatory effect of leptin on IL-10 release. Previous study (22) has shown that an increase in IL-10 level 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 in the course of acute pancreatitis. Moreover, our previous study (41, 42) with EGF administration in the course of acute ischemic pancreatitis has revealed that a decrease in plasma pro-inflammatory IL-1β concentration leads to secondary reduction in plasma anti-inflammatory IL-10. In our present study, leptin reduced serum IL-1β concentration, but it did not lead to a secondary decrease in serum IL-10 concentration. This observation suggests leptin ability to the maintenance of serum anti-inflammatory IL-10 on high level. It may be one of mechanisms responsible for beneficial effect of leptin administration on the course of ischemic pancreatitis.

Clinical and experimental studies have shown that pancreatic ischemia may initiate the acute pancreatitis and always aggravates the severity of pancreatic damage (19-22, 43). On the second hand, vasodilatation and the improvement of pancreatic blood flow has been found to reduce the development of acute pancreatitis (44, 45). In our present study, development of ischemic pancreatitis reduced the pancreatic blood flow. Treatment with leptin caused a partial reversion of pancreatitis-induced fall of pancreatic blood flow, suggesting that the improvement of pancreatic microcirculation contributes to the protective effect of leptin administration. Additional support for this hypothesis is an observation that a similar increase in pancreatic blood flow was observed after leptin administration in caerulein-induced pancreatitis (15).

Previous studies have shown that leptin stimulates a nitric oxide (NO) release from different tissues such as: fat tissue (46), vascular endothelium (47) pituitary gland (48) and also pancreatic acinar cells (15). Nitric oxide is a well known potent vasorelaxant factor. These data suggest that beneficial effect of leptin administration on pancreatic microcirculation is mediated by nitric oxide. The support for this hypothesis are previous finding that inhibition of NO synthase aggravates the pancreatic damage in the course of acute pancreatitis (49, 50).

In the pancreas, leptin mRNA and leptin receptor are mainly expressed in pancreatic islets (31, 32). Hormones of pancreatic islets affect pancreatic exocrine secretion. Somatostatin (51), pancreatic polypeptide (52) and glucagon (53) inhibit secretion of pancreatic enzymes, whereas insulin (54) increases pancreatic exocrine secretion. Administration of leptin inhibits insulin release (32) and reduces pancreatic exocrine secretion (55, 56). Acute pancreatitis is an inflammatory disease associated with autodigestion of the pancreas due to intrapancreatic activation and release of digestive enzymes (57). Inhibition of pancreatic enzyme secretion may reduce intrapancreatic enzyme activity and attenuate pancreatic damage in the course of acute pancreatitis.
In summary, our results provide the evidence that leptin reduces pancreatic damage in the course of ischemic pancreatitis and accelerates the pancreatic regeneration in the course of this disease. These data taken together with a results obtained with a caerulein-induced acute pancreatitis (14, 15) suggest that protective effect of leptin administration is independent of etiology of acute pancreatitis. The protective and healing effects of leptin appears to be dependent on the improvement of pancreatic blood flow, the increase in pancreatic cell growth, and the limitation of pro-inflammatory interleukin-1β release.

REFERENCES


