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European Journal of Pharmacology 430 (2001) 113–121

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Hepatocyte growth factor attenuates pancreatic damage in caerulein-induced pancreatitis in rats

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Received 3 August 2001; accepted 21 August 2001

Abstract

Hepatocyte growth factor (HGF) overexpression was reported in experimental and clinical acute pancreatitis. These observations prompted us to determine the effect of HGF administration on the development of caerulein-induced pancreatitis in rats. Acute pancreatitis was induced by s.c. infusion of caerulein (10 μg/kg/h) for 5 h. HGF was administrated twice (30 min before caerulein or saline infusion and 3 h later) at the doses: 0.4, 2, 10 or 50 μg/kg s.c. Immediately after cessation of caerulein or saline infusion, the pancreatic blood flow, plasma amylase and lipase activity, plasma cytokines concentration, cell proliferation, and morphological signs of pancreatitis were examined. Caerulein administration induced acute edematous pancreatitis manifested by 41% decrease in DNA synthesis, 53% inhibition of pancreatic blood flow, a significant increase in plasma amylase and lipase activity, plasma interleukin-1β and interleukin-6 concentration, as well as, the development of the histological signs of pancreatic damage (edema, leukocyte infiltration, and vacuolization). Administration of HGF without induction of pancreatitis increased plasma interleukin-10. Treatment with HGF, during induction of pancreatitis, increased plasma interleukin-10 and attenuated the pancreatic damage, what was manifested by histological improvement of pancreatic integrity, the partial reversion of the drop in DNA synthesis and pancreatic blood flow, and the reduction in pancreatitis evoked increase in plasma amylase, lipase, and interleukin-1β and interleukin-6 levels. HGF administrated at the dose 2 μg/kg exhibited a similar beneficial effect as administration of HGF at the doses 10 or 50 μg/kg. Treatment with HGF at the dose 0.4 μg/kg was less effective. We conclude that: (1) administration of HGF attenuates pancreatic damage in caerulein-induced pancreatitis; (2) this effect seems to be related to the increase in production of interleukin-10, the reduction in release of interleukin-1β and interleukin-6, and the improvement of pancreatic blood flow.

Keywords: Pancreatitis; HGF, hepatocyte growth factor; Interleukin-1β; Interleukin-10

1. Introduction

Hepatocyte growth factor (HGF), also known as scatter factor (Furlong et al., 1991) is a heterodimer molecule composed of a 69-kDa α subunit and a 34-kDa β subunit (Matsumoto and Nakamura, 1992). HGF is produced by nonparenchymal cells in the liver, kidney, or lung (Gherardi and Stocker, 1991). Parenchymal cells in these tissues express high levels of HGF receptor (Gherardi and Stocker, 1991). Several fibroblast cell lines, which do not express the HGF receptor, also produce HGF, suggesting that this factor acts as paracrine effector in mesenchymal–epithelial interactions (Gherardi and Stocker, 1991). HGF stimulates growth, motility, and morphogenesis of various types of cells, most predominantly of epithelial-origin cells (Matsumoto and Nakamura, 1992). HGF is the most potent mitogen for hepatocytes (Matsumoto and Nakamura, 1992), renal tubular epithelial cells (Matsumoto and Nakamura, 1992), epidermal keratocytes (Matsumoto et al., 1991), and endothelial cells (Bussolino et al., 1992). HGF mRNA and HGF activity increased markedly in the liver after various liver injuries and in the kidney following unilateral nephrectomy or acute renal injury (Nakamura, 1991), and

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01352-8

HGF is responsible for regeneration of these organs. It has been shown that serum HGF levels are increased in patients with various liver diseases and that serum HGF level may be useful in clinical assessment of patients with fulminant hepatic failure (Godha et al., 1988; Tsubouchi et al., 1991; Tomiya et al., 1992).

In experimental studies, HGF prevents the onset or progress of hepatic fibrosis/cirrhosis and is an effective drug for treatment of fatty liver (Matsumoto and Nakamura, 1997). HGF prevents the onset of acute and chronic renal failure, and acting as pulmotrophic factor, enhances lung regeneration, and suppresses the onset of lung fibrosis (Matsumoto and Nakamura, 1997). Moreover, this cytokine plays an important role in the development and maintenance of the nervous system (Sun et al., 1999; Miyazawa et al., 1998).

In the pancreas, HGF is considered as a potent mitogen for normal human pancreas cells (Vila et al., 1995) and pancreatic carcinoma cells (Kiehne et al., 1997). The increased levels of HGF have been found in patients with acute pancreatitis, and the serum HGF levels reflect the clinical severity of this disease (Ueda et al., 1996, 1997). HGF levels increase with complications such as organ failure, infected pancreatic necrosis, and sepsis, and decrease with successful treatment (Ueda et al., 1996). Also in experimental pancreatitis, the increase in plasma HGF level (Ueda et al., 2000) and tissue HGF overexpression was observed (Ueda et al., 2000; Menke et al., 1999). The degree of plasma HGF elevation was correlated with the severity of pancreatitis and the organ dysfunction, and administration of anti-HGF neutralizing antibodies caused the increase in dysfunction of organs and number of apoptotic cells (Ueda et al., 2000). These findings indicate that endogenous HGF is involved in the process of tissue repair in the course of acute pancreatitis and suggest that treatment with HGF may reduce pancreatic damage in this disease. The present study was undertaken to examine this hypothesis.

2. Materials and methods

2.1. Animals and treatment

Studies were performed on 188 male Wistar rats weighing 120–140 g. The animals were housed in cages with wire mesh bottoms, with normal room temperature, and a 12-h light–dark cycle. Drinking water and food were available ad libitum. The study was conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University.

Experiments were carried out in two separate series. The first series was performed with different doses of HGF to evaluate the effect of HGF administration on the development of pancreatitis. Pancreatitis was induced by caerulein infusion in rats kept in individual cages. Caerulein (Takus, Pharmacia & Upjohn, Erlangen, Germany) was diluted in saline and infused s.c. for 5 h at 10 μg/kg/h at a rate of 1.0 ml/h. The first series of experiments was carried out on the following experimental groups: (1) rats infused with 0.9% NaCl s.c. for 5 h to serve as control group; (2) rats infused with 0.9% NaCl s.c. for 5 h and treated twice with HGF (30 min before saline infusion and 3 h later) at the doses: 0.4, 2, 10, or 50 μg/kg s.c.; (3) rats with caerulein-induced pancreatitis; (4) rats with caerulein-induced pancreatitis and treated twice with HGF (30 min before caerulein infusion and 3 h later) at the doses: 0.4, 2, 10, or 50 μg/kg s.c. Animals from the first series of experiments were sacrificed after 5 h of infusion with saline or caerulein.

Human recombinant HGF purified from culture fluids from transformed Chinese hamster ovary (CHO) cells was obtained from Biomedical Research Center, Osaka University Medical School, Osaka, Japan.

The second series of experiment was performed to evaluate the effect of standard dose of HGF (2 μg/kg) on the time course of pancreatic damage development expressed as plasma amylase and lipase activity. Animals from this series were sacrificed at 1, 2, 3, 4, and 5 h after the beginning of saline or caerulein infusion.

2.2. Determination of pancreatic blood flow

Following the infusion of saline or caerulein, the animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened. The pancreata were exposed for the measurement of the pancreatic blood flow by laser Doppler flowmeter using Laserflo, model BPM 402 A Blood Perfusion monitor (Vasamedics, St. Paul, MN, USA), as described previously (Konturek et al., 1994). Pancreatic blood flow was measured in five different portions of the pancreas and the area of laser emission of the probe was about 1 mm^2, while the depth of measurement reached about 3 mm. It was recorded and presented as percentage change from control value obtained in rats infused with saline.

2.3. Determination of plasma amylase and lipase activity, and plasma cytokines concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of plasma amylase, lipase, interleukin-1β, interleukin-6, interleukin-10, and tumor necrosis factor-α (TNF-α). Plasma amylase activity was determined by an enzymatic method (Amylase reagent set [kinetic], Alpha Diagnostic, Warszawa, Poland). Plasma lipase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak, Rochester, NY, USA) using Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Rochester, NY, USA). The values of plasma amylase and lipase activity were

expressed as units per liter (U/l). Plasma interleukin-1β, interleukin-6, interleukin-10, and TNF-α were measured in duplicate using appropriate BioSource Cytoscreen rat kits based on a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (BioSource International, Camarillo, CA, USA). Concentration of interleukins and TNF-α was determined from a standard curve of recombinant interleukins or TNF-α, respectively. The values of plasma cytokines concentration were expressed as picograms per milliliter (pg/ml).

2.4. 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 the procedure of Giles and Myers (1965). 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).

2.5. 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 hematoxylin and eosin. The slides were examined histologically by two experienced pathologists without the knowledge of the treatment given. 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 (absent) to 3 (maximal diffuse infiltration in the entire pancreatic gland). Grading of vacuolization was based on the appropriate percentage of cell involved: 0 = absent, 1 = less than 25%, 2 = 25–50%, and 3 = more than 50%.

2.6. Statistical analysis

Comparison of the differences between the mean values of various groups of experiments 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 statistically significant. Results are expressed as means (±S.E.M.).

3. Results

3.1. The first series of experiments

3.1.1. Morphological features

Pancreata of saline-infused animals showed no tissue alteration macroscopically and at light microscopic level (Table 1). Also treatment with any dose of HGF in saline-infused animals did not affect pancreatic tissue in morphological examination. Infusion with caerulein caused acute edematous pancreatitis in all rats tested (Table 1). The pancreas was grossly swollen and enlarged with a visible collection of edematous fluid. At light microscopic level, interlobular and moderate intralobular edema was accompanied with abundant and diffuse inflammatory leukocyte infiltration. Vacuolization was observed in 25–50% of acinar cells. Treatment with HGF, at the doses 2, 10, or 50 μg/kg before and during caerulein infusion, caused similar beneficial effect on pancreatic tissue, reducing caerulein-evoked pancreatic damage. In most cases, pancreatic edema was limited to intralobular space, inflammatory leukocyte infiltration was scarce, predominantly perivascular. Vacuolization was similar as in caerulein alone-treated rats and included 25–50% of acinar cells. Administration of HGF at the lowest dose (0.4 μg/kg) was without

Table 1
Effect of hepatocyte growth factor (HGF) on histological signs of pancreatic damage in caerulein-induced pancreatitis

	Edema (0–3)	Infiltration (0–3)	Vacuolization (0–3)
Control	0	0	0
Caerulein	2	2/3	2
HGF 0.4 μg	0	0	0
HGF 2.0 μg	0	0	0
HGF 10.0 μg	0	0	0
HGF 50.0 μg	0	0	0
HGF 0.4 μg + caerulein	2	2	2
HGF 2.0 μg + caerulein	1/2	1/2	2
HGF 10.0 μg + caerulein	1/2	1/2	2
HGF 50.0 μg + caerulein	1/2	1/2	2

Numbers represent the predominant histological grading in each group.

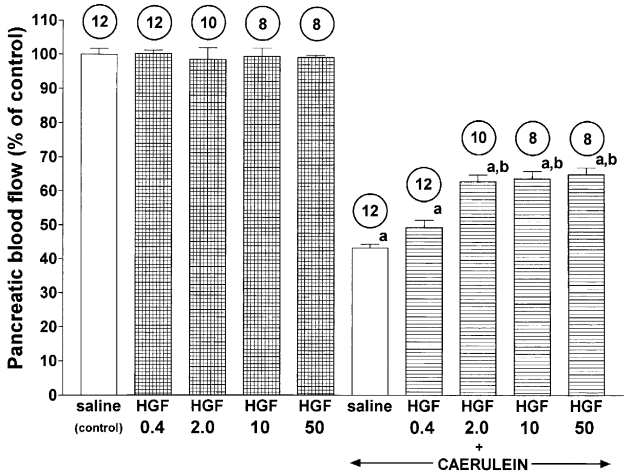

Fig. 1. Pancreatic blood flow in rats without or with caerulein-induced pancreatitis (caerulein 10 μg/kg/h for 5 h), treated with saline (control), or HGF (given twice at the doses 0.4, 2.0, 10, or 50 μg/kg). Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. $^{a}P < 0.05$ compared with control, $^{b}P < 0.05$ compared with caerulein given alone.

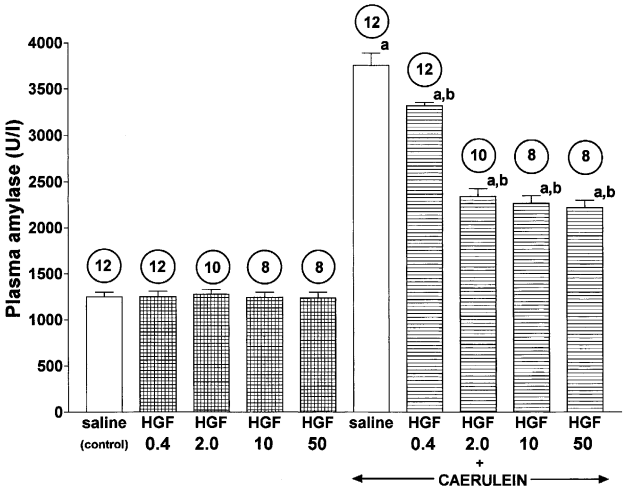

Fig. 3. Plasma amylase activity in groups of rats as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. $^{a}P < 0.05$ compared with control, $^{b}P < 0.05$ compared with caerulein given alone.

marked effect on edema or vacuolization evoked by caerulein infusion. Only leukocyte infiltration was less pronounced.

3.1.2. Pancreatic blood flow

Treatment with any dose of HGF did not affect pancreatic blood flow in animals infused with saline (Fig. 1). Infusion of caerulein for 5 h reduced pancreatic blood flow by 57% when compared to saline-infused control group. In rats with caerulein infusion, administration of HGF at the doses 2, 10, and 50 μg/kg caused significant and similar reversion of caerulein-induced fall of pancreatic blood flow. Effect of HGF, given at the dose 0.4 μg/kg, on pancreatic blood in animals with caerulein infusion was weak and statistically insignificant.

3.1.3. Biochemical parameters

In saline-infused control rats, pancreatic DNA synthesis reached 58.8 ± 1.5 dpm/μg DNA (Fig. 2). Treatment with HGF at any dose did not affect pancreatic DNA synthesis in animals infused with saline. In animals with caerulein-induced pancreatitis, pancreatic DNA synthesis was reduced reaching 34.8 ± 1.1 dpm/μg DNA. In rats with caerulein infusion, administration of HGF at the doses 2, 10, and 50 μg/kg significantly attenuated the reduction in DNA synthesis. HGF given at the dose 0.4 μg/kg was without effect on caerulein-evoked fall in pancreatic DNA synthesis.

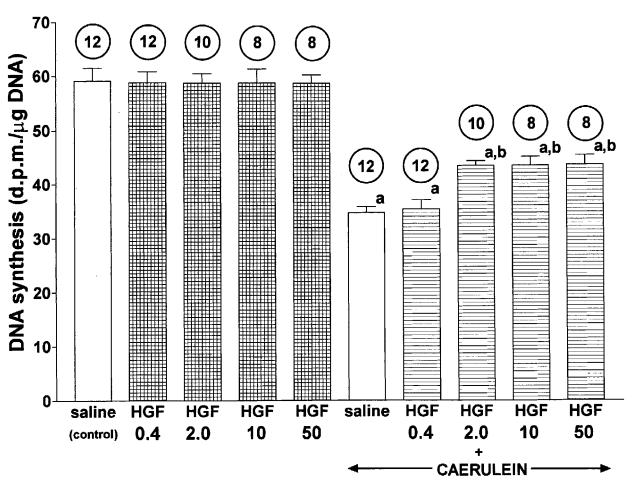

Fig. 2. Pancreatic DNA synthesis in rats in groups as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. $^{a}P < 0.05$ compared with control, $^{b}P < 0.05$ compared with caerulein given alone.

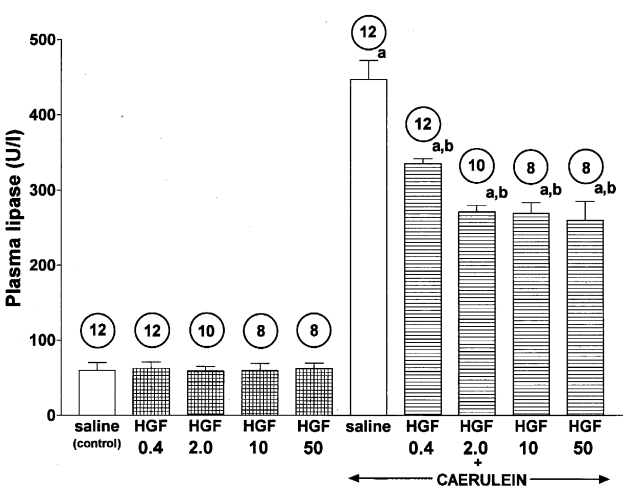

Fig. 4. Plasma lipase activity in groups of rats as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. $^{a}P < 0.05$ compared with control, $^{b}P < 0.05$ compared with caerulein given alone.

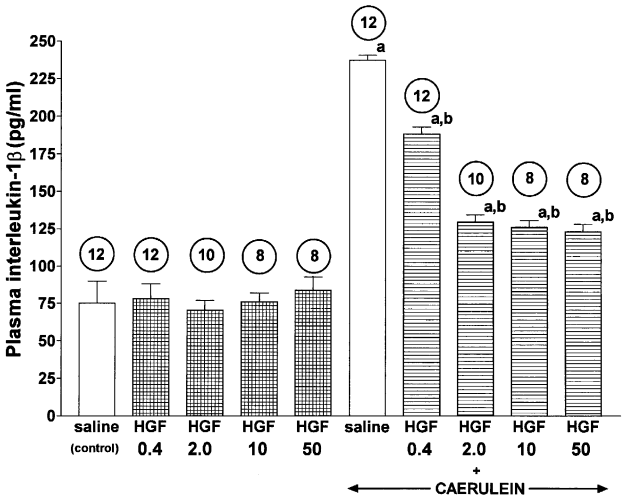

Fig. 5. Plasma interleukin-1β concentration in groups of rats as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein given alone.

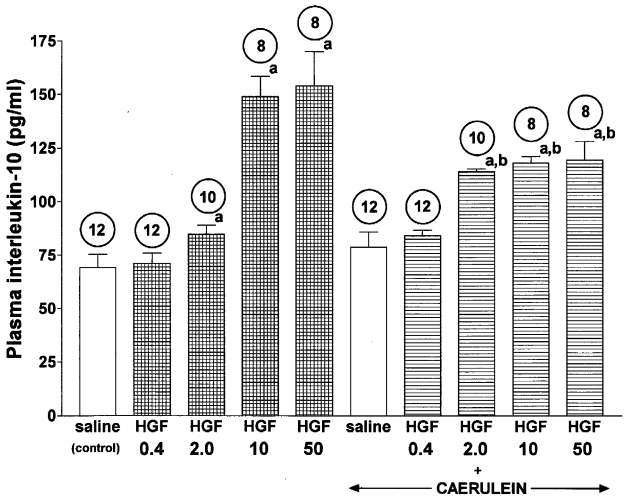

Fig. 7. Plasma interleukin-10 concentration in groups of rats as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein given alone.

Plasma amylase (Fig. 3) and lipase (Fig. 4) activity in control saline-infused rats reached 1193 ± 76 and 52.0 ± 14.4 U/l, respectively. Administration of HGF did not affect plasma amylase and lipase activity in rats infused with saline. Infusion with caerulein for 5 h caused acute pancreatitis and increased plasma amylase and lipase activity by 214% and 757%, respectively, when compared with saline-infused control rats. Treatment with HGF, at the doses 2, 10, and 50 μg/kg, markedly reduced caerulein-evoked increase in plasma amylase and lipase activity by about 40%. Effect of HGF administrated at the dose 0.4 μg/kg on plasma amylase and lipase activity was less pronounced but still statistically significant.

In control rats infused with saline, plasma interleukin-1β concentration was 74.5 ± 11.9 pg/ml (Fig. 5). Treatment with HGF was without effect on plasma interleukin-1β level in saline-infused rats. Caerulein caused threefold increase in plasma interleukin-1β concentration and this increase was diminished by HGF. HGF administrated at the doses 2, 10, or 50 μg/kg exhibited similar and strong effect on caerulein-evoked increase in plasma interleukin-1β concentration. Effect of HGF given at the dose 0.4 μg/kg on plasma interleukin-1β concentration was weaker but still marked in animals with caerulein-induced pancreatitis.

Plasma interleukin-6 in animals treated with saline or HGF alone was not detected (Fig. 6). The ELISA detection limit of interleukin-6 was 8 pg/ml. In animals infused with caerulein for 5 h, plasma interleukin-6 reached a

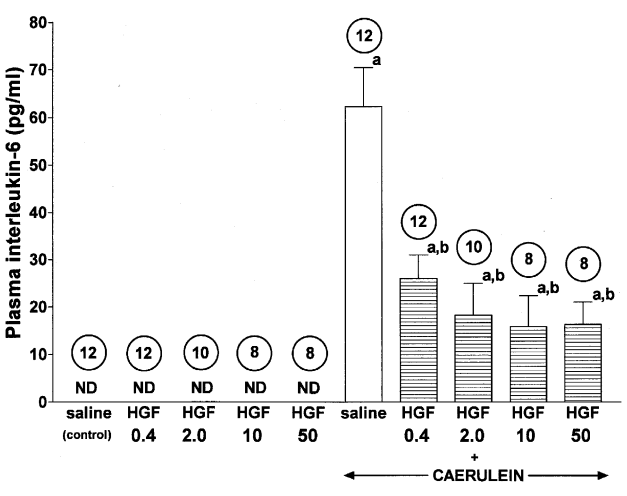

Fig. 6. Plasma interleukin-6 concentration in groups of rats as in Fig. 1. Mean ± S.E.M. Number in circle indicates the number of rats used in each experimental group. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein given alone. ND = not detected.

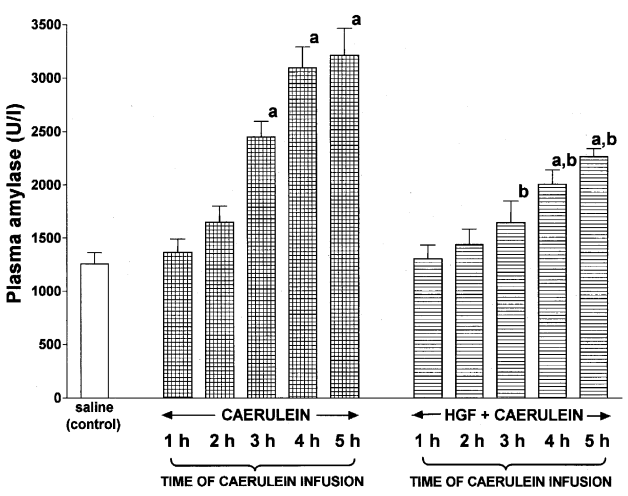

Fig. 8. Time course of plasma amylase activity during infusion of caerulein without or with HGF administration (2 μg/kg). Mean ± S.E.M. of eight observations in each experimental group. [a]$P < 0.05$ compared with control, [b]$P < 0.05$ compared with caerulein given alone at the same time of observation.

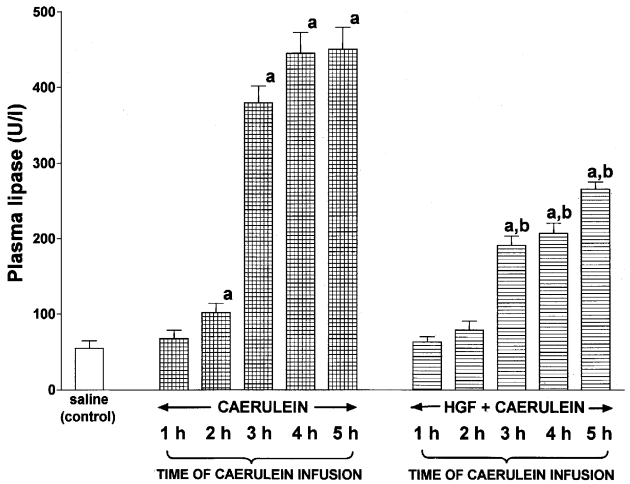

Fig. 9. The time course of plasma lipase activity during infusion of caerulein without or with HGF administration (2 μg/kg). Mean ± S.E.M. of eight observations in each experimental group. [a] $P < 0.05$ compared with control, [b] $P < 0.05$ compared with caerulein given alone at the same time of observation.

value of 62.3 ± 8.2 pg/ml. Treatment with HGF at the doses 0.4, 2, 10, or 50 μg/kg significantly attenuated caerulein-induced increase in plasma interleukin-6 by 58%, 71%, 74%, or 74%, respectively.

In control rats, plasma interleukin-10 reached a value of 68.7 ± 5.9 pg/ml (Fig. 7). Infusion of caerulein for 5 h did not significantly affect the plasma interleukin-10 concentration. Treatment with HGF increased plasma concentration of interleukin-10 in rats infused with saline, as well as, with caerulein. In rats without caerulein-induced pancreatitis, this effect was more pronounced.

Plasma levels of TNF-α in all experimental animals were under the detection limit, which was 4 pg/ml.

3.2. The second series of experiments

Infusion of caerulein resulted in progressive increase in plasma amylase activity (Fig. 8) and plasma amylase level was statistically higher than in control group starting from the third hour of caerulein administration. Treatment with HGF at the dose 2 μg/kg reduced caerulein-evoked increase in plasma amylase activity and this effect became significant from the third hour of caerulein infusion.

Plasma lipase activity rose faster than plasma amylase activity reaching the marked increase after the second hour of caerulein infusion (Fig. 9). Administration of HGF at the dose 2 μg/kg caused the significant attenuation of this caerulein-induced effect starting from the third hour of observation.

4. Discussion

In our present study, we have demonstrated that administration of exogenous HGF before and during induction of acute pancreatitis attenuates the severity of pancreatic damage in caerulein-induced pancreatitis. The beneficial effect of treatment with HGF was manifested by reduction in plasma amylase and lipase activity. This effect was significant starting from the third hour of caerulein infusion. Also plasma interleukin-1β and interleukin-6 levels were reduced. There was a close relationship between the beneficial effect of HGF administration on pancreatic histology, the reduction in biochemical signs of pancreatitis and the improvement of pancreatic blood flow. Morphological features have shown a decrease in tissue edema and leukocyte infiltration.

Previous studies have indicated relationship between inflammation and HGF (Miyazawa et al., 1994). HGF is secreted as a single-chain inactive precursor (pro-HGF) and it is converted to biologically active heterodimer by HGF-converting enzyme (Mizuno et al., 1994). This process is extensively induced in injured tissues (Miyazawa et al., 1994) and HGF-converting enzyme is an acute-phase protein (Okajima et al., 1997). In patients with acute pancreatitis, serum HGF levels are elevated and significantly higher in severe cases with organ failure and in nonsurvivors (Ueda et al., 1996). It has been found that serum HGF levels reflect clinical course of acute pancreatitis and may be a useful clinical parameter for determining the patient prognosis (Ueda et al., 1997). Serum HGF is as useful as C-reactive protein (CRP) and more useful than IL-6 for detecting severe pancreatitis and for predicting hepatic dysfunction. Moreover, it was also shown that serum HGF is more useful than CRP or IL-6 for predicting renal and respiratory dysfunction (Ueda et al., 1997). Similar correlation between plasma HGF level and severity of acute pancreatitis has been observed in animal models of acute pancreatitis (Ueda et al., 2000).

These data and our present findings taken together suggest that HGF is not a factor of acute inflammation but a result of acute pancreatitis. Also, it indicates that the elevation of HGF level during inflammation plays a role in self-defense mechanism reducing tissue damage. This hypothesis is in agreement with data obtained by Ueda et al. (2000). They have shown that neutralization of HGF by anti-HGF antibodies increases the number of apoptotic cells in the course of acute pancreatitis (Ueda et al., 2000).

HGF belongs to growth factors family. On the cellular level of target cells, almost all growth factor receptors belong to the class of transmembrane receptors with intrinsic tyrosine kinase activity. The intracellular signal transduction mechanism by tyrosine kinase receptors includes multiple signaling cascade (Kiehne et al., 2001). Growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or platelet-derived growth factor (PDGF) are active during organ development and are involved in the protection against mucosal damage and tissue repair in the stomach, duodenum, or colon (Szabo and Vincze, 2000; Konturek et al., 1988; Kusstatscher et al., 1995; Piazuelo et al., 2000). In the pancreas, protective

and therapeutic effect in the course of acute pancreatitis was found after administration of EGF (Warzecha et al., 1999; Dembiński et al., 2000) and bFGF (Hosokawa et al., 2000). A beneficial effect of treatment with EGF on the pancreas was shown to be dependent, at least in part, on the improvement of pancreatic blood flow, the increase in pancreatic cell proliferation, and limitation of interleukin-1β release (Warzecha et al., 1999; Dembiński et al., 2000). The similar circulatory, trophic, and anti-inflammatory effect in the course of acute pancreatitis has been observed in our present study after administration of HGF.

In acute pancreatitis, leukocytes adhere to the vascular endothelium reducing pancreatic blood flow (Kusterer et al., 1993), infiltrate pancreatic tissue, and produce pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNF-α within the pancreas (Norman et al., 1994). Interleukin-1β is a well-known mediator of acute inflammation and plays a crucial role in the induction of the release of other members of pro-inflammatory cytokine cascade (Kingsnorth, 1997). On the other hand, interleukin-1 blockade by use of naturally occurring receptor antagonist almost completely attenuates the rise in serum interleukin-6 and TNF-α level and decreases the severity of experimental acute pancreatitis (Norman et al., 1995). These data are in agreement with our present observations and partly elucidate the mechanism of pancreatic protection after HGF administration. Treatment with HGF has reduced the leukocyte infiltration of pancreatic tissue and the production of interleukin-1β and interleukin-6 leading to inhibition of inflammatory process. In contrast to data obtained by Norman et al. (1995), TNF-α has not been detected in any group of animals with acute pancreatitis in our present study. This discrepancy is probably dependent on different animal models used in both studies. Administration of caerulein to mice results in induction of severe necrotizing pancreatitis as in Norman et al.'s study, whereas administration of caerulein to rats leads only to induction of mild edematous pancreatitis. The similar lack of TNF-α detection in caerulein-induced pancreatitis in rats was observed by Sameshima et al. (1993). In their study, the serum TNF-α activity was undetectable in caerulein-induced pancreatitis without addition of lipopolysaccharide that originated from a Gram-negative bacterial wall. They suggest that increased TNF-α production occurs in pancreatitis in the presence of intra-abdominal sepsis, and may contribute to the development of severe acute pancreatitis.

The very important finding of the present study is the observation that treatment with HGF increases plasma interleukin-10 concentration. In contrast to interleukin-1β, interleukin-10 has been found to be a major anti-inflammatory cytokine. Interleukin-10 reduces the activation of macrophages (Moore et al., 1993) and inhibits the production of pro-inflammatory cytokines (De Waal et al., 1991) and reactive oxygen species (Moore et al., 1993). In the pancreas, administration of interleukin-10, before and after induction of acute pancreatitis, attenuates the severity of inflammation (Van Laethem et al., 1995; Rongione et al., 1997). Interleukin-10 is produced relatively late following activation of T cells or monocytes/macrophages, compared to other cytokines (Moore et al., 1993). The relationship between plasma interleukin-10 and interleukin-1β in the course of acute pancreatitis has been found in our previous study (Dembiński et al., 2001). We have observed the increase in plasma interleukin-10 which was 24 h preceded by the increase in plasma interleukin-1β. This shift in the time between an increase in plasma interleukin-1β and interleukin-10 level has suggested that an increase in interleukin-10 is a consequence of an increase in interleukin-1β and interleukin-10 plays a role in self-defense mechanism limiting the intensity of the inflammatory process. In our present study, the increase in plasma interleukin-1 concentration was observed immediately after 5 h of caerulein infusion as a result of tissue damage and leukocyte activation. In contrast to interleukin-1β, plasma interleukin-10 in caerulein-treated rats was still low because of a short time of observation. On the second hand, administration of HGF has caused an increase in interleukin-10 even when HGF was given alone without induction of acute pancreatitis. This observation has suggested that HGF-induced release of interleukin-10 acting on early phase of inflammation causes the strong anti-inflammatory effect and reduction in development of acute pancreatitis.

Clinical and experimental studies have shown that pancreatic ischemia may initiate the acute pancreatitis and always aggravates pancreatic damage (Gullo et al., 1996; Lonardo et al., 1999; Menger and Vollmar, 1999; Klar et al., 1990). The severity of such experimental pancreatitis is closely correlated with tissue ischemia. The moderate and severe pancreatitis was found to be accompanied by progressive decrease in pancreatic blood perfusion (Knoefel et al., 1994). The mild edematous pancreatitis, such as that induced in this study by caerulein infusion, was found to be accompanied by initial hyperemia (Knoefel et al., 1994) but this was followed by severe reduction in pancreatic circulation (Furukawa et al., 1993; Konturek et al., 1992). Additional reduction in the pancreatic circulation by the exposure of animals with caerulein-induced pancreatitis to stress led to augmentation of edematous pancreatitis and hemorrhagic damage of pancreatic tissue (Furukawa et al., 1993). On the other hand, vasodilatation and the improvement of pancreatic blood flow has been found to reduce the development of acute pancreatitis (Warzecha et al., 1997). In our present study, pancreatic overstimulation with caerulein induced acute edematous pancreatitis and reduced the pancreatic blood flow. Pretreatment with an effective dose of HGF caused a partial reversion of caerulein-induced fall of pancreatic blood flow, suggesting that the improvement of pancreatic microcirculation might contribute to the protective effect of HGF administration. The mechanism of vascular effects of HGF administration is unclear. HGF given alone without caerulein infusion has not affected pancreatic blood flow. This observation indi-

cates that HGF acts indirectly on pancreatic blood flow, probably by the limitation of caerulein-induced pancreatic edema and reduction of leukocyte activation.

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