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Inhibition of cyclooxygenase-2 reduces the protective effect of hepatocyte growth factor in experimental pancreatitis

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Abstract

Hepatocyte growth factor (HGF) overexpression is observed in experimental and clinical acute pancreatitis. Moreover, previous studies have shown that administration of HGF reduces pancreatic damage in experimental pancreatitis. The aim of our studies was to determine the role of cyclooxygenase-1 and cyclooxygenase-2 in the protective effect of HGF administration against caerulein-induced pancreatitis. Acute pancreatitis was induced in rats by infusion of caerulein. HGF was administered twice at the dose 10 μg/kg s.c. The activity of cyclooxygenase-1 and cyclooxygenase-2 was inhibited by resveratrol and rofecoxib, respectively (10 mg/kg). Immediately after cessation of caerulein or saline infusion, pancreatic blood flow, pancreatic cell proliferation, pancreatic prostaglandin E_2 generation, plasma lipase activity, plasma interleukin-1β and interleukin-10 concentration were measured and morphological signs of pancreatitis were examined. Expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA transcripts was determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Cyclooxygenase protein production was analyzed by Western blot. Administration of HGF or caerulein alone, or their combination, was without effect on cyclooxygenase-1 mRNA expression in pancreatic tissue. Expression of cyclooxygenase-2 mRNA was increased by HGF and caerulein. The maximal increase in cyclooxygenase-2 mRNA expression was observed when HGF administration was combined with caerulein infusion. A similar effect was observed when we studied the influence of HGF and caerulein on pancreatic cyclooxygenase-2 production, as determined by Western blot. Administration of HGF without induction of acute pancreatitis increased pancreatic prostaglandin E_2 generation and plasma interleukin-10, and this effect was abolished by the cyclooxygenase-2 inhibitor, rofecoxib. Treatment with HGF, during the development of pancreatitis, increased the plasma interleukin-10 concentration and attenuated pancreatic damage, as evidenced by: (a) histological improvement of pancreatic integrity; (b) the partial reversal of the decrease in DNA synthesis and pancreatic blood flow; (c) the reduction in pancreatitis-evoked increase in plasma lipase and interleukin-1β. Administration of resveratrol and rofecoxib alone was without effect on the development of pancreatitis. Combination of rofecoxib with HGF reduced the HGF-evoked increase in plasma interleukin-10 concentration and pancreatic prostaglandin E_2 generation, and abolished the protective effect of HGF against pancreatic damage in pancreatitis. Resveratrol did not affect the protective effect of HGF. We conclude that: (1) HGF induces cyclooxygenase-2 but not cyclooxygenase-1 expression; (2) inhibition of cyclooxygenase-2 in HGF-treated rats decreases the release of anti-inflammatory interleukin-10, increases the production of pro-inflammatory interleukin-1β and reduces pancreatic blood flow; (3) cyclooxygenase-2 activity is necessary for the protective effect of HGF in acute pancreatitis.

Keywords: Pancreatitis; HGF (hepatocyte growth factor); Cyclooxygenase-2; Interleukin-1β; Interleukin-10

1. Introduction

Hepatocyte growth factor (HGF), also known as scatter factor (Furlong et al., 1991), is secreted by cells of mesodermal origin and binds to the c-Met/HGF receptor on neighboring epithelial cells, acting as a paracrine effector in mesenchymal–epithelial interactions (Gherardi and Stocker, 1991). HGF plays an important role in organogenesis. This peptide is involved, among others, in the formation of liver, lung, muscle, teeth, mammary gland and neural tissue (Schmidt et al., 1995; Sun et al., 1999; Funakoshi and Nakamura, 2003). HGF is the most potent mitogen for hepatocytes (Matsumoto and Nakamura, 1992), renal tubu-

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lar epithelial cells (Matsumoto and Nakamura, 1992), epidermal keratocytes (Matsumoto et al., 1991) and endothelial cells (Bussolino et al., 1992). HGF is implicated in the regeneration and protection of various organs, including the liver, lung, stomach, heart, kidney and the brain (Nakamura, 1991; Matsumoto and Nakamura, 1997; Brzozowski et al., 2001; Miyazawa et al., 1998).

HGF is considered a potent mitogen for normal human pancreas cells (Vila et al., 1995) and pancreatic carcinoma cells (Kiehne et al., 1997). Increased levels of HGF have been found in patients with acute pancreatitis, and the increase in serum HGF level reflects the clinical severity of the pancreatitis and organ dysfunction (Ueda et al., 1996; Ueda et al., 1997). Also in experimental pancreatitis, an increase in plasma HGF level (Ueda et al., 2000) and tissue HGF overexpression are observed (Ueda et al., 2000; Menke et al., 1999). Administration of antibodies that neutralize HGF aggravates the organ dysfunction and increases apoptosis in the course of acute pancreatitis (Ueda et al., 2000). Administration of recombinant HGF attenuates the pancreatic damage occurring in the course of experimental pancreatitis and this effect seems to be related to the increase in the production of interleukin-10, to a decrease in the release of interleukin-1β and to an improvement in pancreatic blood flow (Warzecha et al., 2001b).

Cyclooxygenase, the key enzyme of prostaglandin synthesis, exists in two isoforms, cyclooxygenase-1 and cyclooxygenase-2 (Smith et al., 1996). Cyclooxygenase-1 is constitutively expressed in most tissues and has been suggested to mediate the synthesis of prostaglandins required for physiological functions and the maintenance of organ integrity. Cyclooxygenase-2 is undetected in most tissues under normal conditions, but is highly inducible by cytokines, mitogens and endotoxins, and is responsible for the increased production of prostaglandins during inflammation (Masferrer et al., 1994; Smith et al., 1996). Early studies have shown that inhibition of cyclooxygenases activity by nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) leads to gastric ulcers and delays healing of the gastric mucosa (Konturek et al., 1981; Wang et al., 1989). The concept was developed that the maintenance of mucosal integrity in the gastrointestinal tract depends exclusively on cyclooxygenase-1 activity without a contribution of cyclooxygenase-2, and that the ulcerogenic effects of NSAIDs are an effect of cyclooxygenase-1 inhibition (Peskar, 2001). Recent studies, however, indicate that both cyclooxygenases contribute to gastric mucosal defence. Overexpression of cyclooxygenase-2 has been demonstrated at the ulcer edge during the healing of experimental gastric ulcers, and inhibition of cyclooxygenase-2 activity by selective NSAID delays ulcer healing (Mizuno et al., 1997).

In the stomach, there is also evidence that growth factors such as HGF or gastrin can induce prostaglandin synthesis through the up-regulation of cyclooxygenase-2 (Jones et al., 1999; Brzozowski et al., 2000, 2001; Komori et al., 2002), and inhibition of cyclooxygenase-2 activity abolishes the beneficial effect of HGF on gastric ulcer healing (Brzozowski et al., 2001). The effect of HGF on cyclooxygenase expression in the pancreas has not been studied. The aim of present study was to determine whether HGF or induction of acute pancreatitis increases the expression of cyclooxygenases mRNA and the production of cyclooxygenase-2 protein in the pancreas. We also attempted to explore the role of cyclooxygenase-1 and cyclooxygenase-2 activity in the protective effect of HGF on the pancreas during the development of acute pancreatitis.

2. Materials and methods

2.1. 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-h light–dark cycle.

The following experimental groups were used (10 animals in each group): (1) animals infused with 0.9% NaCl s.c. for 5 h (control); (2) animals infused with 0.9% NaCl s.c. for 5 h and treated twice with HGF (the first injection 30 min prior to saline infusion and the second injection 3 h later, 10 μg/kg s.c. per injection); (3) animals infused with 0.9% NaCl s.c. for 5 h and treated with the cyclooxygenase-1 inhibitor, resveratrol (Cayman Chemicals, Ann Arbor, MI, USA, 10 mg/kg i.g. 1 h before saline infusion); (4) animals infused with 0.9% NaCl s.c. for 5 h and treated with the cyclooxygenase-2 inhibitor, rofecoxib (Vioxx, Merc Sharpk and Dohme Idea, Glattbrugg, Switzerland, 10 mg/kg i.g. 1 h before saline infusion); (5) animals with caerulein-induced pancreatitis; (6) animals with caerulein-induced pancreatitis and treated twice with HGF (the first injection 30 min prior to caerulein infusion and the second injection 3 h later, 10 μg/kg s.c. per injection); (7) animals with caerulein-induced pancreatitis and treated with resveratrol (10 mg/kg i.g. 1 h before caerulein infusion); (8) animals with caerulein-induced pancreatitis and treated with rofecoxib (10 mg/kg i.g. 1 h before caerulein infusion); (9) animals with caerulein-induced pancreatitis and treated with resveratrol and HGF at the time and doses as above; (10) animals with caerulein-induced pancreatitis and treated with rofecoxib and HGF at the time and doses as above.

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.

Acute pancreatitis was induced by caerulein infusion in rats kept in individual cages. Caerulein (Takus, Pharmacia and Upjohn, Erlangen, Germany) was diluted in saline and infused s.c. for 5 h at 10 μg/kg/h. Animals were killed after 5 h of saline or caerulein infusion.

2.2. Determination of pancreatic blood flow

After 5 h saline or caerulein infusion, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened. The pancreas was exposed for the measurement of pancreatic blood flow by laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed, Järfälla, Sweden), as described previously (Konturek et al., 1994). Pancreatic blood flow was measured in five different portions of the pancreas. The area of laser emission of the probe was about 1 mm^2, while the depth of measurement reached about 3 mm. Data are presented as percent change from control value obtained in rats infused with saline.

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

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of plasma lipase, interleukin-1β and interleukin-10. 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 and Johnson Clinical Diagnostic, Rochester, NY, USA). Plasma lipase activity is expressed as units/liter (U/l). Plasma interleukin-1β and interleukin-10 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). The concentration of interleukins was determined from standard curves for recombinant interleukin-1β or interleukin-10, respectively. Plasma interleukin concentration is expressed as pg/ml.

2.4. Reverse transcriptase-polymerase chain reaction for detection of mRNA for cyclooxygenase-1, cyclooxygenase-2 and β-actin

After the collection of blood samples, the pancreas was dissected out and divided into samples for analysis of pancreatic mRNA, pancreatic protein, measurement of pancreatic DNA synthesis and histological examination. Samples of pancreatic tissue for transcriptase-polymerase chain reaction (RT-PCR) were snap-frozen in liquid nitrogen, and then stored at −80 °C until the time of RNA extraction. Total RNA was extracted from samples by the method of Chomczynski and Sacchi (1987), using extraction kit from Stratagene (Heidelberg, Germany). Following precipitation, RNA was resuspended in RNAse-free water and its concentration was estimated by absorbance at 260 nm wavelength. RNA samples were stored at −80 °C until analysis.

Single-stranded cDNA was generated from 5 μg of total cellular RNA using StrataScript reverse transcriptase (Stratagene) and oligo-(dT)-primers (Stratagene). Briefly, 5 μg of total RNA was uncoiled by heating (65 °C for 5 min) and then reverse transcribed into complementary DNA (cDNA) in a 50-μl reaction mixture that contained 50U Moloney murine leukemia virus reverse transcriptase (MMLV-RT), 0.3 μg oligo-(dT)-primer, 1 μl RNAse Block Ribonuclease Inhibitor (40 U/μl), 2 μl of a 100 mM mixture of deoxyadenosine triphosphate (dATP), deoxyribothymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 5 μl 10× RT buffer (10 mM Tris–HCl, pH = 8.3, 50 mM KCl, 5 mM $MgCl_2$). The resultant cDNA (2 μl) was amplified in a 50-μl reaction volume containing 2 U Taq polymerase, 200 μM (each) dNTP (Pharmacia, Germany), 1.5 mM $MgCl_2$, 5 μl 10× polymerase chain reaction buffer (50 μM KCl, 10 μM Tris–HCl, pH = 8.3) and specific primers used at a final concentration of 0.5 μM. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT). The cyclooxygenase-1 primer sequences were: sense, 5′-AGC CCC TCA TTC ACC CAT TT-3′, and antisense, 3′-CAC GGA CGC CTG TTC TAC GG-5′; the expected length of the product was 561 bp. The cyclooxygenase-2 primer sequences were: sense, 5′-ACA ACA TTC CCT TCC TTC-3′, and antisense, 3′-CCT TAT TTC CTT TCA CAC C-5′; the expected length of the product was 201 bp. The β-actin primer sequences were: sense, 5′-TTG TAA CCA ACT GGG ACG ATA TGG-3′, and antisense, 3′-GAT CTT GAT CTT CAT GGT GCT AGG-5′; the expected length of the product was 764 bp. The nucleotide sequences of the primers for cyclooxygenase-1, cyclooxygenase-2 and β-actin were based on the sequences of the published cDNAs (Xie et al., 1991; Kennedy et al., 1993; O'Banion et al., 1992). The primers were synthesized by GIBCO BRL/Life Technologies (Eggenstein, Germany). Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted product was confirmed by using a 100-bp ladder (Takara, Shiga, Japan) as a standard size marker. Expression of the product was quantified using a video image system (Kodak, USA). The polymerase chain reaction product signal was standardized against the β-actin signal for each sample and expressed as cyclooxygenase-1 and cyclooxygenase-2/β-actin ratio.

2.5. Protein extraction and analysis of cyclooxygenase-2 expression in pancreatic tissue by Western blot

Shock-frozen tissue from rat pancreas was homogenized in lysis buffer (100 mM Tris–HCl, pH 7.4, 15% glycerol, 2 mM EDTA, 2% sodium dodecyl sulfate (SDS), 100 mM D,L-dithiothreitol) by the addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mM phenylmethylsulfonyl fluoride. Insoluble material was removed by centrifugation at 12 000 × *g* for 15 min. Approximately 50 μg of the total protein extract was loaded on SDS-polyacrylamide gels and run 40 mA, followed by transfer on nitrocellulose membrane (Protran, Schleicher and Schuell, Germany) by

electroblotting. Bovine serum albumin (3%w/v) (Sigma Aldrich, Germany) in Tris-buffered saline (TBS)-Tween-20 buffer (137 mmol NaCl, 20 mmol Tris–HCl, pH 7.4, 0.1% Tween-20) was used to block filters for at least 1 h at room temperature. Specific primary antibody against cyclooxygenase-2 (rabbit polyclonal, 1:200 dilution; Santa Cruz, USA) or β-actin (mouse monoclonal, dilution 1:5000; Sigma Aldrich) was added to the membrane, followed by an antirabbit-immunoglobulin G (IgG) or antimouse IgG horseradish peroxidase-conjugated secondary antibody (dilution 1:20 000; Promega, WI, USA) dissolved in 1% nonfat milk in TBS-Tween-20 buffer. Incubation of primary antibody was followed by three washes with TBS-Tween-20 buffer for 10 min. Incubation of the secondary antibody was followed by five washes for 10 min. Immunocomplexes were detected by the SuperSignal West Pico Chemiluminescent Kit (Pierce, USA). Thereafter, the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparison between different treatment groups was made by determining the cyclooxygenase-2 protein/β-actin ratio of the immunoreactive area by densitometry.

2.6. Determination of pancreatic DNA synthesis

The rate of DNA synthesis in samples of pancreatic tissue was determined as described previously (Warzecha et al., 1999a). Briefly, the minced pancreatic tissue was incubated 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). DNA concentration was determined by the Giles and Myers (1965) procedure. The incorporation of [^{3}H]thymidine into DNA was measured by counting DNA containing solution in a liquid scintillation system. DNA synthesis is expressed as [^{3}H]thymidine disintegrations per minute per microgram DNA (dpm/μg DNA).

2.7. Determination of pancreatic prostaglandin E_2 generation

The samples of fresh pancreatic tissue (weighing about 50 mg) were placed in Eppendorf vials with 1 ml of Tris buffer (50 mM, pH 9.5), minced with scissors for 15 s, washed and centrifuged for 10 s. The pellets were resuspended in 1 ml of Tris buffer and each sample was incubated on a Vortex mixer for 1 min and centrifuged for 15 s. The supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) to block any further generation of prostaglandins, and kept at −80 °C until the radioimmunoassay (RIA). The DNA content in the pellet was determined by the Giles and Myers (1965) procedure. Finally, the prostaglandin E_2 content in the supernatant was measured in duplicate using Prostaglandin E_2 [^{125}I] RIA kit (NEN™ Life Science Products, Boston, MA, USA). The capacity of pancreatic tissue to generate prostaglandin E_2 is expressed in nanograms of prostaglandin E_2 per milligram of pancreatic DNA (ng/mg DNA).

2.8. Histological examination

Samples of pancreatic tissue for histological examination were fixed in 10% formalin, embedded in paraffin and sections were sliced and stained with hematoxylin and eosin. Slides were examined by two experienced pathologists without knowledge of the treatment given (four slides per animal). The histological grading of edema was made using our scale ranging from 0 to 3: 0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = severe interlobular and intralobular edema. Hemorrhage was graded: 0 = absent, 1 = from 1 to 2 foci per slide, 2 = from 3 to 5 foci per slide, 3 = more than 5 foci per slide. Leukocyte infiltration was graded: 0 = absent, 1 =scare perivascular infiltration, 2 = moderate perivascular and scare diffuse infiltration, 3 = abundant diffuse infiltration. Acinar necrosis was graded: 0 = absent, 1 = less than 15% of cells involved, 2 = from 15% to 35% of cells involved, 3 = more than 35% of cells involved. Grading of vacuolization was based on the percentage of cells involved: 0 = absent, 1 = less than 25%, 2 = 25–50% and 3 = more than 50%.

2.9. 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. Pancreatic expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA by reverse transcriptase-polymerase chain reaction and cyclooxygenase-2 protein synthesis by Western blot

As shown in Fig. 1, cyclooxygenase-1 mRNA was detected in the pancreas of all groups of animals. Administration of HGF, caerulein-induced pancreatitis and their combination were without significant effect on cyclooxygenase-1 mRNA expression in pancreatic tissue.

The pancreatic expression of cyclooxygenase-2 mRNA in the control saline-treated group was weak and the ratio of cyclooxygenase-2/β-actin mRNA reached a value 0.13 ± 0.02 (Fig. 2). Administration of HGF and induction of acute pancreatitis caused a significant increase in the pancreatic expression of cyclooxygenase-2 mRNA and the ratio of cyclooxygenase-2/β-actin mRNA reached 0.84 ± 0.06 and 0.52 ± 0.04, respectively. The maximal increase

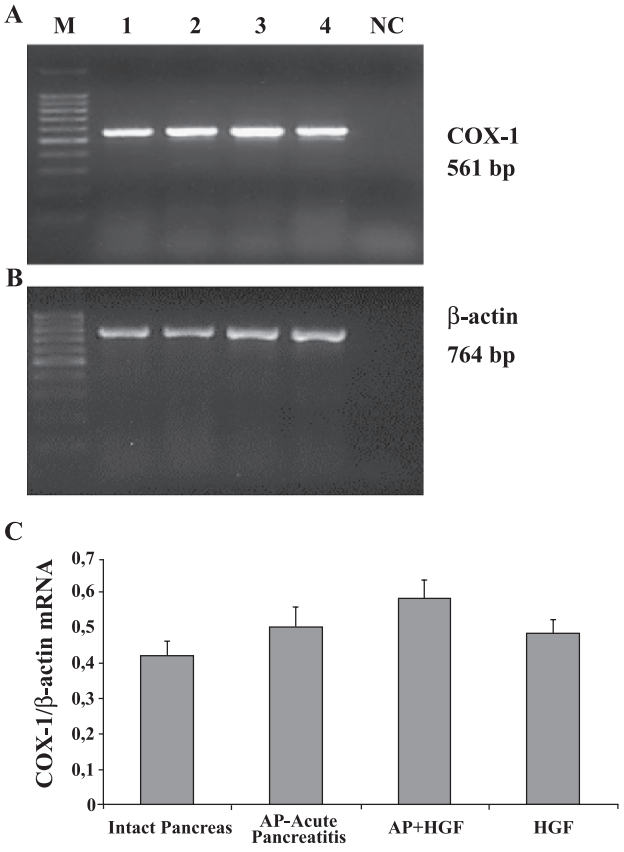

Fig. 1. Determination of cyclooxygenase-1 mRNA (A) and β-actin mRNA (B) by RT-PCR, and the ratio of cyclooxygenase-1 mRNA over β-actin mRNA (C) in pancreatic tissue of control animals (lane 1), animals with caerulein-induced pancreatitis (line 2), animals with caerulein-induced pancreatitis and treated with HGF (line 3), and animals treated with HGF without induction of acute pancreatitis (line 4).

in pancreatic cyclooxygenase-2 mRNA expression was observed when treatment with HGF was combined with caerulein-induced pancreatitis: the cyclooxygenase-2/β-actin ratio reached a value 1.19 ± 0.13.

Fig. 3 shows the effect of acute pancreatitis and treatment with HGF on the production of cyclooxygenase-2 protein in the pancreatic tissue. In control animals with an intact pancreas, the synthesis of cyclooxygenase-2 protein was weak and the ratio of cyclooxygenase-2 protein over β-actin protein reached a value 0.16 ± 0.01. Induction of acute pancreatitis and treatment with HGF significantly increased the ratio of cyclooxygenase-2 protein over β-actin protein in pancreatic tissue. The highest ratio of cyclooxygenase-2 protein to β-actin protein in pancreatic tissue was observed in animals with acute pancreatitis given HGF.

3.2. Effect of HGF, resveratrol, rofecoxib and caerulein administration on pancreatic generation of prostaglandin E_2

In saline-infused control rats, the pancreatic generation of prostaglandin E_2 reached 18.9 ± 1.7 ng/mg DNA (Fig. 4). Treatment with resveratrol alone slightly, but significantly, reduced pancreatic prostaglandin E_2 generation, whereas rofecoxib alone did not affect pancreatic prostaglandin E_2 generation in animals infused with saline. Treatment with HGF or induction of pancreatitis by caerulein significantly increased pancreatic prostaglandin E_2 generation. Administration of HGF combined with caerulein infusion caused a maximal increase in prostaglandin E_2 generation in the pancreas, reaching a value of 60.7 ± 5.2 ng/mg DNA. Administration of resveratrol did not significantly affect pancreatic prostaglandin E_2 generation in animals treated with caerulein or with the combination of caerulein and HGF. In contrast, pretreatment with rofecoxib significantly reduced pancreatic prostaglandin E_2 generation in animals treated with caerulein alone or with the combination of HGF plus caerulein.

3.3. Effect of HGF administration and inhibition of cyclooxygenase-1 and cyclooxygenase-2 on the biochemical parameters of acute pancreatitis

Plasma lipase activity in control saline-infused rats reached 60.0 ± 4.0 U/l (Fig. 5). Administration of HGF,

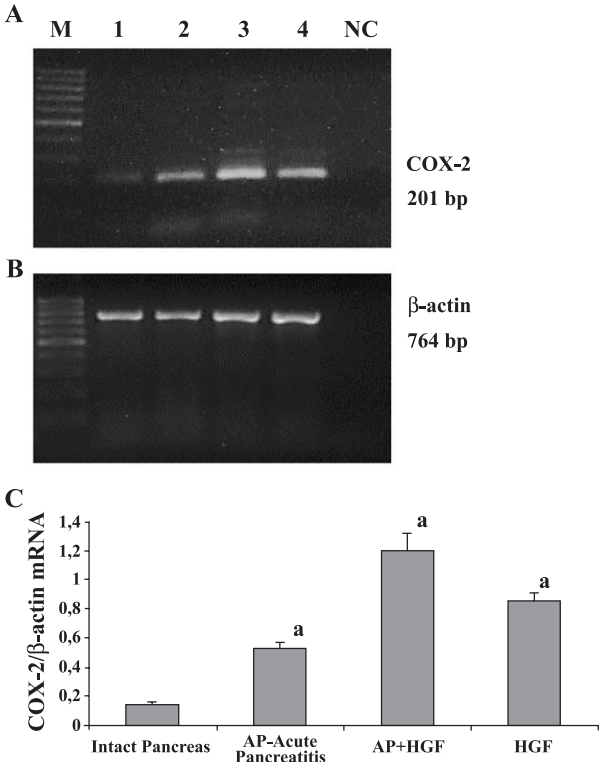

Fig. 2. Determination of cyclooxygenase-2 mRNA (A) and β-actin mRNA (B) by RT-PCR, and the ratio of cyclooxygenase-2 mRNA over β-actin mRNA (C) in pancreatic tissue from control animals (lane 1), animals with caerulein-induced pancreatitis (line 2), animals with caerulein-induced pancreatitis and treated with HGF (line 3), and animals treated with HGF without induction of acute pancreatitis (line 4). The ratio of COX-2 mRNA over β-actin mRNA is expressed as the mean ± S.E.M. $^{a}P < 0.05$ compared to control (intact pancreas).

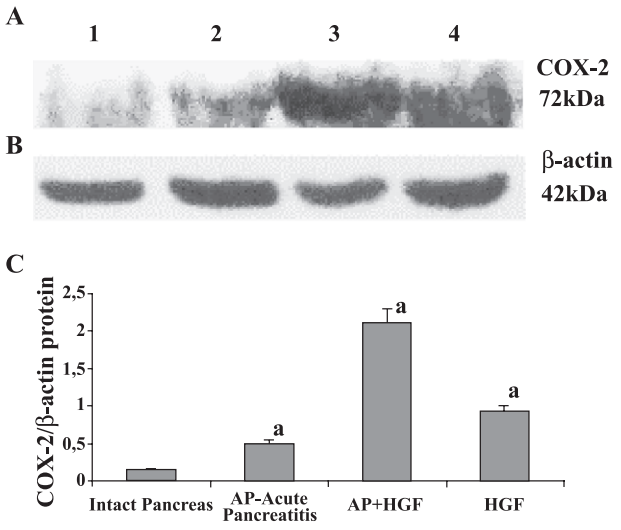

Fig. 3. Representative Western blot analysis of cyclooxygenase-2 protein (A) and β-actin protein (B), and the ratio of cyclooxygenase-2 protein over β-actin protein (C) in pancreatic tissue of control animals (lane 1), animals with caerulein-induced pancreatitis (line 2), animals with caerulein-induced pancreatitis and treated with HGF (line 3), and animals treated with HGF without induction of acute pancreatitis (line 4). The ratio of COX-2 protein over β-actin protein is expressed as the mean ± S.E.M. $^{a}P < 0.05$ compared to control (intact pancreas).

the cyclooxygenase-1 inhibitor-resveratrol or the cyclooxygenase-2 inhibitor-rofecoxib did not affect plasma lipase activity in rats infused with saline. Infusion with caerulein for 5 h increased plasma lipase activity by 625%, when compared with that of saline-infused control rats. Treatment with HGF markedly reduced the caerulein-evoked increase in plasma lipase activity by 37%. Resveratrol and rofecoxib given alone did not affect plasma lipase activity in animals with caerulein-induced pancreatitis. Pretreatment with resveratrol was without effect on the HGF-evoked decrease in plasma lipase activity in animals with acute pancreatitis. In contrast, pretreatment with rofecoxib abolished the HGF-evoked reduction in plasma lipase activity in animals with caerulein-induced pancreatitis.

In control rats infused with saline, the plasma interleukin-1β concentration was 72.0 ± 6.0 pg/ml (Fig. 6). Treatment with HGF, resveratrol or rofecoxib was without effect on the plasma interleukin-1β level in saline-infused rats. Caerulein caused a three-fold increase in plasma interleukin-1β concentration and this increase was significantly diminished by HGF. Resveratrol given alone or in combination with HGF was without effect on the caerulein-evoked increase in plasma interleukin-1β concentration. Rofecoxib given alone was without effect on caerulein-evoked increase in plasma interleukin-1β concentration, but administration of rofecoxib in combination with HGF significantly reversed the HGF-evoked decrease in plasma interleukin-1β concentration in animals with caerulein-induced pancreatitis.

In control rats, the plasma interleukin-10 level reached 67.5 ± 6.5 pg/ml (Fig. 7). Infusion of caerulein for 5 h did not significantly affect the plasma interleukin-10 concentration. Treatment with HGF increased the plasma concentration of interleukin-10 in rats infused with saline and in those infused with caerulein. In rats without caerulein-induced pancreatitis, this effect was more pronounced. Administration of resveratrol or rofecoxib alone to saline or caerulein-treated animals was without effect on the plasma interleukin-10 concentration. Also, pretreatment with resveratrol did not affect the plasma interleukin-10 concentration in animals treated with HGF plus caerulein. In animals treated

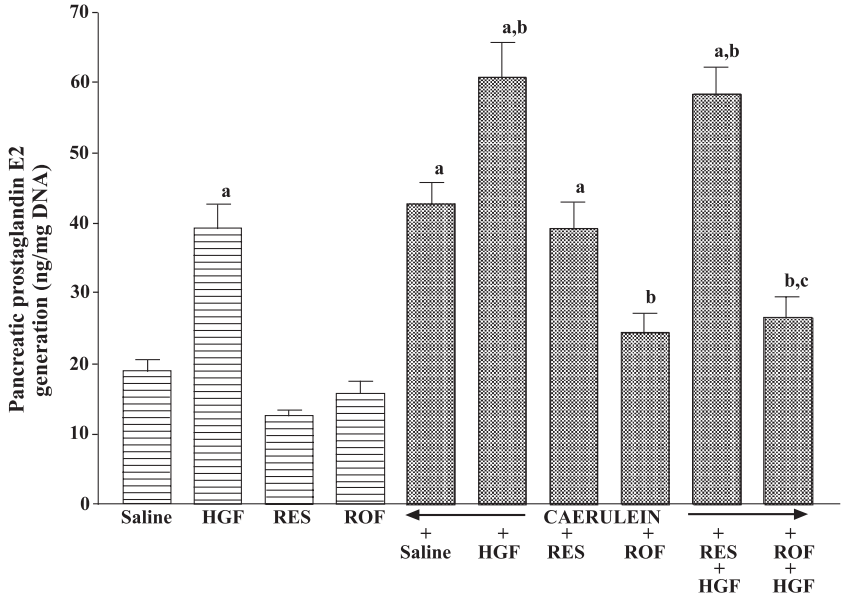

Fig. 4. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on pancreatic generation of prostaglandin E_2. Mean ± S.E.M. $^{a}P < 0.05$ compared with saline-infused control, $^{b}P < 0.05$ compared with caerulein given alone, $^{c}P < 0.05$ compared with combination of HGF plus caerulein.

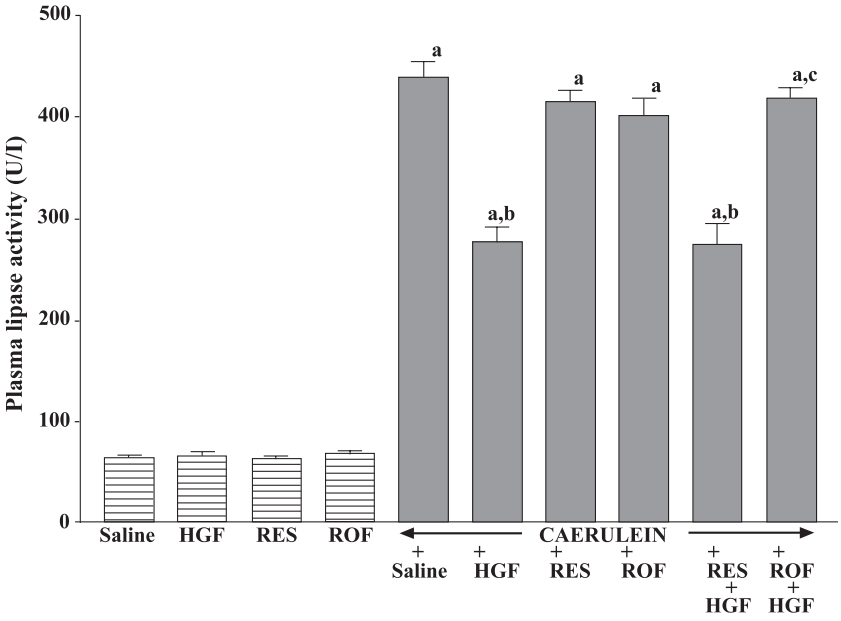

Fig. 5. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on plasma lipase activity. Mean ± S.E.M. [a]$P<0.05$ compared with saline-infused control, [b]$P<0.05$ compared with caerulein given alone, [c]$P<0.05$ compared with combination of HGF plus caerulein.

with a combination of HGF and caerulein, administration of rofecoxib abolished the HGF-evoked increase in plasma interleukin-10 concentration.

In saline-infused control rats, pancreatic DNA synthesis reached 60.6 ± 2.8 dpm/μg DNA (Fig. 8). Treatment with HGF, resveratrol or rofecoxib alone did not affect pancreatic DNA synthesis in animals infused with saline. In animals with caerulein-induced pancreatitis, pancreatic DNA synthesis was reduced, reaching 33.2 ± 1.3 dpm/μg DNA. In rats infused with caerulein, administration of HGF significantly attenuated the reduction in pancreatic DNA synthesis. Resveratrol and rofecoxib given alone did not affect pancreatic DNA synthesis in animals given a caerulein infusion. Also, pretreatment with resveratrol was without effect on pancreatic DNA synthesis in animals treated with a combination of HGF and caerulein. In contrast, in animals treated with HGF plus caerulein, pretreatment with rofecoxib reduced pancreatic DNA syn-

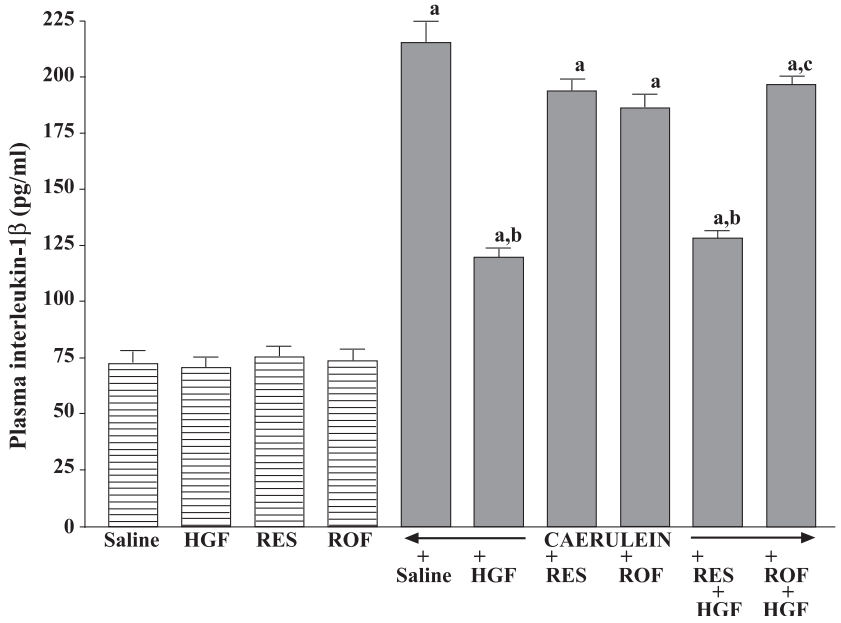

Fig. 6. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on plasma interleukin-1β concentration. Mean ± S.E.M. [a]$P<0.05$ compared with saline-infused control, [b]$P<0.05$ compared with caerulein given alone, [c]$P<0.05$ compared with combination of HGF plus caerulein.

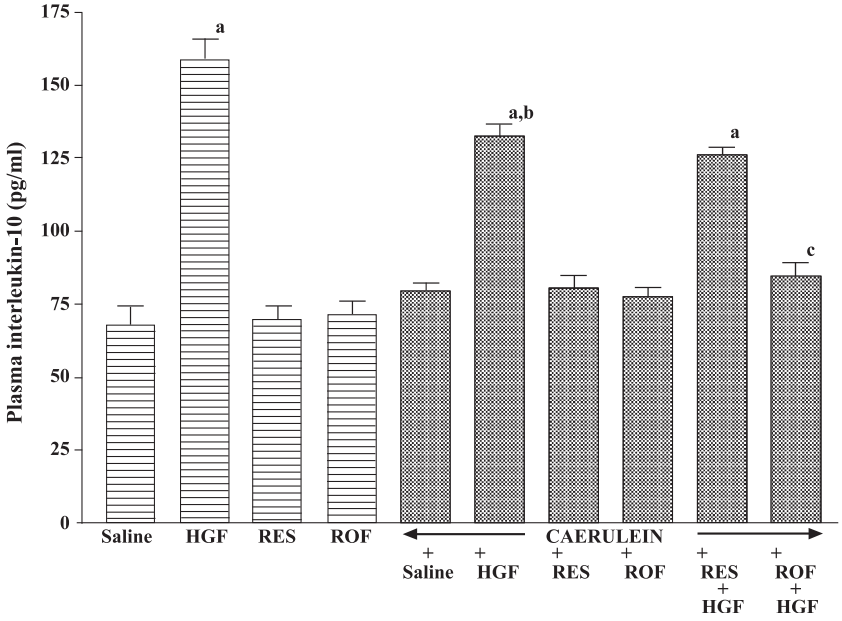

Fig. 7. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on plasma interleukin-10 concentration. Mean ± S.E.M. $^{a}P<0.05$ compared with saline-infused control, $^{b}P<0.05$ compared with caerulein given alone, $^{c}P<0.05$ compared with combination of HGF plus caerulein.

thesis to the level observed in animals with caerulein-induced pancreatitis not given HGF.

3.4. Effect of HGF administration, inhibition of cyclooxygenase-1 and cyclooxygenase-2, and caerulein infusion on pancreatic blood flow

Treatment with HGF, resveratrol or rofecoxib did not affect pancreatic blood flow in animals infused with saline (Fig. 9). Infusion of caerulein for 5 h reduced pancreatic blood flow by 60% when compared to that of saline-infused control animals. In rats given a caerulein infusion, administration of HGF significantly reversed the caerulein-induced fall in pancreatic blood flow. Resveratrol or rofecoxib given alone was without effect on pancreatic blood flow in animals with caerulein-induced pancreatitis. In contrast, rofecoxib abolished the HGF-induced improvement in pancreatic blood flow in animals infused with caerulein.

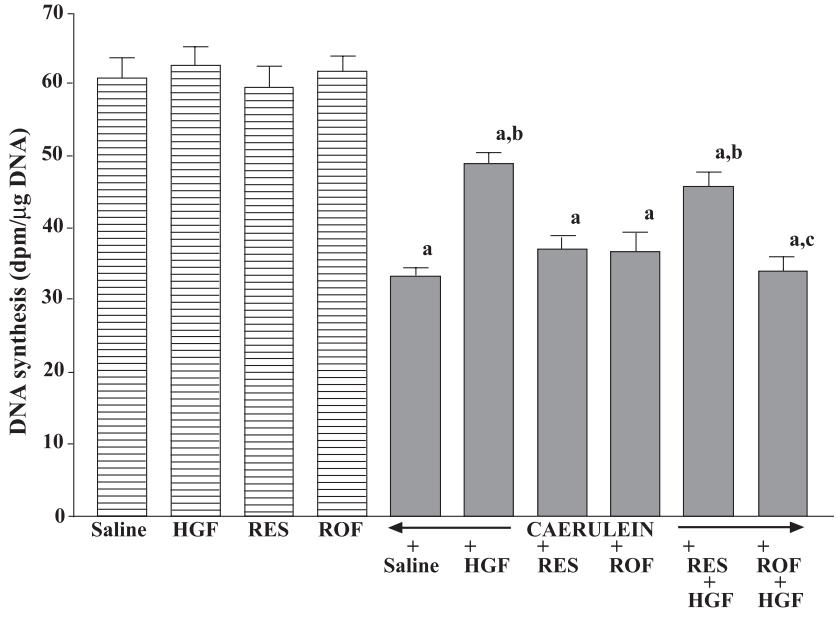

Fig. 8. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on pancreatic DNA synthesis. Mean ± S.E.M. $^{a}P<0.05$ compared with saline-infused control, $^{b}P<0.05$ compared with caerulein given alone, $^{c}P<0.05$ compared with combination of HGF plus caerulein.

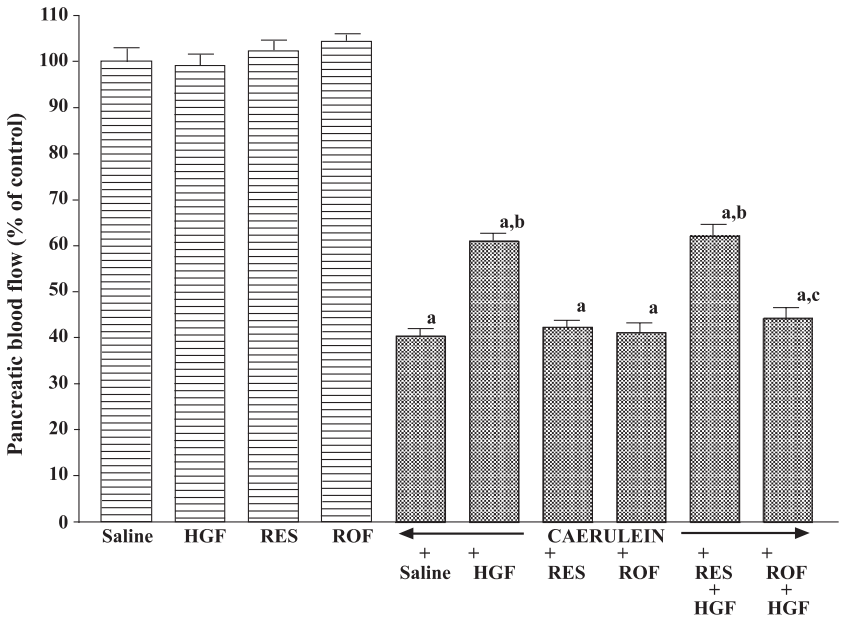

Fig. 9. Effect of HGF, resveratrol (RES) rofecoxib (ROF) and caerulein applied alone or in combination on pancreatic blood flow. Mean ± S.E.M. $^{a}P<0.05$ compared with saline-infused control, $^{b}P<0.05$ compared with caerulein given alone, $^{c}P<0.05$ compared with combination of HGF plus caerulein.

3.5. Morphological features of pancreatic tissue

The pancreas of saline-infused animals showed macroscopically and at light microscopic level no tissue alteration (Table 1). Also treatment with HGF, resveratrol or rofecoxib in saline-infused animals did not affect pancreatic tissue morphology. 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 by moderate perivascular and scare diffuse inflammatory leukocyte infiltration. Vacuolization in half of the cases was observed in 25–50% of acinar cells; in the remaining cases, vacuolization was found in more than 50% of acinar cells. Treatment with HGF before and during caerulein infusion reduced the caerulein-evoked pancreatic damage. Treatment with HGF caused a reduction in pancreatic edema, inflammatory infiltration and vacuolization of acinar cells. Administration of resveratrol or rofecoxib alone was without effect on caerulein-evoked damage. In animals treated with the combination of HGF and caerulein, administration of rofecoxib abolished the beneficial effect of HGF. Pancreatic damage was similar to that in animals treated with caerulein alone (Table 1).

4. Discussion

Previous studies have shown that growth factors such as epidermal growth factor (EGF) (Warzecha et al., 1999b; Dembiński et al., 2000), basic fibroblast growth factor (bFGF) (Hosokawa et al., 2000) or insulin-like growth factor-1 (IGF-1) (Dembiński et al., 2002; Warzecha et al.,

Table 1
Examination of pancreatic tissue of animals treated with HGF, resveratrol, rofecoxib or caerulein alone or in combination

	Histology				
	Edema (0–3)	Hemorrhages (0–3)	Inflammatory infiltration (0–3)	Vacuolization (0–3)	Necrosis (0–3)
Control	0	0	0	0	0
HGF	0	0	0	0	0
Resveratrol	0	0	0	0	0
Rofecoxib	0	0	0	0	0
Caerulein	2	0	2	2/3	0
HGF + caerulein	1/2	0	1	2	0
Resveratrol + caerulein	2	0	1	2/3	0
Rofecoxib + caerulein	2	0	2	2/3	0
Resveratrol + HGF + caerulein	1/2	0	1	2	0
Rofecoxib + HGF + caerulein	2	0	2	2/3	0

Numbers represent the predominant histological grading in each group.

2003) have protective and therapeutic effects in the course of acute pancreatitis. Also, HGF has been shown to attenuate pancreatic damage in the course of acute pancreatitis (Warzecha et al., 2001b). Our present study confirms and extends previous findings. Administration of HGF reduced pancreatic tissue damage and plasma lipase activity. These effects were associated with a reduction in plasma interleukin-1β concentration, an increase in plasma interleukin-10 concentration and an improvement in pancreatic blood flow.

Interleukin-1β is a well-known mediator of acute inflammation and plays a crucial role in the release of other members of pro-inflammatory cytokine cascade (Kingsnorth, 1997). In acute pancreatitis, interleukin-1β and other pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α (TNF-α), are produced within the pancreas and subsequently within distant organs, which become dysfunctional during severe pancreatitis (Norman et al., 1997). Pro-inflammatory cytokines are responsible for local pancreatic injury and the development of systemic inflammatory response syndrome and multiple organ failure (Frossard and Past, 2002), and the level of pro-inflammatory cytokines is correlated with the severity of acute pancreatitis (Norman et al., 1997). Experimental study has shown that blockade of interleukin-1β by naturally occurring receptor antagonist prevents the rise in serum interleukin-6 and tumor necrosis factor-α level, and decreases pancreatic damage in experimental acute pancreatitis (Norman et al., 1995). These observations are in agreement with our present data and partly explain the mechanism of protective activity of HGF on the pancreas. Treatment with HGF reduced the leukocyte infiltration of pancreatic tissue and the production of interleukin-1β, leading to a reduction of pancreatic damage.

The new finding of our present study is that the development of acute pancreatitis, as well as administration of HGF, causes an increase in the expression of cyclooxygenase-2 mRNA and production of cyclooxygenase-2 protein in pancreatic tissue. In contrast, both above factors were without effect on the pancreatic expression of cyclooxygenase-1 mRNA. This finding suggests the involvement of cyclooxygenase-2 and cyclooxygenase-2-derived prostaglandins in the protective effects of HGF in the pancreas. This concept is supported by studies performed by Robert et al. (1989) and Buscail et al. (1990). They found that administration of prostaglandins reduces pancreatic damage in experimental caerulein-induced pancreatitis. Also in other organs, cyclooxygenase-2 and cyclooxygenase-2-derived prostaglandins are involved in the maintenance of tissue integrity. Blockade of cyclooxygenase-2 by selective non-steroidal anti-inflammatory drugs delays the healing of experimental gastric ulcers (Halter et al., 2001) and inhibits liver regeneration (Rudnick et al., 2001) and bone fracture healing (Simon et al., 2002).

There are some reports that inhibition of cyclooxygenase-2 activity reduces the severity of acute experimental pancreatitis. Studies performed by Song et al. (2002) and Ethridge et al. (2002) with mice have shown that pharmacological inhibition of cyclooxygenase-2 or cyclooxygenase-2 gene disruption ameliorates the severity of pancreatitis and pancreatitis-associated lung injury. In contrast, in the rat model of pancreatitis, Foitzik et al. (2003) have found some beneficial systemic effects of cyclooxygenase-2 inhibition in acute pancreatitis, such as an improvement of renal and respiratory function, but they have not observed any significant effect of cyclooxygenase-2 inhibition on histological score of pancreatic damage or plasma level of trypsinogen activation peptides. Similar effects were observed by us in our present study. In animals not given HGF, pretreatment with refecoxib before administration of caerulein tended to reduce the plasma level of pro-inflammatory interleukin-1β and plasma lipase activity, but these results were not statistically significant. Also, a study performed by Jaworek et al. (2002) has shown a lack of significant effect of cyclooxygenase-2 inhibitor applied alone on pancreatic damage in the rat model of acute pancreatitis. The reason for the difference between the potent protective effect of cyclooxygenase-2 inhibition against pancreatic damage in the mouse model of acute pancreatitis and the weak effect in the rat model of acute pancreatitis is unclear. There may be differences in the role of cyclooxygenase-2 activity in the development of inflammation in the two species.

The important finding of the present study is that blockade of cyclooxygenase-2 activity by rofecoxib abolished the protective effect of HGF on the pancreas during acute pancreatitis, leading to an increase in pancreatic damage, plasma lipase activity, and pro-inflammatory interleukin-1β, and a decrease in pancreatic DNA synthesis, pancreatic blood flow and plasma interleukin-10. Interleukin-10 is a major anti-inflammatory cytokine. This interleukin reduces the activation of macrophages and inhibits the production of pro-inflammatory cytokines (De Waal et al., 1991) and reactive oxygen species (Moore et al., 1993). In the course of acute pancreatitis, interleukin-10 plays a role in the self-defense mechanism, limiting the intensity of the inflammatory process (Dembiński et al., 2001) and the increase in interleukin-10 level is a consequence of an increase in interleukin-1β. Administration of IL-10 before and during induction of acute pancreatitis decreases the severity of pancreatitis (Van Laethem et al., 1995). In our present study, treatment with HGF-1 increased the pancreatic generation of prostaglandin E_2 and the plasma interleukin-10 concentration in animals infused with saline, as well as in animals infused with caerulein or the combination of caerulein plus resveratrol. In animals treated with HGF in combination with caerulein and rofecoxib, the pancreatic generation of prostaglandin E_2 was strongly reduced and the plasma interleukin-10 concentration reached the same value as in animals not administered HGF. This observation indicates that cyclooxygenase-2 and cyclooxygenase-2-derived prostaglandins are involved in the HGF-induced increase in plasma interleukin-10 concentration.

Pancreatic microvascular failure and pancreatic ischemia may be a primary cause of clinical (Fernandez-Cruz et al., 1993; Sakorafas et al., 1998; Lonardo et al., 1999) and experimental acute pancreatitis (Menger and Vollmar, 1999; Dembiński et al., 2001), but an early disturbance of pancreatic circulation is also observed in acute pancreatitis caused by other nonvascular factors (Kusterer et al., 1991; Menger and Vollmar, 1999; Warzecha et al., 1997). The severity of experimental pancreatitis is closely correlated with tissue ischemia (Knoefel et al., 1994). An additional reduction in pancreatic circulation aggravates pancreatic damage during acute pancreatitis (Furukawa et al., 1993; Klar et al., 1990; Dembiński et al., 1996), whereas vasodilatation and an improvement of pancreatic blood flow have been found to reduce the development of acute pancreatitis (Klar et al., 1990; Warzecha et al., 2001a). In our present study, treatment with HGF caused an increase in the pancreatic generation of prostaglandin E_2 and led to a partial reversal of the pancreatitis-induced decrease in pancreatic blood flow, whereas inhibition of cyclooxygenase-2 activity by rofecoxib abolished the pancreatic generation of prostaglandin E_2 and decreased pancreatic blood flow to a level observed in animals with caerulein-induced pancreatitis and not administered HGF. This effect indicates that an increase in cyclooxygenase-2 activity is involved in the improvement of pancreatic microcirculation produced by HGF in acute pancreatitis. This is a rapid effect, and for this reason, it seems to be due to a direct vasodilating effect of cyclooxygenase-2-derived prostaglandins. Long-lasting inhibition of cyclooxygenase-2 activity has been shown to exhibit the anti-angiogenic effect during tissue regeneration (Guo et al., 2002).

Previous studies (Abe et al., 1995; Jaworek et al., 2002) have shown that pretreatment with a low dose of bacterial lipopolysaccharide (a component of the bacterial cell wall) protects the pancreas from the injury produced by caerulein overstimulation. The protective effect of lipopolysaccharide administration involves, among others, induction of cyclooxygenase-2 expression, generation of prostaglandin E_2 and improvement of pancreatic blood flow; and the inhibition of cyclooxygenase-2 abolishes this protective effect of lipopolysaccharide administration on the pancreas (Jaworek et al., 2002). This observation suggests that the protective effect of HGF and of lipopolysaccharide is based on the same mechanism.

In summary, we have demonstrated that HGF administration and induction of acute pancreatitis induce cyclooxygenase-2 expression without affecting cyclooxygenase-1 expression. Also, we found that cyclooxygenase-2 activity is necessary, but is not sufficient, for the protective effect of HGF on the pancreas during the development of acute caerulein-induced pancreatitis. Inhibition of cyclooxygenase-2 activity abolishes the beneficial effect of HGF, such as the increased release of anti-inflammatory interleukin-10, the decreased production of pro-inflammatory inflammatory-1β and the improvement of pancreatic blood flow.

References

Abe, R., Shimosegawa, T., Moriizumi, S., Kikuchi, Y., Kimura, K., Satoh, A., Koizumi, M., Toyota, T., 1993. Lipopolysaccharide induces manganese superoxide dismutase in the rat pancreas: its role in caerulein pancreatitis. Biochem. Biophys. Res. Commun. 217, 1216–1222.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Pajdo, R., Schuppan, D., Drozdowicz, D., Ptak, A., Pawlik, M., Nakamura, T., Hahn, E.G., 2000. Involvement of cyclooxygenase (COX)-2 products in acceleration of ulcer healing by gastrin and hepatocyte growth factor. J. Physiol. Pharmacol. 51, 751–773.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Schuppan, D., Drozdowicz, D., Kwiecień, S., Majka, J., Nakamura, T., Hahn, E., 2001. Effect of local application of growth factors on gastric ulcer healing and mucosal expression of cyclooxygenase-1 and -2. Digestion 64, 15–29.

Buscail, L., Bussenot, I., Bouisson, M., Senegas-Balas, F., Bertrand, C., Balas, D., Vaysse, N., Ribet, A., 1990. Protective effect of misoprostol, a synthetic prostaglandin E_1 analog, on caerulein-induced acute pancreatitis in rats. Pancreas 5, 171–176.

Bussolino, F., Di Renzo, M.F., Ziche, M., Bocchietto, E., Olivero, M., Naldini, L., Gaudino, G., Tamagnone, L., Coffer, A., Comoglio, P.M., 1992. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J. Cell Biol. 119, 629–641.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chlorophorm extraction. Ann. Biochem. Exp. Med. 162, 156–159.

De Waal, M.R., Abrams, J., Bennett, B., Figdor, C.G., De Vries, J.E., 1991. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174, 1209–1220.

Dembiński, A., Warzecha, Z., Konturek, P.C., Ceranowicz, P., Konturek, S.J., 1996. Influence of capsaicin sensitive afferent neurons and nitric oxide (NO) on caerulein induced pancreatitis in rats. Int. J. Pancreatol. 19, 179–189.

Dembiński, A., Warzecha, Z., Konturek, P.C., Ceranowicz, P., Stachura, J., Tomaszewska, R., Konturek, S.J., 2000. Epidermal growth factor accelerates pancreatic recovery after caerulein-induced pancreatitis. Eur. J. Pharmacol. 398, 159–168.

Dembiński, A., Warzecha, Z., Ceranowicz, P., Tomaszewska, R., Stachura, J., Konturek, S.J., Sendur, R., Dembiński, M., Pawlik, W.W., 2001. Pancreatic damage and regeneration in the course of ischemia-induced acute pancreatitis in rats. J. Physiol. Pharmacol. 52, 221–236.

Dembiński, A., Warzecha, Z., Ceranowicz, P., Sendur, R., Knafel, A., Dembiński, M., Jaworek, J., Pawlik, W.W., Bilski, J., Konturek, S.J., 2002. IGF-1 stimulates production of interleukin-10 and inhibits development of caerulein-induced pancreatitis. Gut 51 (Suppl. III), A171.

Ethridge, R.T., Chung, D.H., Slogoff, M., Ehlers, R.A., Hellmich, M.R., Rajaraman, S., Saito, H., Uchida, T., Evers, B.M., 2002. Cyclooxygenase-2 gene disruption attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. Gastroenterology 123, 1311–1322.

Fernandez-Cruz, L., Sabater, L., Gilberg, R., Ricart, M.J., Saens, A., Astudillo, E., 1993. Native and graft pancreatitis following combined pancreas-renal transplantation. Br. J. Surg. 80, 1429–1432.

Foitzik, T., Hotz, H.G., Hotz, B., Witting, F., Buhr, H.J., 2003. Selective inhibition of cyclooxygenase-2 (COX-2) reduces prostaglandin E_2 production and attenuates systemic disease sequelae in experimental pancreatitis. Hepato-gastroenterology 50, 1159–1162.

Frossard, J.L., Past, C.M., 2002. Experimental acute pancreatitis: new insight into the pathophysiology. Front. Biosci. 7, d275–d287.

Funakoshi, H., Nakamura, T., 2003. Hepatocyte growth factor: from diagnosis to clinical applications. Clin. Chim. Acta 327, 1–23.

Furlong, R.A., Takehara, T., Taylor, W.G., Nakamura, T., Rubin, J.S., 1991. Comparison of biological and immunochemical properties indicates that scatter factor and hepatocyte growth factor are indistinguishable. J. Cell. Sci. 100, 173–177.

Furukawa, M., Kimura, T., Sumii, T., Yamaguchi, H., Nawata, H., 1993.

Role of local pancreatic blood flow in development of hemorrhagic pancreatitis induced by stress in rats. Pancreas 8, 499–505.
Gherardi, E., Stocker, M., 1991. Hepatocyte growth factor-scatter factor: mitogen, motogen and met. Cancer Cells 3, 227–232.
Giles, K.W., Myers, A., 1965. An improvement diphenylamine method for the estimation of deoxyribonucleic acid. Nature 206, 93.
Guo, J.S., Cho, C.H., Lam Liu, E.S., Choy, H.T., Wang, J.Y., Leung Koo, M.W., 2002. Antiangiogenic effect of a highly selective cyclooxygenase-2 inhibitor on gastric ulcer healing in rats. Toxicol. Appl. Pharmacol. 183, 41–45.
Halter, F., Tarnawski, A.S., Schmassmann, A., Peskar, B.M., 2001. Cyclooxygenase-2 implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. Gut 49, 443–453.
Hosokawa, M., Tsukada, H., Fukuda, F., Oya, M., Onomura, M., Nakamura, H., Kodama, M., Fujita, J., Seino, Y., 2000. Therapeutic effect of basic fibroblast growth factor on experimental pancreatitis in rat. Pancreas 20, 373–377.
Jaworek, J., Bonior, J., Tomaszewska, R., Jachimczak, B., Kot, M., Bielański, W., Pawlik, W.W., Sendur, R., Stachura, J., Konturek, P.C., Konturek, S.J., 2002. Involvement of cyclooxygenase-derived prostaglandin E_2 and nitric oxide in the protection of rat pancreas afforded by low dose of lipopolysaccharide. J. Physiol. Pharmacol. 52, 107–126.
Jones, M.K., Sasaki, E., Halter, F., Pai, R., Nakamura, T., Arakawa, T., Kuroki, T., Tarnawski, A.S., 1999. HGF triggers activation of the COX-2 gene in rat gastric epithelial cells: action mediated through the ERK2 signaling pathway. FASEB J. 13, 2186–2194.
Kennedy, B.P., Chan, C.C., Culp, S.A., Cromlish, W.A., 1993. Cloning and expression of rat prostaglandin endoperoxide synthase (cyclooxygenase)-2 cDNA. Biochem. Biophys. Res. Commun. 197, 494–500.
Kiehne, K., Herzig, K.H., Fölsch, U.R., 1997. C-met expression in pancreatic cancer and effects of hepatocyte growth factor on pancreatic cancer cell growth. Pancreas 15, 35–40.
Kingsnorth, A., 1997. Role of cytokines and their inhibitors in acute pancreatitis. Gut 40, 1–4.
Klar, E., Messmer, K., Warshaw, A.L., Herfarth, C., 1990. Pancreatic ischemia in experimental acute pancreatitis: mechanism, significance and therapy. Br. J. Surg. 77, 1205–1210.
Knoefel, W.T., Kollias, N., Warshaw, A.L., Waldner, H., Nishioka, N.S., Rattner, D.W., 1994. Pancreatic microcirculatory changes in experimental pancreatitis of graded severity in the rat. Surgery 116, 904–913.
Komori, M., Tsuji, S., Sun, W.H., Tsujii, M., Kawai, N., Yasumaru, M., Kakiuchi, Y., Kimura, A., Sasaki, Y., Higashiyama, S., Kawano, S., Hori, M., 2002. Gastrin enhances gastric mucosal integrity through cyclooxygenase-2 upregulation in rats. Am. J. Physiol. 283, G1368–G1378.
Konturek, S.J., Piastucki, I., Brzozowski, T., Radecki, T., Dembinska-Kiec, A., Zmuda, A., Gryglewski, R., 1981. Role of prostaglandins in the formation of aspirin-induced gastric ulcers. Gastroenterology 80, 4–9.
Konturek, S.J., Szlachcic, A., Dembiński, A., Warzecha, Z., Jaworek, J., Stachura, J., 1994. Nitric oxide in pancreatic secretion and hormone-induced pancreatitis in rats. Int. J. Pancreatol. 15, 19–28.
Kusterer, K., Enghofer, M., Zendler, S., Blöchle, C., Usadel, K.H., 1991. Microcirculatory changes in sodium taurocholate-induced pancreatitis in rats. Am. J. Physiol. 260, G346–G351.
Lonardo, A., Grisendi, A., Bonilauri, S., Rambaldi, M., Selmi, I., Tondelli, E., 1999. Ischemic necrotizing pancreatitis after cardiac surgery. A case report and review of the literature. Ital. J. Gastroenterol. Hepatol. 31, 872–875.
Masferrer, I.L., Zweifel, B.S., Maming, P.T., Hauser, S.D., Eaky, K.M., Smith, W.G., Isakson, P.C., Siebert, K., 1994. Selective inhibition of inducible cyclooxygenase-2 in vivo is anti-inflammatory and ulcerogenic. Proc. Natl. Acad. Sci. U. S. A. 9, 3228–3232.
Matsumoto, K., Nakamura, T., 1992. Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions. Crit. Rev. Oncog. 3, 27–54.
Matsumoto, K., Nakamura, T., 1997. HGF: its organotrophic role and therapeutical potential. Ciba Found. Symp. 212, 198–211.
Matsumoto, K., Hashimoto, K., Yoshikawa, K., Nakamura, T., 1991. Marked stimulation of growth and motility of human keratocytes by hepatocyte growth factor. Exp. Cell Res. 196, 114–120.
Menger, M.D., Vollmar, B., 1999. Microcirculation: initiating or aggravating factor. In: Büchler, M.W., Uhl, W., Friess, H., Malfertheiner, P. (Eds.), Acute pancreatitis. Novel concepts in biology and therapy. Blackwell, Berlin-Vienna, pp. 63–70.
Menke, A., Yamaguchi, H., Giehl, K., Adler, G., 1999. Hepatocyte growth factor and fibroblast growth factor 2 are overexpressed after cerulein-induced acute pancreatitis. Pancreas 18, 28–33.
Miyazawa, T., Matsumoto, K., Ohmichi, H., Katoh, H., Yamashima, T., Nakamura, T., 1998. Protection of hippocampal neurons from ischemia-induced delayed neuronal death by hepatocyte growth factor: a novel neurotrophic factor. J. Cereb. Blood Flow Metab. 18, 345–348.
Mizuno, H., Sakamoto, C., Matsuda, K., Wada, K., Uchida, T., Noguchi, H., Akamatsu, T., Kasuga, M., 1997. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. Gastroenterology 112, 387–397.
Moore, K.W., O'Garra, A., de Waal, M.R., Vieira, P., Mosmann, T.R., 1993. Interleukin-10. Annu. Rev. Immunol 11, 165–190.
Nakamura, T., 1991. Structure and function of hepatocyte growth factor. Prog. Growth Factor Res. 3, 67–85.
Norman, J., Franz, M., Messina, J., Riker, A., Fabri, P.J., Rosemurgy, A.S., Gower Jr., W.R., 1995. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery 117, 648–655.
Norman, J.G., Fink, G.W., Denham, W., Yang, J., Carter, G., Sexton, C., Falkner, J., Gower, W.R., Franz, M.G., 1997. Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. Dig. Dis. Sci. 42, 1783–1788.
O'Banion, M.K., Winn, V.D., Young, D.A., 1992. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc. Natl. Acad. Sci. USA 89, 4888–4892.
Peskar, B.M., 2001. Role of cyclooxygenase isoforms in gastric mucosal defence. J. Physiol. (Paris) 95, 3–9.
Robert, A., Lum, J.T., Lancaster, C., Olafsson, A.S., Kolbasa, K.P., Nezamis, J.E., 1989. Prevention by prostaglandins of caerulein-induced pancreatitis in rats. Lab. Invest. 60, 677–691.
Rudnick, D.A., Perlmutter, D.H., Muglia, L.J., 2001. Prostaglandins required for CREB activation and cellular proliferation during lever regeneration. Proc. Natl. Acad. Sci. U. S. A. 98, 8885–8890.
Sakorafas, G.H., Tsiotos, G.G., Bower, T.C., Sarr, M.G., 1998. Ischemic necrotizing pancreatitis. Two case reports and review of literature. Int. J. Pancreatol. 24, 117–121.
Schmidt, C., Bladt, F., Goedecke, S., Brinkmann, V., Zschiesche, W., Sharpe, M., Gherardi, E., Birchmeier, C., 1995. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 373, 699–702.
Simon, A.M., Manigrasso, M.B., O'Connor, J.P., 2002. Cyclo-oxygenase 2 function is essential for bone fracture healing. J. Bone Miner. Res. 17, 963–976.
Smith, W.L., Garavito, R.M., DeWitt, D.L., 1996. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and 2. J. Biol. Chem. 271, 33157–33160.
Song, A.M., Bhagat, L., Singh, V.P., van Acker, G.G.D., Steer, M.L., Saluja, A.K., 2002. Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury. Am. J. Physiol. 283, G1166–G1174.
Sun, W., Funakoshi, H., Nakamura, T., 1999. Differential expression of hepatocyte growth factor and its receptor, c-Met in the rat retina during development. Brain Res. 851, 46–53.
Ueda, T., Takeyama, Y., Toyokawa, A., Kishida, S., Yamamoto, M., Saitoh, Y., 1996. Significant elevation of serum human hepatocyte growth factor levels in patients with acute pancreatitis. Pancreas 12, 76–83.
Ueda, T., Takeyama, Y., Hori, Y., Nishikawa, J., Yamamoto, M., Saitoh, Y.,

1997. Hepatocyte growth factor in assessment of acute pancreatitis: comparison with C-reactive protein and interleukin-6. J. Gastroenterol. 32, 63–70.

Ueda, T., Takeyama, Y., Hori, Y., Shinkai, M., Takase, K., Goshima, M., Yamamoto, M., Kuroda, Y., 2000. Hepatocyte growth factor increases in injured organs and functions as an organotrophic factor in rats with experimental acute pancreatitis. Pancreas 20, 84–93.

Van Laethem, J.L., Marchant, A., Delvaux, A., Goldman, M., Robberecht, P., Velu, T., Devière, J., 1995. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. Gastroenterology 108, 1917–1922.

Vila, M.R., Nakamura, T., Real, F.X., 1995. Hepatocyte growth factor is a potent mitogen for normal human pancreas cells in vitro. Lab. Invest. 73, 409–418.

Wang, J.Y., Yamasaki, S., Takeuchi, K., Okabe, S., 1989. Delayed healing of acetic acid-induced gastric ulcers in rats by indomethacin. Gastroenterology 96, 393–402.

Warzecha, Z., Dembiński, A., Ceranowicz, P., Konturek, P.C., Stachura, J., Konturek, S.J., Niemiec, J., 1997. Protective effect of calcitonin gene-related peptide against caerulein-induced pancreatitis in rats. J. Physiol. Pharmacol. 48, 775–787.

Warzecha, Z., Dembiński, A., Ceranowicz, P., Konturek, P.C., Stachura, J., Tomaszewska, R., Konturek, S.J., 1999a. Calcitonin gene-related peptide can attenuate or augment pancreatic damage in caerulein-induced pancreatitis in rats. J. Physiol. Pharmacol. 50, 49–62.

Warzecha, Z., Dembiński, A., Konturek, P.C., Ceranowicz, P., Konturek, S.J., 1999b. Epidermal growth factor protects against pancreatic damage in cerulein-induced pancreatitis. Digestion 60, 314–323.

Warzecha, Z., Dembiński, A., Ceranowicz, P., Stachura, J., Tomaszewska, R., Konturek, S.J., 2001a. Effect of sensory nerves and CGRP on the development of caerulein-induced pancreatitis and pancreatic recovery. J. Physiol. Pharmacol. 52, 679–704.

Warzecha, Z., Dembiński, A., Konturek, P.C., Ceranowicz, P., Konturek, S.J., Tomaszewska, R., Schuppan, D., Stachura, J., Nakamura, T., 2001b. Hepatocyte growth factor attenuates pancreatic damage in caerulein-induced pancreatitis in rats. Eur. J. Pharmacol. 430, 113–121.

Warzecha, Z., Dembiński, A., Ceranowicz, P., Konturek, S.J., Tomaszewska, R., Stachura, J., Konturek, P.C., 2003. IGF-1 stimulates production of interleukin-10 and inhibits development of caerulein-induced pancreatitis. J. Physiol. Pharmacol. 54, 575–590.

Xie, W.L., Chipman, J.G., Robertson, D.L., Erikson, R.L., Simmons, D.L., 1991. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Natl. Acad Sci. USA 88, 2692–2696.