Inhibition of bradykinin B2 receptor preserves microcirculation in experimental pancreatitis in rats

CHRISTIAN BLOECHLE, KLAUS KUSTERER, REGINA M. KUEHN, CLAUS SCHNEIDER, WOLFRAM T. KNOEFEL, AND JAKOB R. IBZICKI
Department of Surgery, University of Hamburg, 20251 Hamburg; and Department of Endocrinology, Center of Internal Medicine, University of Frankfurt, 60590 Frankfurt, Germany

Bloechle, Christian, Klaus Kusterer, Regina M. Kuehn, Claus Schneider, Wolfram T. Knoefel, and Jakob R. Izbicki. Inhibition of bradykinin B2 receptor preserves microcirculation in experimental pancreatitis in rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G42–G51, 1998.—The effect of B2 receptor bradykinin antagonist icatibant on postcapillary leukostasis, microcirculatory stasis, and tissue necrosis was studied in acute pancreatitis. In rats, pancreatitis was induced by intraductal injection of sodium taurocholate (ST), intravenous caerulein and intraductal infusion of glucodeoxycholic acid (GDOC), or intravenous caerulein infusion alone. Intravital pancreatic microcirculation was observed. Icatibant or vehicle was given 30 min before induction of pancreatitis. In ST pancreatitis, the number of perfused capillaries increased in icatibant-pretreated rats (77% vs. 0% for controls, P < 0.001). Capillary flow was preserved in icatibant-treated rats; total stasis was observed in controls. Mean venular leukocyte adherence decreased in icatibant-treated rats (26% vs. 74% for controls, P < 0.001), and median histopathologic score was reduced (icatibant vs. controls, 5.0 vs. 12 points, respectively; P < 0.01). Kinase II inhibitor captopril or exogenous bradykinin resulted in microcirculatory stasis, extensive venular leukocyte adherence, and severe histological damage. With a 10 times greater icatibant dosage, this adverse effect was compensated. The beneficial effects of icatibant were also observed in intermediate pancreatitis (caerulein + GDOC). In ST and intermediate pancreatitis, icatibant preserved microcirculation, reduced venular leukocyte adherence, and prevented pancreatic tissue damage. B2 receptor bradykinin-mediated postcapillary leukostasis plays an important role in the pathogenesis of severe forms of acute pancreatitis.

in vivo microscopy; hemorrhagic; intermediate; edematous pancreatitis; pancreatic microcirculation; postcapillary leukocyte adherence; bradykinin B2 receptor antagonist; icatibant investigators to further examine the effects of bradykinin in acute pancreatitis. Some derivatives of bradykinin antagonists did not reveal protective action in acute pancreatitis (4). Others, like icatibant, a selective bradykinin B2 receptor antagonist, have been shown to prevent pancreatic injury in caerulein-induced pancreatitis (5, 6).

Microcirculatory changes and the extent of tissue injury differ greatly among mild, modest, and severe pancreatitis (12, 24). Therefore, the effect of this new bradykinin antagonist needs to be characterized in different models of pancreatitis. This study focuses on the effect of icatibant on microcirculatory changes and histopathologic alterations in a model of hemorrhagic necrotizing pancreatitis induced by sodium taurocholate (ST). Chemotaxis of neutrophil leukocytes is one of the well-known actions of bradykinin mediated by the bradykinin B2 receptor (18). Furthermore, postcapillary leukostasis and leukocyte infiltration of pancreatic tissue are characteristic features of the severe course of acute pancreatitis (1, 9, 14, 24). Therefore, the effect of icatibant on leukocyte adherence to the venular endothelium was a major point of interest in this study.

MATERIALS AND METHODS

This study had been approved by the Ethical Committee of the Hamburg Federal Board of Veterinary Medicine and Animal Care. Female Lewis rats with a body weight of 200–230 g were fasted overnight but had free access to water containing 20% glucose. Two sets of experiments were performed.

Experimental Protocol

According to a block design, 100 (5 × 20) animals were randomly allocated to 10 groups (groups I-X; n = 10). Ringer lactate was infused for fluid resuscitation, keeping the central venous pressure between 4 and 6 mmHg and heart rate (HR) as well as mean arterial pressure (MAP) within 10% of baseline frequency throughout the experiment. In addition, inspiratory air was enriched with oxygen (500 ml/min), ensuring steady peripheral arterial oxygen saturation (SaO2). Animals allocated to groups I–VI were subjected to acute hemorrhagic pancreatitis, whereas in animals in groups VII and VIII an intermediate pancreatitis was induced, and in animals in groups IX and X an edematous pancreatitis was induced (1, 13, 15, 24).

Among other peptides, bradykinin may play an essential role in the pathogenesis of acute pancreatitis (17, 20–23, 25). The synthesis and pharmacological characterization of bradykinin antagonists have led
Correspondingly, animals in groups V and VI received intravenous injections of bradykinin at intervals of 10 min (1 nmol/kg body wt; Bachem, Bâle, Switzerland).

Surgery

The animals were anesthetized with intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt) and ketamine (10 mg/kg body wt), allowing spontaneous breathing. Rectal temperature was maintained at 37°C throughout the operation and the entire experiment by placing rats on a heating pad. A tracheostomy was performed to ensure a free airway. The left carotid artery and the left internal jugular vein were cannulated using soft plastic tubing (Cavafix, G20; Braun, Melsungen, Germany). MAP, HR, central venous pressure, and SaO2 were measured continuously (Datex AS/3 monitoring system; Hoyer, Bremen, Germany). The pulsoximetry device was clamped to the right upper paw of the animal.

Induction of Hemorrhagic Pancreatitis

In animals subjected to hemorrhagic pancreatitis, a midline laparotomy was performed, and the duodenal loop was mobilized. The pancreatic duct was cannulated transduodenally via the papilla of Vater with plastic tubing (3 cm long, outer diameter of 0.8 mm, volume <0.01 ml). The tip of the tube was inserted 2 mm into the common biliary-pancreatic duct. The common bile duct was ligated close to the liver. The head and the corpus of the pancreas were placed on a plastic stage. The duodenal loop was fixed on the stage with tissue glue (Histoacyrl; Braun). The pancreas was covered by a polyethylene film (Flexithen; Plastica, Renningen, Germany). After surgery and 15 min of equilibration, ST (4%, 0.4 ml) was infused in the pancreatic duct for 300 s (infusion pressure 25–30 mmHg; see Refs. 1, 13).

Induction of Intermediate Pancreatitis

After surgery to include pancreatic ductal tubing as outlined above, an equilibration period of 15 min was allowed. Intermediate pancreatitis was induced by the combination of intravenous caerulein infusion (5 µg·kg–1·h–1; Takus; Pharmaceuticals, Erlangen, Germany) at a rate of 1 ml/h over 6 h and intraductal infusion of glucodeoxycholic acid (10 mmol/l; 1.0 ml/kg for 300 s; infusion pressure 25–30 mmHg; see Ref. 24).

Induction of Edematous Pancreatitis

After an equilibration period of 15 min after surgery, edematous pancreatitis was induced by intravenous caerulein infusion (5 µg·kg–1·h–1; Takus; Pharmaceuticalia, Erlangen, Germany) at a rate of 1 ml/h over 6 h (15, 24). Laparotomy was not performed in caerulein-induced pancreatitis.

Test Substance

Icatibant (HOE-140: d-Arg-Hyp3,Thi3,Tic7,Oic8-bradykinin) was kindly provided by Hoechst (Frankfurt, Germany). Animals in groups II, III, V, VIII, and X were given 100 nmol/kg body wt of icatibant subcutaneously, which was shown to be effective in baseline dose effectiveness experiments. Animals in groups IV and VI received 10 µmol/kg body wt of icatibant 30 min before induction of pancreatitis. Controls (groups I, VII, and IX) received an equivalent volume of saline (0.9%, 0.2 ml).

In Vivo Microscopy

Intravital microscopy was performed with an epiluminescent microscope (Olympus BH-12). The light of the mercury vapor lamp passed through a filter system (excitation filter 450–490 nm, dichroic mirror 510 nm, barrier filter 515 nm). Fluotar objectives 10/0.45 and 20/0.6 were used, which resulted in a final magnification on the video monitor of ×365 and ×692. Acridine orange (1%, 1.2 ml/kg body wt, injection within 30 s; Sigma Chemical, St. Louis, MO) was injected intravenously, labeling leukocytes (27). The experiments were recorded on videotape with an attached video line (camera: AV-1001, AVT; monitor: TC-1100 SDN; and recorder: NV-180; Panasonic).

Each pancreas was subjected to a thorough examination. Impaired flow or intrapancreatic hemorrhage at baseline examination led to exclusion after entry. Thus the final population of the different groups in both experimental series ranged from n = 7 to n = 10.

The head of the pancreas was defined to be the region of interest. One arterial, one venular, and three capillary sites were studied at each time point of examination (in ST pancreatitis: −15, ±0, +2, +4, +6, +8, +10, +15, +30, ±45, +60 min; in intermediate and in caerulein-induced pancreatitis: −15, ±0, +2, +4, +6, +8, +10, +15, +30, ±45, +60, +90, ±120, +150, ±180, +210, ±240, ±270, ±300, +330, +360 min; where 0 min is defined as the intraductal injection of ST or glucodeoxycholic acid or the start of intravenous caerulein infusion). Six hours after induction of pancreatitis, all animals were killed.

Arterial Constriction

The diameter of interlobular arterioles was measured 15 min before induction of acute pancreatitis. Changes in interlobular arterial diameters were related to the baseline diameter. Arterial vasconstriction or vasodilatation was expressed as the percentage of change in vessel diameter (14).

Number of Perfused Capillaries and Capillary Blood Flow

The number of perfused capillaries per observation site was counted and related to the total number of capillaries (10). Capillary blood flow was assessed using a semiquantitative scale from zero to four (0 = stasis, 1 = flow with clear particle identification and intermittent stops, 2 = flow with clear particle identification without any stops, 3 = particle identification just possible, 4 = no particle identification possible; see Ref. 13).

Leukocyte Adherence

A leukocyte was considered to be adherent to the venular endothelial wall of the interlobular venules if it did not move for at least 30 s. Leukocyte adherence was expressed as the area of adherent leukocytes in a percentage of the vein cross section (14).

The light from the microscope and administration of the fluorescent tracer in sham-operated animals induced a maximal leukocyte adherence of ~12% of the venous cross section (baseline experiments and previous studies (14)).

Histopathologic Scoring

The specimens were fixed in neutral phosphate-buffered 3.5% Formalin, routinely processed, and paraffin embedded. Two 5-µm slices were taken from each specimen. The slices were stained with hematoxylin and eosin. The slides were examined in blinded fashion using light microscopy. The histopathologic findings were scored as previously described (24).

Baseline Experiments

In two additional groups, animals (n = 5/group) were subjected to subcutaneous administration of either vehicle...
Furthermore, mean interlobular arteriolar diameter, significantly higher compared with controls (data not shown). Furthermore, dose effectiveness of icatibant (doses; 1, 10, and 100 nmol/kg body wt) was studied in ST-induced pancreatitis. Control animals received an equivalent volume of vehicle (0.9% saline, 0.2 ml). In these experiments, macrocirculatory hypotension was not corrected.

Further baseline experiments included intraperitoneal injection of the α-adrenergic blocker phentolamine (7 µmol/kg body wt; Ciba-Geigy) 15 min before induction of hemorrhagic ST-induced pancreatitis.

Experimental Series

Arterial constriction. Mean interlobular arteriolar diameter of all animals was 45.7 ± 7.3 µm at baseline measurement. In control animals (group I) with intraductal infusion of ST, a partially reversible segmental vasoconstriction of the interlobular arteriole was observed, reaching a maximum of 42% in reference to the baseline arteriolar diameter 4 min after induction of pancreatitis (P < 0.05). Icatibant pretreatment (group II), captopril (groups IV and V), and exogenous bradykinin (groups VI and VII) administration did not cause significant differences in segmental arterial vasocostriction (Figs. 1A, 2A, and 3A).

In the model of intermediate pancreatitis, mean arterial diameter decreased to 64% of the baseline value in control animals (group VII). After icatibant pretreatment, this arterial vasoconstriction was also observed (Fig. 4A).

In caerulein-induced pancreatitis, mean interlobular arterial diameter increased to 118% of baseline within 15 min after the beginning of caerulein infusion (group IX). Icatibant (group X) did not have any effect on this arterial dilatation (Fig. 5A).

Correspondingly, total stasis developed during ST pancreatitis in controls (group I), whereas icatibant pretreatment (group II) resulted in preserved capillary flow (P < 0.01, Fig. 1C).

With captopril and exogenous bradykinin administration, the microcirculation preserving effect of icatibant in an otherwise effective dosage (100 nmol/l) was not observed (groups III and V). However, this captopril and exogenous bradykinin effect was overcome with an increased dosage of icatibant (10 µmol/l; groups IV and VI; P < 0.001 each; Fig. 2B and C, and Fig. 3B and C).

Induction of intermediate pancreatitis resulted in a partial breakdown of pancreatic microcirculation in control animals (group VII), which was less pronounced in comparison with ST-induced pancreatitis. Icatibant pretreatment (group VIII) preserved pancreatic microcirculation in reference to the number of perfused capillaries and capillary blood flow (P < 0.01 and P < 0.05, respectively; Fig. 4B and C).

In caerulein-induced pancreatitis, capillary microcirculation was preserved during the entire observation period (groups IX and X, each not significant; Fig. 5B and C).

Leukocyte adherence. The mean diameter of interlobular venules was 54.1 ± 7.8 µm as measured at baseline in all animals. Baseline leukocyte adherence in interlobular venules was <5% before induction of pancreatitis. Venular diameter did not change significantly during the observation period.

In controls (group I), ST pancreatitis resulted in venular leukocyte adherence of 74% of vein cross...
section 60 min after intraductal ST injection. Icatibant pretreatment (group II) caused a significant reduction in postcapillary venular leukocyte adherence to 26% of vein cross sections ($P < 0.001$; Fig. 1).

In animals receiving captopril or exogenous bradykinin, leukocyte adherence was 79% and 81% of baseline values 60 min after induction of ST pancreatitis, despite icatibant pretreatment (dosage: 100 nmol/l; groups III and V). With an increased dosage of icatibant (dosage: 10 µmol/l; groups IV and VI) venular leukocyte adherence was significantly reduced ($P < 0.001$; Figs. 2D and 3D).

Intermediate pancreatitis led to a postcapillary leukocyte adherence of 43% of baseline vein cross-sectional diameter in controls (group VII). Icatibant pretreatment (group VIII) reduced leukocyte adherence to 21% of baseline values ($P < 0.05$; Fig. 4D).

In caerulein-induced pancreatitis, venular leukocyte adherence reached a maximum of 14% in controls (group IX) compared with 8% in icatibant-pretreated animals (group X, not significant; Fig. 5D).

Histopathologic scoring. ST-induced pancreatitis caused a median histopathologic damage score of 12.0 points (range 10–14) in controls (group I). Acinar necrosis, hemorrhage, and inflammatory infiltrates were the predominant histological features, whereas only moderate edema was present. An overall reduction of histopathologic damage with a median score of 5.0 points (range 4.0–6.0) was observed in group II ($P < 0.01$; Fig. 6). This beneficial effect of icatibant included reduction of acinar necrosis, hemorrhage, and inflammatory infiltrate, whereas the effect on edema was only moderate.

Despite icatibant treatment at an otherwise effective dosage (100 nmol/l), captopril and exogenous bradykinin administration (groups III and V) resulted in histological tissue damage comparable to that in controls (group I). This negative effect of captopril and exogenous bradykinin on pancreatic tissue damage was compensated with an increased dosage of icatibant (dosage: 10 µmol/l; groups IV and VI; $P < 0.01$; Fig. 6).

Intermediate pancreatitis resulted in a median histopathologic pancreatic injury score of 9.0 points (range 7.5–10) in controls (group VII). Again, acinar necrosis, hemorrhage, and inflammatory infiltrates were the predominant histological features, whereas edema was
less pronounced (Fig. 6). Icatibant (group VIII) caused an overall reduction of pancreatic injury to a median score of 4.5 points (range 3.0–6.5; \( P < 0.01 \); Fig. 6). Reduction of pancreatic injury was related equally to decreased extent of acinar necrosis, hemorrhage, and inflammatory infiltrates.

In caerulein-induced pancreatitis, median histopathologic tissue damage was 6.0 points (range 5.0–6.5) in controls (group IX) compared with 3.5 (range 3.0–4.0) in icatibant-pretreated animals (group X; \( P < 0.05 \); Fig. 6). In caerulein-induced pancreatitis, edema was the predominant histological feature in both controls and icatibant-pretreated animals.

**DISCUSSION**

Acute pancreatitis remains an important problem, both medical and surgical, with considerable morbidity and mortality. Its treatment is largely empirical, and its pathogenesis is poorly understood (3). Among other factors, bradykinin has been discussed as playing an important role in the initiation of the pathophysiologic cascade leading to acute pancreatitis (17). Bradykinin concentrations have been found to be elevated in plasma, lymph, and peritoneal exudate during experimental acute biliary pancreatitis (20, 23). Recently, a close correlation between plasma bradykinin levels and edema formation in caerulein-induced pancreatitis has been reported (25, 29). Furthermore, elevated levels of bradykinin have been found in portal vein plasma after induction of acute pancreatitis (30). Bradykinin is also known to be a key enzyme in the activation of phospholipase A2 and in the generation of leukotrienes and prostaglandins, which in turn promote arteriolar vasoconstriction and leukocyte adhesion to and migration across the endothelial wall of postcapillary venules (9). Furthermore, mediated by bradykinin B2 receptor, bradykinin causes vasodilatation and hypotension, increased vascular permeability, polymorphonuclear leukocyte accumulation, and pain (17, 18, 22, 26), all of which are characteristic features in the course of acute pancreatitis (13, 14, 24). Based on this evidence, bradykinin has also been suggested to be a crucial factor in the pathogenesis of acute pancreatitis and systemic changes during the course of the disease.

The development of a potent and highly selective competitive \( B_2 \) receptor antagonist, icatibant, has recently offered the chance to inhibit \( B_2 \) receptor-mediated bradykinin action. Recently, icatibant has...
been shown to prevent pancreatic edema, loss of plasma fluid, and systemic hypotension in caerulein-induced acute pancreatitis (5,6). However, caerulein-induced pancreatitis is a model of the edematous form of pancreatitis, which is clinically characterized by its mild self-limited course (24). In contrast, hemorrhagic necrotizing pancreatitis, which represents the other edge of the wide spectrum of courses that acute pancreatitis may take, remains a therapeutic challenge. This severe form of acute pancreatitis is still a lifethreatening disease; because its pathogenesis remains elusive, merely symptomatic treatment is possible.

To delineate the different pathways of acute pancreatitis, a variety of experimental models have been proposed. Experimental in vivo models of hemorrhagic necrotizing pancreatitis in small animals should allow a graded response and at the same time be suitable for microcirculatory studies, which have recently been suggested to be causative in the pathogenesis of the severe course of acute pancreatitis (10–13, 19). Experimental in vivo models in small animals, which meet these requirements, are limited to ductal bile injection (glucodeoxycholic acid) and temporary vascular occlusion and causing ischemia-induced hemorrhagic necrotizing pancreatitis (3, 9). Ductal infusion of ST is a well-defined and highly standardized model of bile salt-induced hemorrhagic necrotizing pancreatitis (1). More recently, the combination of ductal bile salt injection (glucodeoxycholic acid) and intravenous caerulein infusion has been described to cause an intermediate course of acute pancreatitis (24). This model of intermediate pancreatitis has been proposed to provide superior opportunity to study innovative therapy. However, in this study focusing on the pathogenesis of hemorrhagic necrotizing pancreatitis, we preferred to use the more severe model of ST-induced pancreatitis. At the other edge of the wide spectrum that the course of acute pancreatitis may take, caerulein-induced pancreatitis reflects edematous pancreatitis, as mentioned above.

One minute after intraductal infusion of ST, the walls of the pancreatic ducts are destroyed, and necrosis of neighboring acini develops subsequently (1, 2). The early changes in pancreatic microcirculation during the course of pancreatitis have recently been described (10–13, 19). Within 2–4 min after intraductal ST injection, reversible vasoconstriction of interlobular arterioles occurs. In the head of the pancreas, microcirculatory breakdown with capillary stasis and postcapillary venular leukocyte adherence is observed within 1 h.
after onset of pancreatitis. Although only moderate pancreatic edema develops, hemorrhagic necrosis is the predominant feature in ST pancreatitis (1, 24). In our present study, the sequence of partially reversible, segmental vasoconstriction of interlobular arterioles followed by capillary stasis and hemorrhagic necrosis has been confirmed. This arterial vasoconstriction could not be inhibited by the $\alpha$-adrenergic receptor blocker phentolamine.

Postcapillary venular leukocyte adherence has been shown to be an essential factor in ischemia-reperfusion injuries of the liver, striated muscle, and the pancreas (9, 16, 28). From muscular ischemia-reperfusion studies, it was concluded that postcapillary leukostasis is a pressure-related phenomenon (8). Despite maintenance of macrohemodynamic pressure, initial arterial vasoconstriction may cause pancreatic arterial hypotension triggering postcapillary leukocyte adherence. Radical scavenger pretreatment, which inhibited ST-induced reversible arterial constriction, led to preservation of capillary microcirculation and reduction of venular leukocyte adherence (14).

In contrast, icatibant pretreatment did not influence the reversible vasoconstriction of the interlobular arterioles in ST-induced pancreatitis. Nevertheless, the breakdown of capillary pancreatic perfusion was prevented, and postcapillary leukocyte adherence was significantly reduced, thus leading to a tremendous decrease of pancreatic tissue damage. As macrocirculatory hypotension and hypoxia were corrected, the positive effect of icatibant with regard to preservation of pancreatic microcirculation and reduction of pancreatic tissue injury was shown to be independent from its action on macrohemodynamics and oxygen delivery. With regard to histopathologic damage, the protective effect of icatibant predominantly referred to reduction of acinar necrosis, hemorrhage, and inflammatory infiltrate. The decreased inflammatory infiltration corresponded well with reduced venular leukocyte adherence. The icatibant effect on edema formation was only moderate.

Furthermore, an increase of bradykinin levels, either of endogenous origin by action of the kinase II inhibitor captopril or exogenously administered, caused microcir-
culatory breakdown, extensive venular leukocyte adherence, and pancreatic tissue injury as seen in controls, despite icatibant pretreatment in an otherwise effective dosage. This adverse effect was overcome by a 100-fold increase of the icatibant dosage. In context, with the dose-dependent action of icatibant observed in baseline experiments, this effect adds evidence for the competitive action of the bradykinin antagonist icatibant.

In the model of acute intermediate pancreatitis, icatibant pretreatment resulted in preservation of capillary perfusion and reduction of postcapillary venular...
leukocyte adherence, whereas initial arterial vasoconstriction, which was moderate compared with that observed in ST-induced pancreatitis, still occurred. Again, inhibition of microcirculatory disorders reduced significant histopathologic pancreatic tissue damage. In contrast, icatibant exerted only moderate improvement of histological damage by reduced edema formation in caerulein-induced pancreatitis, whereas the microcirculation was not altered significantly.

In line with the results of the present study, icatibant has recently been reported to preserve pancreatic microcirculation and to reduce postcapillary venular leukocyte adherence in a pancreatitis model based on ischemia and reperfusion (9). The mechanism of ischemia-reperfusion with subsequent capillary stasis and leukocyte accumulation at the endothelium of interlobular venules, which also applies in ST pancreatitis (7, 9, 14), has been interrupted by inhibition of bradykinin B₂ receptors with icatibant. Although arterial vasoconstriction still took place, capillary perfusion was preserved, and venular leukocyte adherence was inhibited.

Based on the results of the present study and in line with previous reports, postcapillary venular leukocyte adherence and capillary microcirculatory breakdown are confirmed to be important pathogenetic factors in the development of hemorrhagic necrotizing pancreatitis. Inhibition of postcapillary leukostasis and preservation of capillary microcirculation mediated by bradykinin B₂ receptor blockade with icatibant prevented hemorrhagic necrosis in ST-induced pancreatitis, adding to the evidence that bradykinin plays an essential role in the pathogenesis of severe acute pancreatitis.

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Address for reprint requests: J. R. Izbicki, Dept. of Surgery, Univ. Hospital Eppendorf, Univ. of Hamburg, Martinistrasse 52, 20251 Hamburg, Germany.

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