Effect of CP-96,345 on the expression of adhesion molecules in acute pancreatitis in mice

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Lau HY, Bhatia M. Effect of CP-96,345 on the expression of adhesion molecules in acute pancreatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 292: G1283–G1292, 2007. First published January 11, 2007; doi:10.1152/ajpgi.00429.2006.—We investigated the effect of a specific neurokinin-1 receptor (NK1R) antagonist, CP-96,345, on the regulation of the expression of adhesion molecules ICAM-1, VCAM-1, E-selectin, and P-selectin as well as leukocyte recruitment during acute pancreatitis (AP). AP was induced in male Balb/C mice by 10 consecutive hourly intraperitoneal injections of caerulein. In the treatment groups, CP-96,345 was administered at 2.5 mg/kg ip every 30 min before or 1 h after the first caerulein injection. Animals were killed, and the lungs and pancreas were isolated for RNA extraction and RT-PCR or for immunohistochemical staining. mRNA expression of the four adhesion molecules was upregulated in the pancreases during AP. Treatment with CP-96,345 effectively reduced the mRNA expression of P-selectin and E-selectin but not ICAM-1 and VCAM-1. In the lung, ICAM-1, E-selectin, and P-selectin mRNA expression increased during AP. Antagonist treatment suppressed this elevation. Similar expression patterns were seen in the immunohistochemical stainings. Intravital microscopy of the pancreatic microcirculation revealed the effect of CP-96,345 on leukocyte recruitment. The present study provides important information on the relationship between NK1R activation and the regulation of adhesion molecules. Also, this study points to the differential regulation of inflammation in the pancreas and lung with AP.

ACUTE PanCREATITIS (AP) is an acute inflammatory process of the pancreas, with variable involvement of other regional tissues or remote organ systems. It is a common inflammatory disorder with incidences that have increased significantly over the past few decades (1). Most cases are developed as a result of biliary disease or excess alcohol consumption. The severity of this disease ranges from mild, localized, self-limited pancreatic inflammation to fatal systemic inflammatory response syndrome. Present knowledge on the pathophysiology of AP has been reviewed recently (4). Despite advances in the knowledge of the etiologies and pathophysiology of AP, as well as advances in intensive care management for patients with severe AP, the mortality rate is still high, and improvement in treatment is still unsatisfactory (14).

Substance P (SP) and its receptor, neurokinin-1 receptor (NK1R), have been implicated in the pathogenesis of AP by mediating neurogenic inflammation (2–4, 18). Studies have shown that neurogenic inflammation mediated by the binding of SP to NK1R plays a crucial role in the pathogenesis of AP and pancreatitis-associated lung injury in mice and rats (12, 18, 23). Knockout mice deficient in SP and NK1R have been found to be resistant to the development of severe AP (2, 3). In addition, treatment with a NK1R antagonist has been shown to be effective in suppressing the progression of the inflammatory condition (17, 18). Recently, our laboratory has demonstrated that the NK1R antagonist CP-96345 was capable of suppressing several chemokine and cytokines levels in AP (33). Nevertheless, the understanding of the exact mechanism by which NK1R and SP amplifies the severity of pancreatitis remains to be elucidated and requires further investigations of the other components in the inflammation cascade.

Leukocyte recruitment is a hallmark feature of inflammation and is characterized by a sequence of events that bring about the extravasation of leukocytes through leukocyte-endothelial interactions. Upon activation, leukocytes roll and adhere to the endothelium through interactions between selectins (E-selectin and P-selectin) or adhesion molecules (ICAM-1 and VCAM-1) with their respective counterligands. This is followed by the emigration of leukocytes to the site of injury under the influence of chemotactic agents. It has been demonstrated that levels and expressions of ICAM-1, VCAM-1, P-selectin, and E-selectin are upregulated and enhanced in AP (21, 22, 34, 35). Immunoneutralization of adhesion molecules has been proven to be effective in the treatment of AP (37). The administration of monoclonal antibody against ICAM-1 to rats with acute severe pancreatitis significantly enhanced capillary blood flow in the pancreas, reduced leukocyte rolling, and stabilized capillary permeability (10). Moreover, blocking VCAM-1 decreased leukocyte adherence and recruitment into the lung, therefore reducing lung injury in severe AP (6). However, the relationship between NK1R blockage and leukocyte activation during AP is largely unknown.

In the present study, we investigated the effect of the NK1R antagonist CP-96,345 on mRNA and protein expressions of various adhesion molecules implicated during AP. In addition, we also studied the changes in leukocyte-endothelial interactions by intravital microscopy. This provided important information for the understanding of the role of SP and NK1R in the regulation of the downstream inflammatory cascades mediated by adhesion molecules during AP.

METHODS

Induction of AP. AP was induced in mice as previously described (17, 18). All experiments were approved by the animal ethics committee of the National University of Singapore and were performed in accordance with established International Guiding Principles for Animal Research. Caerulein was obtained from Bachem (Bubendorf, Switzerland). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Amylase activity. For the determination of MPO activity, plasma. These plasma samples were then kept for the determination of ventricles using a heparinized syringe and centrifuged to obtain surgical anaesthesia). Blood samples were drawn from the right caerulein injection, animals were killed by a lethal dose of pentobarbital (2, 3). Briefly, tissue samples were homogenized in 20 mM phosphate pancreas and lung was quantitated by measuring tissue MPO activity and optimal incubation conditions described (5). Total RNA from the pancreas and lungs was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol with modifications. Briefly, pancreatic or pulmonary tissues were isolated, immediately mounted and embedded in OCT matrix and further disrupted by sonication (40 s). The sample was then subjected to four cycles of freezing and thawing. The suspension was subjected to four cycles of freezing and thawing (70°C). For samples taken for immunohistochemistry, whole animals were perfused with PBS.

**Determination of plasma amylase activity.** Amylase activity was measured using a kinetic spectrophotometric assay as previously described using 4,6-ethylidene(G)-p-nitrophenyl(G)-1′-d-maltohexoside (Sigma, St. Louis, MO) as the substrate (2, 3).

**Determination of MPO activity.** Neutrophil sequestration in the pancreas and lung was quantitated by measuring tissue MPO activity (2, 3). Briefly, tissue samples were homogenized in 20 mM phosphate buffer (pH 7.4) and centrifuged (13,000 g, 10 min, 4°C), and the resulting pellet was resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecltrimethylammonium bromide (Sigma). The suspension was subjected to four cycles of freezing and thawing and further disrupted by sonication (40 s). The sample was then centrifuged (13,000 g, 5 min, 4°C), and the supernatant was used for the MPO assay. MPO activity was determined as previously described using tetramethylbenzidine (Sigma) as the substrate (2, 3). Results were expressed as activity per unit of dry weight (fold increase over control).

**RT-PCR.** RT-PCR experiments were carried out as previously described (5). Total RNA from the pancreas and lungs was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol with modifications. Briefly, pancreatic or pulmonary tissues were isolated and rapidly ground and homogenized in TRIzol reagent using a Potter homogenizer. The aqueous layer was obtained in several minutes (28). However, the alveolar thickening in inflamed lungs was slightly reduced due to the pressure exerted on the lung tissue during the infusion of the diluted OCT compound.

Sections of 7 μm of the pancreas or lungs were fixed in ice-cold acetone for 10 min, washed, and air dried. Sections were then incubated with blocking buffer [3% BSA in Tris-buffered saline (TBS)] for 1 h, and washed. This was followed by an incubation with the primary antibody diluted to the optimal working concentrations and in the optimal conditions (Table 2). After that, sections were reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) at 25°C for 5 min and 42°C for 30 min, followed by 85°C for 5 min. cDNA was used as a template for PCR amplification by iQTM Supermix (Bio-Rad). PCR primers (Table 1) for the detection of ICAM-1, VCAM-1, E-selectin, and P-selectin were synthesized by Prologo. The primers were intron spanning such that genomic DNA contamination was excluded. cDNA synthesized from 1 μg total RNA was included in a typical PCR. The reaction mixture was first subject to 95°C for 3 min for the activation of polymerase. This was followed by an optimal cycles of amplifications (Table 1), consisting of 95°C for 30 s, the optimal annealing temperature (Table 1) for 30 s, and 72°C for 30 s. PCR amplification was performed in MyCycler (Bio-Rad). PCR products were separated on 1% (wt/vol) agarose gels containing 0.05 mg/100 ml ethidium bromide. Images of the agarose gels were taken in a UVP GelDoc-It Imaging System (Upland) under an ultraviolet lamp. The semiquantitative analysis of the density of the cDNA bands was carried out using LabWorks software (Upland).

**Immunohistochemistry.** Immediately after PBS perfusion, the pancreas was harvested, mounted and embedded in OCT compound (Ames, Division of Miles Laboratories, Elkhart, IN) in liquid nitrogen, and stored at −70°C until being used. On the other hand, a mixture of OCT compound and PBS (1:2) was slowly infused into the trachea until the lungs were completely inflated, whereupon the lungs were isolated, immediately mounted and embedded in an OCT matrix in liquid nitrogen, and stored at −70°C until being used. Using this simple approach, sections of the lungs in the expanded state could be obtained in several minutes (28). However, the alveolar thickening in inflamed lungs was slightly reduced due to the pressure exerted on the lung tissue during the infusion of the diluted OCT compound.

Sections of 7 μm of the pancreas or lungs were fixed in ice-cold acetone for 5 min and 42°C for 30 min, followed by 85°C for 5 min. cDNA was used as a template for PCR amplification by iQTM Supermix (Bio-Rad). PCR primers (Table 1) for the detection of ICAM-1, VCAM-1, E-selectin, and P-selectin were synthesized by Prologo. The primers were intron spanning such that genomic DNA contamination was excluded. cDNA synthesized from 1 μg total RNA was included in a typical PCR. The reaction mixture was first subject to 95°C for 3 min for the activation of polymerase. This was followed by an optimal cycles of amplifications (Table 1), consisting of 95°C for 30 s, the optimal annealing temperature (Table 1) for 30 s, and 72°C for 30 s. PCR amplification was performed in MyCycler (Bio-Rad). PCR products were separated on 1% (wt/vol) agarose gels containing 0.05 mg/100 ml ethidium bromide. Images of the agarose gels were taken in a UVP GelDoc-It Imaging System (Upland) under an ultraviolet lamp. The semiquantitative analysis of the density of the cDNA bands was carried out using LabWorks software (Upland).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Optimal Conditions</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA</td>
<td>Sense: 5′-GTAACCGGTTGAAAGCCGATT-3′</td>
<td>Lung: 22 cycles</td>
<td>150</td>
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<td></td>
<td>Antisense: 5′-CCATCCCAATCGGATAGGCG-3′</td>
<td>Pancreas: 22 cycles</td>
<td></td>
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<tr>
<td>ICAM-1</td>
<td>Sense: 5′-CAACTGGAAGCTTGGAGCTG-3′</td>
<td>Lung: 33 cycles</td>
<td>437</td>
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<tr>
<td></td>
<td>Antisense: 5′-TAGCTGGAAGATGCAAAGTCG-3′</td>
<td>Pancreas: 35 cycles</td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Sense: 5′-CTCTGAGGTGACAGCTACGACGGT-3′</td>
<td>Annealing: 60°C</td>
<td>441</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5′-TTTCTGGCAATTCGTCAATGACGGG-3′</td>
<td>Pancreas: 36 cycles</td>
<td></td>
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<tr>
<td>E-selectin</td>
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<td>622</td>
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<tr>
<td></td>
<td>Antisense: 5′-TGCAAGCTAAGCGCCCTCATT-3′</td>
<td>Pancreas: 36 cycles</td>
<td></td>
</tr>
<tr>
<td>P-selectin</td>
<td>Sense: 5′-TACGAGCTTGACGAGGGACCCG-3′</td>
<td>Lung: 34 cycles</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5′-GGCTGAGCTACATTATTTTACAGC-3′</td>
<td>Pancreas: 36 cycles</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Immunohistochemistry antibody working dilutions and optimal incubation conditions**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Working Dilution</th>
<th>Optimal Conditions</th>
<th>Duration, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>1:200 (pancreas) and 1:8,000 (lung)</td>
<td>Humidified chamber at room temperature</td>
<td>2</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>1:40</td>
<td>Humidified chamber at room temperature</td>
<td>2</td>
</tr>
<tr>
<td>E-selectin</td>
<td>1:20</td>
<td>Humidified chamber at 4°C</td>
<td>24</td>
</tr>
<tr>
<td>P-selectin</td>
<td>1:20</td>
<td>Humidified chamber at 4°C</td>
<td>24</td>
</tr>
</tbody>
</table>
washed and incubated in horseradish peroxidase-conjugated second-
ary antibodies for 30 min at room temperature. Finally, slides were
treated with the chromogen 3,3-diaminobenzidine for 10 min, rinsed,
counterstained with hematoxylin, dehydrated in Histoclear, and cov-
erslipped. Negative controls were included by replacing the primary
antibody with nonimmune serum. All incubations and washes were
performed with TBS (3$	ext{H}11003$) and carried out at room temperature
(25°C). Sections were then examined under a light microscope.

Rat anti-mouse ICAM-1 (CD54) monoclonal antibody was pur-
chased from Chemicon. Rat anti-mouse VACM-1 (CD106) polyclonal
antibody was purchased from Serotec. Rabbit anti-mouse E-selectin
polyclonal antibody and goat anti-mouse P-selectin polyclonal anti-
body were obtained from Biovision.

Intravital microscopy. Microvascular changes of the pancreas dur-
ing AP were assessed by intravital microscopy as previously de-
scribed with modifications (13). Briefly, after 10 hourly caerulein
injections, animals were injected 0.1 ml of 0.01% rhodamine 6G
(Sigma) intravenously for the in vivo staining of leukocytes. Surgical
anesthesia was then induced by an intraperitoneal injection of a
mixture of ketamine and medetomindine (56 mg/kg ketamine and 0.75
mg/kg medetomindine). A midline laparotomy was performed, and
the pancreas with the duodenal loop was gently exteriorized and
placed in an immersion chamber containing Ringer solution main-
tained at 37°C. The pancreatic microcirculation was then examined
in epi-illumination under a fluorescence microscope (Eclipse PhysioSta-
tion E600FN, Nikon). Postcapillary venules (18–40 µm diameter) in
three different areas on the same pancreas were examined. Cells that
did not move or detach from the endothelium within a period of 30 s
were defined as adherent leukocytes (stickers), whereas those white
cells moving at a velocity less than two-thirds of that of the majority
of the cells were defined as rolling leukocytes (rollers). Videos were
captured via a CoolSNAP HQ monochrome camera (Roper Scientific)
and were analyzed using the Metamorph Imaging System (Molecular
Devices).

Statistics. Data are expressed as means ± SE. In all figures, vertical
error bars denote SEs. The absence of such error bars indicates that
the SE falls within the dimensions of the data bar. The significance of
differences between groups was evaluated by ANOVA with post hoc
Tukey’s test when three or more groups were compared. A P value
of <0.05 was considered to indicate a statistically significant differ-
ence.

RESULTS

Effect of CP-96,345 treatment on plasma amylase activity
and MPO levels in the pancreas and lungs. Evidence of
pancreatic injury in AP induced by an intraperitoneal admin-
istration of caerulein at a dose of 50 µg·kg$^{-1}$·h$^{-1}$ for 10 h was
confirmed by an increase in plasma amylase (Fig. 1A) and
an increase in the MPO activity in the pancreas and lungs

![Fig. 1. Effect of CP-96,345 administration on plasma amylase levels (A) and
MPO activity (B) in the pancreas (open bars) and lungs (shaded bars) in the
four treatment groups. The following groups are shown: control (saline
only), caerulein [10 consecutive hourly caerulein (50 µg·kg$^{-1}$·h$^{-1}$) injec-
tions], CP-96,345 prophylactic [CP-96,345 (2.5 mg/kg) 30 min before caer-
ulein injections], and CP-96,345 therapeutic [CP-96,345 (2.5 mg/kg) 60 min
after the first caerulein injection]. Histograms show mean values ± SE of at
least 8 animals. *Significantly different from the control group (P < 0.05);#
significantly different from the caerulein group (P < 0.05).

Fig. 2. Adhesion molecule mRNA expression in the pancreas (open bars) and
lungs (shaded bars) in the four treatment groups (control, caerulein, CP-96345
prophylactic, and CP-96345 therapeutic). A: ICAM-1; B: VCAM-1. Histograms
show mean values ± SE of at least 8 animals. *Significantly different from the
control group (P < 0.05); #significantly different from the caerulein group
(P < 0.05).
(Fig. 1B) in mice treated with caerulein compared with control mice treated with intraperitoneal normal saline. In animals administered CP-96,345 either 30 min before the first dose of caerulein or 1 h after the first injection of caerulein, plasma amylase levels and pancreatic water content were significantly attenuated compared with animals treated with caerulein alone. MPO activity in the pancreas was also reduced in both groups of mice treated with CP-96,345.

Effect of CP-96,345 treatment on pancreatic and pulmonary ICAM-1 mRNA and protein expression in AP. Figure 2A shows relative ICAM-1 mRNA levels in the pancreas and lungs. Hyperstimulation of the pancreas by caerulein resulted in an increase in ICAM-1 mRNA expression in both organs. Administration of CP-96,345 significantly suppressed elevated mRNA levels in the lung but had no effect on mRNA expression in the pancreas. This is in contrast with the immunohistochemistry staining results (Fig. 3), whereas, with prophylactic CP-96,345 treatment, there was an apparent reduction in the immunoreactivity at the endothelial layer of blood vessels in both the pancreas and lungs.

Effect of CP-96,345 treatment on pancreatic and pulmonary VCAM-1 mRNA and protein expression in AP. In the pancreas, 10 consecutive hourly injections of caerulein resulted in a 2.5-fold increase in VCAM-1 mRNA expression (Fig. 2B). When CP-96,345 was administered prophylactically, only a minimal, if any, decrease in VCAM-1 expression was observed. Immunohistochemistry staining, however, demonstrated a significant decline in VCAM-1 immunoreactivity.

Fig. 3. Immunohistochemistry staining of pancreas (A–D) and lung (E–H) sections (7 μm) using monoclonal anti-ICAM-1 antibody. A and E: control group; B and F: caerulein group; C and G: CP-96,345 prophylactic group; D and H: CP-96,345 therapeutic group. Scale bars = 20 μm. Arrowheads point to the areas where significant up/downregulation could be easily observed.
in the prophylactic treatment group (Fig. 4). In the lungs, RT-PCR and immunohistochemistry (Fig. 4) showed that VCAM-1 levels were not affected by both caerulein and CP-96,345 treatments.

Effect of CP-96,345 treatment on pancreatic and pulmonary E-selectin mRNA and protein expression in AP. E-selectin mRNA expression (Fig. 5A) correlated well with protein expression (Fig. 6). E-selectin mRNA and protein expression were not detected in the normal pancreas. During AP, E-selectin was upregulated and localized extensively on the endothelial layer of blood vessels in the pancreas and lungs as well as connective tissues between acinar cells in the pancreas. Reductions of E-selectin mRNA and protein expression in the pancreas and lungs were observed in the CP-96,345 prophylactic treatment group. Therapeutic treatment with the antagonist significantly reduced E-selectin mRNA expression in the lungs but not pancreas.

Effect of CP-96,345 treatment on pancreatic and pulmonary P-selectin mRNA and protein expression in AP. A twofold increase in P-selectin mRNA expression was observed in the pancreas during AP (Fig. 5B). Significant reductions in P-selectin expression were detected in mice treated with CP-96,345. Immunohistochemistry of P-selectin revealed a similar trend (Fig. 7). Increased P-selectin immunoreactivity was observed particularly in the endothelial layer and the surrounding connective tissues of blood vessels in the pancreas. In the lungs, P-selectin mRNA levels increased threefold during AP, and prophylactic antagonist treatment significantly

![Fig. 4. Immunohistochemistry staining of pancreas (A–D) and lung (E–H) sections (7 μm) using polyclonal anti-VCAM-1 antibody. A and E: control group; B and F: caerulein group; C and G: CP-96,345 prophylactic group; D and H: CP-96,345 therapeutic group. Scale bars = 20 μm. Arrowheads point to the areas where significant up/downregulation could be easily observed.](http://ajpgi.physiology.org/)
lowered mRNA expression (Fig. 5B). Increased expression of P-selectin in the blood vessel endothelial layer and surrounding connective tissue was noticed during AP, and the antagonist reduced P-selectin protein expression (Fig. 7).

Effect of CP-96,345 treatment on leukocyte activation in the pancreas in AP. Table 3 summarizes leukocyte rolling and adherent properties obtained through intravital microscopy examination of postcapillary venules in the pancreas. Caerulein hyperstimulation of the pancreas increased the numbers of rolling leukocytes by >25 times. In addition, these leukocytes were rolling at a significantly slower speed compared with the control group. As a result, more leukocytes adhered to the endothelium of the blood vessel.

On the other hand, prophylactic and therapeutic treatments with CP-96,345 have been shown to effectively suppress these proinflammatory behaviors of leukocytes. Both treatments successfully reduced the rolling and adherent leukocytes by ∼50%.

DISCUSSION

The recruitment of leukocytes to the site of tissue injury is an important component of inflammation. It is achieved by a complex interaction between adhesion molecules under the influence of various chemoattractants. This multistep process involves leukocyte rolling, adhesion, and emigration on the endothelium to reach the site of tissue injury. It is believed that leukocyte rolling is mediated by selectins (31) and leukocyte adhesion is regulated by integrins (36). Since treatment with CP-96,345, an NK1R-specific antagonist, has been effective in reducing the severity of AP (17, 18), it is likely that the treatment with the NK1R antagonist may affect the regulation of integrin-associated ligands and selectins. Therefore, we investigated the effect of NK1R blockage on the expression of integrin-associated ligands (ICAM-1 and VCAM-1) and selectins (E-selectin and P-selectin) in the pancreas and lungs during AP.

In the pancreas, ICAM-1, VCAM-1, E-selectin, and P-selectin mRNA and protein expression were upregulated during caerulein-induced AP. Previous studies have demonstrated similar findings (8, 11, 19). mRNA expression of ICAM-1 and VCAM-1 were not affected by the antagonist treatment. However, their immunoreactivities were slightly but significantly reduced with the antagonist treatment. ICAM-1 and VCAM-1 expression are known to link to the severity of organ injury in animal models of AP (21, 10, 38). ICAM-1, in particular, plays a crucial part in neutrophil adhesion to the endothelium (20, 39). On the other hand, both prophylactic and therapeutic treatments with CP-96,345 significantly reduced mRNA and protein expression of E-selectin and P-selectin, with prophylactic administration of the antagonist producing a stronger effect. Selectins have been identified as important markers of AP (24, 27). The E-selectin level has been shown to correlate well with the degree of organ dysfunction in AP patients, whereas the P-selectin level is significantly higher in the nonsurvivors (27). Together, these results may imply that the blockage of NK1R has a significant role on leukocyte rolling and adhesion in the pancreatic microcirculation. Therefore, it is clear that CP-96,345 treatment is effective in preventing the progression of the disease by interfering the expression of proinflammatory molecules.

To demonstrate the relationship between the expression of these adhesion molecules and the process of leukocyte recruitment into the inflamed pancreas, we studied rhodamine 6G-labeled leukocytes in the pancreatic microcirculation using intravital microscopy. Upon hyperstimulation by caerulein, the amount of rolling leukocytes in postcapillary venules increased tremendously (Table 3). These leukocytes have slower rolling speed and therefore a higher chance to adhere to the endothelium. CP-96,345 treatment has been shown to significantly reduce the number of rolling leukocytes and restore the rolling speed to normal. Although the number of adherent leukocytes was also lowered in the antagonist-treated groups, the reduction was more or less proportioned to the smaller number of rolling leukocytes. These results suggest that NK1R signaling has a more pronounced effect on leukocyte rolling than on leukocyte adherence.

One of the major causes of death in AP patients is lung injury that clinically manifests as acute respiratory distress syndrome. Therefore, we went on to study the effect of

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Fig. 5. E-selectin (A) and P-selectin (B) mRNA expression in the pancreas (open bars) and lungs (shaded bars) in the four treatment groups (control, caerulein, CP-96,345 prophylactic, and CP-96,345 therapeutic). Histograms show mean values ± SE of at least 8 animals. ND, not detected. *Significantly different from the control group (P < 0.05); #significantly different from the caerulein group (P < 0.05).
CP-96,345 treatment on the expression of four adhesion molecules in the lungs. ICAM-1, E-selection, and P-selectin mRNA and protein expression were significantly elevated during AP. Treatment with the NK1R antagonist effectively reduced the elevated levels, with prophylactic administration of the compound offering a stronger effect. However, pulmonary VCAM-1 expression was not affected during AP and CP-96,345 administration. It has been shown in a previous study that the treatment of another NK1R receptor antagonist, SR-140333, did not affect the VCAM-1 level in the lungs in a rat model of airway inflammation (16). We have previously shown evidence of a differential regulation of the neurokinin system in the pancreas and lungs in caerulein-induced AP (17). The results of the present study provide further evidence of the differential regulation of inflammatory responses in the pancreas and lungs in AP, especially in terms of the role of different adhesion molecules in the pancreas and lung.

The highly regulated process of leukocyte recruitment involves the participation of a wide range of adhesion proteins and signaling molecules. In the present study, there is a discrepancy between the increase in rolling leucocytes (observed in intravital microscopy) and the increase in the adhesion molecules expression (measured using immunohistochemistry and RT-PCR). It has been suggested that the protective effect of CP-96,345 is mediated by the change in the regulation of a number of adhesion molecules, and ICAM-1, VCAM-1, E-selectin, and P-selectin are only a few of the major contributors of the recruitment process. In fact, SP has

Fig. 6. Immunohistochemistry staining of pancreas (A–D) and lung (E–H) sections (7 μm) using polyclonal anti-E-selectin antibody. A and E: control group; B and F: caerulein group; C and G: CP-96,345 prophylactic group; D and H: CP-96,345 therapeutic group. Scale bars = 20 μm. Arrowheads point to the areas where significant up/downregulation could be easily observed.
Fig. 7. Immunohistochemistry staining of pancreas (A–D) and lung (E–H) sections (5 μm) using polyclonal anti-P-selectin antibody. A and E: control group; B and F: caerulein group; C and G: CP-96,345 prophylactic group; D and H: CP-96,345 therapeutic group. Scale bars = 20 μm. Arrowheads point to the areas where significant up/downregulation could be easily observed.

Table 3. Leukocyte rolling and adherent properties in postcapillary venules during acute pancreatitis and the effect of CP-96,345

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Caerulein</th>
<th>CP-96,345 Prophylactic</th>
<th>CP-96,345 Therapeutic</th>
</tr>
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<tbody>
<tr>
<td>Rolling leukocyte flux, cells/min</td>
<td>9.13±2.15</td>
<td>232.50±11.50*</td>
<td>102.00±12.42†</td>
<td>132.75±10.48†</td>
</tr>
<tr>
<td>Rolling velocity, μm/s</td>
<td>21.53±1.09</td>
<td>10.56±1.21*</td>
<td>25.32±6.08†</td>
<td>23.88±3.92†</td>
</tr>
<tr>
<td>Adherent leukocytes, cells/100 μm</td>
<td>0.33±0.21</td>
<td>3.67±0.33†</td>
<td>4.67±0.91†</td>
<td></td>
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</table>

Values are means ± SE. The following groups are shown: control (saline only), caerulein [10 consecutive hourly caerulein (50 μg · kg⁻¹ · h⁻¹) injections], CP-96,345 prophylactic [CP-96,345 (2.5 mg/kg) 30 min before caerulein injections], and CP-96,345 therapeutic [CP-96,345 (2.5 mg/kg) 60 min after the first caerulein injection]. *Significantly different from the control group (P < 0.05); †significantly different from the caerulein group (P < 0.05).
been shown to affect levels of other adhesion molecules such as lymphocyte function-associated antigen-1 (15, 29), α5-integrin (25), and complement receptor-associated OKM1 molecule (9). The role and regulation of these molecules are being studied in other inflammatory conditions, and further investigations on these molecules in AP are required.

In conclusion, the present results have shown that the NK1R antagonist CP-96,345 is effective in reducing pancreas and lung expression of adhesion molecules implicated in AP. It is clear that SP and NK1R play an important role in AP, and the NK1R antagonist represents a promising therapeutic tool for the treatment of this condition, an incurable clinical condition (2, 3, 18). In addition, these results have also demonstrated differential regulation of the expression of adhesion molecules in the pancreas and lungs in AP.

ACKNOWLEDGMENTS

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GRANTS

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