Cholecystokinin-8 Treatment of Pigs with Induced Acute Pancreatitis Significantly Reduces Acinar Necrosis and Edema of Pancreatic Tissue

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Abstract

Objective: Acute pancreatitis is an inflammatory process of the pancreas and a leading cause of hospitalization amongst gastrointestinal disorders. Previously, cholecystokinin (CCK) has been described to play a role in regeneration of pancreas. This study was undertaken to get more insights in the function of cholecystokinin octapeptide (CCK-8) during induced pancreatitis in an animal model.

Methods: In this study, acute pancreatitis was induced in 38 pigs. Two hours after the induction of acute pancreatitis, the animals were grouped according to the melatonin treatment into the following two groups: group 1/CCK - 8 group and group 2/non-CCK - 8 groups. Intraoperative clinical data, postoperative blood parameters and 'Porcine Well-Being' (PWB) score, as well as post-mortem histopathological data were analysed.

Results: At baseline, physiologically parameters of the pigs of both groups were comparable. No differences were observed regarding the overall survival of animals (p=0.97). Postoperative PWB score were significantly better in animals treated with CCK - 8 as compared to the control group (p=0.029). Moreover, histopathological analysis of the pancreatic tissue revealed that acinar necrosis and edema were significant reduced in the CCK - 8 group in comparison to the control group (p=0.016 and p=0.019).

Conclusion: CCK - 8 treatments reduces acinar necrosis and edema of pancreatic tissue after induction of an acute pancreatitis in pigs. Thus, it can be speculated that CCK - 8 may be useful as a therapeutic medical treatment of severe acute pancreatitis.

Keywords: Acute pancreatitis; Experimental model; Cholecystokinin - 8; CCK-8

Introduction

Acute pancreatitis is the leading gastrointestinal cause of hospitalization. In the majority of cases, acute pancreatitis manifests as a mild, self-limited course. However, in 15% to 25% of patients, it leads to tissue necrosis and infection with severe complications including endocrine and exocrine pancreatic insufficiency, organ failure, fistulae, bleeding, and death [1]. The gastrointestinal peptide cholecystokinin (CCK) acts physiologically on CCK receptors initiating various intracellular signaling pathways, which in turn result in enzyme/acid secretion, cellular proliferation and anti-apoptosis, and cell migration [2-6]. Intracellular signaling pathways activated involve the hydrolysis of phosphatidylinositol biphosphate by phospholipase C to generate inositol trisphosphate and diacylglycerol, which subsequently induce calcium mobilization and activation of protein kinase C[7]. Several of these pathways involve activation or cross talk with tyrosine kinase receptors and proliferative pathways associated with cell growth including mammalian target of rapamycin, Akt, and extracellular signal-regulated kinases [2]. In addition, CCK has been shown to play an important role in regulating pancreatic growth in animals and pancreatic regeneration [8-11]. This study was undertaken to get more insights in the clinical effect of CCK - 8 treatments in pigs after induced acute pancreatitis. Our data demonstrate that CCK - 8 reduces acinar necrosis and edema of pancreatitis tissue in pigs. Thus, it can be speculated that CCK - 8 may be useful as a therapeutic...
treatment of patients with severe acute pancreatitis.

**Research Design and Methods**

**Study design**

The study was approved by the Governmental Commission on the Care and Use of Animals of the City of Hamburg. The animals received care in compliance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 86 - 23, revised 1996). 38 pigs (German Hybrid) were included and randomized to two different treatment groups: group 1 (CCK - 8, n=18) and group 2 (non - CCK - 8; control group; n=20). Acute pancreatitis was induced in both groups but only the animals of the CCK – 8 groups were treated with CCK - 8.

**Surgical preparation**

After fasting overnight with free access to water, ketamine (10 mg/kg), midazolam (0.5 mg/kg), azapropazone (4 mg/kg) and atropine (0.0015 mg/kg) were administered for premedication. For monitoring of heart rate and oxygen saturation a 5-lead electrocardiogram and pulsed oximetry were used. After preoxygenation anaesthesia was induced by intravenous injection of 0.5 mg/kg midazolam. The animals were intubated and ventilated in a pressure - controlled mode assuring tidal volumes of 8 – 12 ml/kg and an endexpiratory pCO2 of 35 mmHg to 40 mmHg using an inspiratory oxygen concentration of 0.35 (Zeus, Draeger Medical Systems, Lübeck, Germany). Continuous infusion
of fentanyl (0.05 mg/kg/h) and sevoflurane (Fet 2.0) was used for balanced anaesthesia. After cleaning, shaving, disinfection and sterile draping, the femoral artery was cannulated using a 5 F thermistor tipped arterial catheter (PICCO, PV 2015L20, Pulsion, Germany) to avoid pancreatic pressure necrosis [17-19]. After a few minutes of equilibration the baseline values of all parameters (M0) were measured. According to the protocol the measurements includes blood gas analysis, measurement of tissue oxygenation (tpO2) and the microcirculation in the pancreatic head using a PiCCO plus monitoring system (version 6.0, Pulsion Medical Systems, Munich, Germany). Fluid management was identical for all animals. A basal infusion rate of 13 ml/kgBW/h was administered using hydroxyethyl starch 6% and Ringer’s solution at a fixed ratio of 1:2. Macrocirculation measurements includes blood gas analysis, measurement of tissue oxygenation (tpO₂) and the microcirculation in the pancreatic head using a PiCCO plus monitoring system (version 6.0, Pulsion Medical Systems, Munich, Germany). Fluid management was identical for all animals. A basal infusion rate of 13 ml/kgBW/h was administered using hydroxyethyl starch 6% and Ringer’s solution at a fixed ratio of 1:2. Macrocirculation

**Table 4: Results of blood test of animals.**

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
<th>CCK-8 group</th>
<th>non-CCK-8 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>8.7 ± 1.0</td>
<td>8.8 ± 0.6</td>
<td>8.6 ± 0.9</td>
<td>8.7 ± 1.0</td>
<td>9.1 ± 1.2</td>
<td>9.0 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>9.1 ± 1.2</td>
<td>9.0 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
</tr>
<tr>
<td>M2</td>
<td>8.7 ± 0.5</td>
<td>8.7 ± 0.7</td>
<td>8.5 ± 0.9</td>
<td>9.6 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>8.9 ± 1.1</td>
<td>8.9 ± 1.3</td>
<td>9.1 ± 0.92</td>
<td>8.9 ± 1.4</td>
<td>27.1 ± 2.1</td>
<td>26.9 ± 2.3</td>
<td>25.8 ± 2.5</td>
<td>29.5 ± 2.4</td>
<td>27.2 ± 2.2</td>
<td>26.8 ± 3.3</td>
</tr>
<tr>
<td>M8</td>
<td>8.7 ± 0.5</td>
<td>8.7 ± 0.7</td>
<td>8.5 ± 0.9</td>
<td>9.6 ± 0.8</td>
<td>9.1 ± 0.8</td>
<td>9.0 ± 0.8</td>
<td>8.9 ± 1.1</td>
<td>8.9 ± 1.3</td>
<td>9.1 ± 0.92</td>
<td>8.9 ± 1.4</td>
<td>27.1 ± 2.1</td>
<td>26.9 ± 2.3</td>
<td>25.8 ± 2.5</td>
<td>29.5 ± 2.4</td>
<td>27.2 ± 2.2</td>
<td>26.8 ± 3.3</td>
</tr>
<tr>
<td>day 1</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 2</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 3</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 4</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 5</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 6</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
<tr>
<td>day 7</td>
<td>15.3 ± 7.1</td>
<td>21.6 ± 10.1</td>
<td>13.8 ± 6.7</td>
<td>23.9 ± 12.8</td>
<td>25.9 ± 10.1</td>
<td>26.8 ± 6.0</td>
<td>26.3 ± 6.9</td>
<td>29.0 ± 8.9</td>
<td>28.8 ± 10.1</td>
<td>26.9 ± 8.3</td>
<td>16.2 ± 6.0</td>
<td>21.4 ± 9.0</td>
<td>15.9 ± 7.2</td>
<td>29.9 ± 10.1</td>
<td>26.8 ± 11.1</td>
<td>25.0 ± 8.3</td>
</tr>
</tbody>
</table>

**Table 5: Histopathological analysis of pancreatic tissue.**

<table>
<thead>
<tr>
<th>Acinar necrosis</th>
<th>Fatty tissue necrosis</th>
<th>Inflammation</th>
<th>Edema</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>group 1 (CCK-8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>2.5 (0.0-3.0)</td>
<td>0.7 (0.0-3.0)</td>
<td>1.7 (0.0-3.0)</td>
<td>2.7 (1.7-3.0)</td>
</tr>
<tr>
<td>M2</td>
<td>2.7 (0.3-3.0)</td>
<td>3.0 (1.0-3.0)</td>
<td>2.5 (1.0-3.0)</td>
<td>3.0 (2.0-3.0)</td>
</tr>
<tr>
<td><strong>group 2 (non-CCK-8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>2.5 (0.0-3.0)</td>
<td>0.7 (0.0-3.0)</td>
<td>1.7 (0.0-3.0)</td>
<td>2.7 (1.7-3.0)</td>
</tr>
<tr>
<td>M2</td>
<td>2.5 (0.3-3.0)</td>
<td>1.7 (0.0-3.0)</td>
<td>2.5 (1.0-3.0)</td>
<td>2.5 (1.0-3.0)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td><strong>p=0.016</strong></td>
<td><strong>p=0.068</strong></td>
<td><strong>p=0.305</strong></td>
<td><strong>p=0.019</strong></td>
</tr>
</tbody>
</table>
period of 30 min was allowed before the effects were measured every 60 min (M3 - 8). After the last intraoperative measurement (M8) all catheters were removed except the central venous catheter, that was subcutaneously tunneled to the dorsal neck of the pig for application of analgesic medication and blood gas testing in the postoperative course. The abdominal cavity and incision of the neck were closed and anesthesia was terminated. The animals were extubated and if sufficient spontaneous breathing was assured, they were transferred to heated boxes in the animal facility. For 7 days the animals were closely monitored and analgesics were given every 4 hr to 6 hr (piritramide 15 mg, equivalent to 10 mg morphine). Once a day blood samples and blood gas analysis were performed and the animals were evaluated for their fitness using two scores that had been used earlier by our group[20]. Animals surviving the observation period were re-anesthetized on the 7th postoperative day, and sacrificed by fast injection of potassium chloride during anesthesia. The pancreas was removed for histopathologic examination and molecular biological analysis. In animals that died during the postoperative course the pancreas was removed directly after death. Representative specimens of the pancreas were taken. Parts of each pancreatic area, that is, head, corpus, and tail were stored in 3.5% buffered formalin, separately. The tissues were then processed, embedded in paraffin and 5 µm slices were stained with hematoxylin and eosin. The slices were examined by an experienced pathologist. Specimens were examined by a treatment - blinded experienced pathologist. The histopathologic evaluation of the pancreatic lesions based on a previous publication[21]. Histopathologic changes were evaluated for each pancreatic area, that is, head, corpus, and tail, separately, and for each anatomic region a total score ranging from 0 (no alterations) to 12 (severe pancreatitis) was determined (Table 6).

### Statistical analysis

Statistical analysis was performed with SPSS® for Windows® (Version 22.0) (SPSS Inc., Chicago, IL). Descriptive analysis of parametric parameters is expressed as means and standard deviation. Ordinal data were expressed as median and range. For analysis of the difference between the groups in repeated measurements the variance analysis for repeated measurements (ANOVA) followed by a time - by - treatment -interaction test was used. Additionally, the area under curve was calculated during the intraoperative treatment (M2 to M8). Differences between the treatment groups were analysed using one-way ANOVA. Significance statements refer to p values of two-tailed tests that were less than 0.05.

### Results

#### Baseline characteristics

The animals were grouped according to the operative procedure into the following two groups: group 1/CCK - 8 group and group 2/non - CCK - 8 groups. A total of 18 animals were treated with CCK - 8, while a total of 20 animals were grouped to the control cohort. At baseline, the clinical characteristics of the animals of both groups were similar as demonstrated in Table 1. In detail, the mean length and weight were 98.4 cm and 30.2 kg of the CCK - 8 group and 98.3 cm and 30.7 kg of the control group 98.3 (p=0.95 and p=0.62).

#### Overall survival

No differences were observed regarding the overall survival of the pigs (CCK-8 group: 153h versus non-CCK-8 group: 144h; p=0.97) as demonstrated in (Table 1) and (Figure 1).

#### Tissue oxygenation of the pancreatic tissue

(Figure 2) and (Table 2) show the tissue oxygenation of the pancreas during the operative course of both groups (M0-M8). The oxygenation data were comparable in both analyzed groups (p=0.547).

#### Intraoperative hemodynamic data and blood test results

All animals were kept in stable hemodynamic conditions during the operation. The hemodynamic data are shown in Table 3 and the results of the blood tests in (Table 4). These data demonstrate that there were no significant differences concerning the analysed parameters of both animal cohorts.

<table>
<thead>
<tr>
<th>Table 6: Histopathological analysis of pancreatic tissue.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acinar necrosis</strong></td>
</tr>
<tr>
<td>0 = none</td>
</tr>
<tr>
<td>1 = &lt;10 single necrosis/lobule</td>
</tr>
<tr>
<td>2 = ≥10 single necrosis/lobule</td>
</tr>
<tr>
<td>3 = ≥2/3 of plane</td>
</tr>
</tbody>
</table>

*Figure 1: The overall survival of the pigs.*

*Figure 2: The tissue oxygenation of the pancreas during the operative course of both groups (M0-M8).*
Postoperative fitness and PWB score of the animals

As shown in (Table 5), there were no significant differences regarding the fitness score of animals which were treated with CCK - 8 compared to the animals of the control group (p=0.093), only at M10 significant advantages were detected. Interestingly, pigs of the CCK - 8 group had an increased PWB score as compared to the pigs of the control group (p=0.029). In detail, significant advantages were present at M10, M11 and M12.

Histopathological analysis

The histopathological analysis revealed that acinar necrosis and edema were significant reduced in the CCK - 8 group as compared to control group (p=0.016 and p=0.019) as demonstrated in (Table 6). The overall score showed a tendency to favourable results in the CCK - 8 group but missed statistical significance (p=0.062).

Discussion

CCK has been described to play a role in regeneration of pancreas. This study was undertaken to get more insights in the function of CCK - 8 during induced pancreatitis in an animal model. In summary, our data demonstrate that the treatment with CCK - 8 reduces acinar necrosis and edema of the pancreatic tissue and reduce the severity of the disease after experimental induction of acute pancreatitis. The majority of acute pancreatitis is mild and associated with a short time of hospitalization[1]. This mild form of acute pancreatitis is characterized by the absence of organ failure and/or pancreatic necrosis, while the severe form is associated with a systemic inflammatory response syndrome and/or organ failure [22]. The presence of organ failure and infected pancreatic necrosis is strongly correlated with the prognosis of patients[23]. Our data demonstrated that the overall survival rate was comparable in both analysed subgroups of pigs after the induction of acute pancreatitis. However, we observed an increased PWB score in pigs, which were treated with CCK - 8 as compared to the pigs of the control group. Previously, Jia et al. [24] had demonstrated that the most favorable strategy for the treatment of acute pancreatitis is to maintain the pancreas at rest during an early stage for only a short period, followed by pancreatic stimulation. Thus, it can be speculated that the treatment of the animals with CCK - 8 enhanced the recovery of pancreatic function. Pathophysiologically, inappropriate activation of pancreatic proenzymes within the gland itself leads to tissue and microvascular injury, release of pro-inflammatory mediators, and local inflammation [1]. During earlier stages of acute pancreatitis pro-inflammatory cytokines such as tumor necrosis factor α are produced by the pancreatic acinar cells and Interleukin -6 and -10 are expressed on the cells surface [25-27]. Moreover, anti - inflammatory cytokines are produced to inhibit the immune response, rendering the host at risk for systemic infection [28]. Interestingly, in our study the treatment of the animals with CCK - 8 was linked to a reduction of acinar necrosis and edema of the pancreatic tissue. Previously, Elsässer et al. [11] had demonstrated that CCK plays an important role in regulating pancreatic regeneration. Our data underline the assumption that CCK - 8 may have a positive effect on the recovery of the pancreas after the induction of an acute pancreatitis. However, the underlying biological mechanism remains elusive. An issue, worth to be discussed is the interval between induction of pancreatitis and beginning of treatment. In our study, the interval chosen was rather short. However to our understanding this seems to be adequate, because the direct intraductal injection of bile acid induces an acute pancreatitis within a few minutes, which is much faster than acute biliary pancreatitis found in the clinical situation [29,30]. In our experimental setting a severe acute pancreatitis was observed macroscopically in all animals prior to beginning of therapeutic intervention. If the interval between induction and beginning of the treatment is too long, the effect of improvement of the pancreatic microcirculation may fail to appear when fulminate necrosis are already present, as the rationale for the treatment approach is to improve microcirculatory perfusion and thereby save not yet irreversible injured tissue from infarction and necrosis [31,32]. In summary, we demonstrated that CCK-8 reduces acinar necrosis and edema of pancreatic tissue after induction of an acute pancreatitis. Thus, it can be speculated that CCK-8 may be useful as a therapeutic medical treatment of severe acute pancreatitis.

References