Clinics in Surgery

Research Article
Published: 20 Jun, 2018

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 - mortal 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

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Received Date: *24 May 2018*
Accepted Date: *11 Jun 2018*
Published Date: *20 Jun 2018*

Citation:
Grupp K, Bonk S, Poppe A, Seifert L, Wodack K, Trepte C, et al. Cholecystokinin-8 Treatment of Pigs with Induced Acute Pancreatitis Significantly Reduces Acinar Necrosis and Edema of Pancreatic Tissue. Clin Surg. 2018; 3: 1988.

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 bisphosphate 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

Table 1: Characteristics of animals.

Characteristics of animals	group 1 (CCK-8)	group 2 (non-CCK-8)	p
length (mean (SD))	98.4 (4.3)	98.3 (3.7)	0.94549
weight (kg)	30.2 (3.1)	30.7 (2.7)	0.615715
survival (h)	153 (138-168)	144 (119-168)	0.97

Table 2: Intraoperative tissue oxygenation (tp O2 mmHg) of the pancreas.

	Intraoperative										
	M0	M1	M2	M3	M4	M5	M6	M7	M8	AUC	MW
group 1 (CCK-8)	54.5 ± 20.3	17.4 ± 8.4	17.4 ± 20.0	15.4 ± 11.6	14.9 ± 9.3	14.6 ± 9.3	14.4 ± 9.4	17.6 ± 12.2	16.1 ± 11.3	93.7 ± 56.5	15.8 ± 9.8
group 2 (non-CCK-8)	54.9 ± 22.9	16.5 ± 13.4	14.6 ± 12.1	18.2 ± 13.4	19.1 ± 13.0	18.4 ± 12.7	17.9 ± 12.6	17.4 ± 12.3	17.3 ± 11.6	106.9 ± 71.6	17.5 ± 11.7

Table 3: Intraoperative hemodynamtic data.

		M0	M1	M2	M3	M4	M5	M6	M7	M8
HR	CCK8 group	70.57 ± 9.562623	73.533333 ± 10.899286	80 ± 19.271247	77.944467 ± 15.288512	75.422222 ± 12.909002	78.0 ± 13.8	78.1 ± 14.1	80.8 ± 19.9	87.3 ± 18.6
	non-CCK8 group	75.02 ± 9.301	83.7604 ± 14.75202	87.2031 ± 24.68215	83.75 ± 22.65866	82.2396 ± 15.56087	87.9 ± 20.9	91.0 ± 18.5	86.9 ± 16.0	94.7 ± 19.7
MAP	CCK8 group	55.177778 ± 6.301759	55.577778 ± 5.131937	56.644444 ± 7.047984	66.455555 ± 7.761951	70.916667 ± 11.510046	64.5 ± 6.6	67.0 ± 7.4	63.1 ± 7.2	61.4 ± 8.5
	non-CCK8 group	53.9531 ± 4.79486	55.8333 ± 5.98145	56.2552 ± 8.83121	68.4583 ± 9.96354	64.75 ± 7.26381	63.7 ± 6.9	60.9 ± 5.4	62.4 ± 5.4	63.0 ± 8.8
SVV	CCK8 group	8.577778 ± 3.300954	8.633333 ± 3.261244	9.733333 ± 4.42073	6.061111 ± 3.72258	5.161111 ± 1.970131	6.4 ± 2.1	5.7 ± 2.4	6.0 ± 3.0	7.3 ± 2.8
	non-CCK8 group	7.7708 ± 3.12154	8.6667 ± 4.66984	9.9167 ± 5.47249	5.3125 ± 2.89436	5.5417 ± 4.33397	5.5 ± 3.7	6.8 ± 3.3	5.9 ± 2.6	6.4 ± 2.8
PPV	CCK8 group	10.322222 ± 3.082507	10.233333 ± 3.147057	11.711111 ± 3.845976	7.2 ± 3.481927	6.572222 ± 2.106035	7.8 ± 2.0	7.4 ± 3.1	7.6 ± 3.0	8.7 ± 2.7
	non-CCK8 group	9.3385 ± 2.62969	10.2187 ± 4.90001	11.6302 ± 5.51286	6.625 ± 2.89796	6.9687 ± 3.87596	7.0 ± 3.1	7.9 ± 2.7	7.5 ± 2.5	7.9 ± 2.7
CVP	CCK8 group	4.3333 ± 3.35469	3.8444 ± 2.9083	3.5111 ± 2.61518	4.8667 ± 2.91901	5.2333 ± 2.86869	4.6 ± 3.0	5.1 ± 3.5	5.4 ± 3.3	4.4 ± 2.7
	non-CCK8 group	3.3958 ± 2.56029	2.7708 ± 1.75	3.4635 ± 2.62272	4.7708 ± 2.49954	4.1875 ± 2.55884	4.1 ± 2.6	3.9 ± 1.8	4.3 ± 2.1	4.6 ± 2.4
CI	CCK8 group	3.0939 ± 0.63962	2.9572 ± 0.58821	3.0769 ± 0.74022	3.9323 ± 0.69164	4.2896 ± 1.13674	4.1 ± 1.0	4.5 ± 1.0	4.3 ± 1.0	4.3 ± 1.2
	non-CCK8 group	3.3726 ± 0.37145	3.3786 ± 0.44087	3.4421 ± 0.46401	4.5444 ± 0.86329	4.4297 ± 0.73437	4.7 ± 1.0	4.6 ± 0.7	4.5 ± 0.6	4.9 ± 1.0
GEDI	CCK8 group	602.7 ± 139.65493	567.7889 ± 142.43827	548 ± 113.92458	631.5611 ± 137.39474	676.5111 ± 201.0056	657 ± 143	682 ± 124	675 ± 153	643 ± 132
	non-CCK8 group	610.2333 ± 77.71723	581.6 ± 78.37681	575.6111 ± 99.52793	656.7111 ± 93.29624	653.744 ± 77.355674	650 ± 76	638 ± 100	642 ± 99	642 ± 93
ELWI	CCK8 group	19.3778 ± 5.18678	19.9778 ± 5.33886	19.9111 ± 5.51112	19.8056 ± 5.27457	20.8667 ± 6.47192	21.4 ± 4.3	21.3 ± 3.3	21.2 ± 3.4	21.0 ± 3.6
	non-CCK8 group	19.0167 ± 1.72637	19.4667 ± 1.69406	19.3444 ± 1.87132	20.4889 ± 2.13388	20.4222 ± 1.37706	21.0 ± 1.1	20.4 ± 1.9	21.3 ± 2.3	20.4 ± 1.7
SVI	CCK8 group	44.4 ± 8.62715	41.4667 ± 7.3212	39.1556 ± 7.56125	52.3111 ± 9.76751	56.7889 ± 13.10132	55.5 ± 11.3	59.5 ± 10.5	56.3 ± 11.4	52.1 ± 9.7
	non-CCK8 group	44.6042 ± 4.40575	41.2083 ± 6.17627	40.7604 ± 8.71907	55.0625 ± 7.27372	55.0938 ± 5.13303	55.4 ± 5.8	54.9 ± 9.0	54.9 ± 6.8	52.6 ± 6.0
SVRI	CCK8 group	1362.6333 ± 256.7463	1427.2111 ± 277.35371	1458.8 ± 340.47142	1272.1778 ± 281.21285	1269.9667 ± 324.90056	1176 ± 246	1117 ± 237	1088 ± 220	1084 ± 215
	non-CCK8 group	1258.2396 ± 180.98867	1281.5938 ± 184.42518	1270.9427 ± 222.04696	1156.5625 ± 179.18609	1097.5885 ± 161.15545	1014 ± 183	949 ± 209	1009 ± 141	994 ± 237
PVPI	CCK8 group	3.0956 ± 0.57788	3.3878 ± 0.40161	3.4689 ± 0.48263	2.9794 ± 0.33054	2.9244 ± 0.337	3.1 ± 0.5	3.0 ± 0.4	3.0 ± 0.4	3.1 ± 0.4
	non-CCK8 group	2.9974 ± 0.4549	3.2051 ± 0.40135	3.2417 ± 0.52319	2.9718 ± 0.43266	3.0276 ± 0.4514	3.1 ± 0.4	3.1 ± 0.5	3.1 ± 0.4	3.1 ± 0.4

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), azaperone (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 endexperiatory pCO_2 of 35 mmHg to 40 mmHg using an inspiratory oxygen concentration of 0.35 (Zeus, Draeger Medical Systems, Lübeck, Germany). Continuous infusion

Table 4: Results of blood test of animals.

		Intraoperative			Postoperative						
		M0	M2	M8	day 1	day 2	day 3	day 4	day 5	day 6	day 7
Hemoglobin	CCK-8 group	8,7 ± 1,0	8,8 ± 0,6	8,6 ± 0,9	9,7 ± 0,9	9,5 ± 1,1	9,8 ± 1,7	9,2 ± 0,7	9,1 ± 0,7	8,9 ± 0,6	9,0 ± 0,8
	non-CCK-8 group	8,7 ± 0,5	8,7 ± 0,7	8,5 ± 0,9	9,6 ± 0,8	9,1 ± 0,8	9,0 ± 0,8	8,9 ± 1,1	8,9 ± 1,3	9,1 ± 0,92	8,9 ± 1,4
Hematocrit	CCK-8 group	27 ± ,5	27,5 ± 2,8	26,1 ± 3,1	29,2 ± 2,8	28,2 ± 3,5	29,3 ± 5,1	28,2 ± 2,5	27,5 ± 3,0	28,5 ± 1,4	27,6 ± 1,9
	non-CCK-8 group	27,1 ± 2,1	26,9 ± 2,3	25,6 ± 2,5	29,5 ± 2,4	27,2 ± 2,2	27,0 ± 2,3	26,8 ± 3,3	26,8 ± 4,3	28,5 ± 3,0	28,3 ± 4,0
Leuco-cytes	CCK-8 group	15,3 ± 7,1	21,6 ± 10,1	13,8 ± 6,7	23,9 ± 12,8	25,9 ± 10,1	26,6 ± 8,0	26,3 ± 6,9	29,0 ± 8,9	28,8 ± 10,1	26,9 ± 8,3
	non-CCK-8 group	16,2 ± 6,0	21,4 ± 9,0	15,9 ± 7,2	29,9 ± 10,1	26,8 ± 11,1	25,0 ± 8,3	23,1 ± 10,8	29,0 ± 12,6	29,9 ± 12,7	25,2 ± 9,6
Thrombo-cytes	CCK-8 group	343 ± 73	328 ± 269	331 ± 88	347 ± 104	364 ± 138	373 ± 127	413 ± 112	466 ± 197	501 ± 122	395 ± 195
	non-CCK-8 group	411 ± 121	406 ± 113	388 ± 97	426 ± 91	355 ± 131	457 ± 179	446 ± 117	477 ± 161	542 ± 130	488 ± 160
Amylase	CCK-8 group	1757 ± 524	3729 ± 1026	5156 ± 1849	12005 ± 4554	8723 ± 3210	6951 ± 6144	6015 ± 5914	5261 ± 6849	5939 ± 7786	3703 ± 2459
	non-CCK-8 group	1725 ± 438	3129 ± 745	4285 ± 1364	12019 ± 8253	6642 ± 2608	4521 ± 2542	3471 ± 1885	3227 ± 2303	2809 ± 2176	3978 ± 5137
Lipase	CCK-8 group	13 ± 0	155 ± 75	290 ± 134	455 ± 256	262 ± 141	176 ± 96	180 ± 167	186 ± 298	216 ± 298	179 ± 248
	non-CCK-8 group	13 ± 0	119 ± 58	232 ± 97	435 ± 401	208 ± 122	120 ± 83	109 ± 95	127 ± 143	106 ± 132	82 ± 93
Lactate	CCK-8 group	1.9 ± 0.7	1.5 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.2 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
	non-CCK-8 group	1.9 ± 0.7	1.5 ± 0.5	1.4 ± 0.3	1.2 ± 0.4	1.2 ± 0.3	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.3
Calcium	CCK-8 group	1.35 ± 0.06	1.22 ± 0.06	1.31 ± 0.07	1.30 ± 0.06	1.24 ± 0.06	1.39 ± 0.04	1.40 ± 0.01	1.34 ± 0.04	1.30 ± 0.05	1.33 ± 0.06
	non-CCK-8 group	1.31 ± 0.07	1.24 ± 0.09	1.32 ± 0.08	1.31 ± 0.05	1.36 ± 0.06	1.38 ± 0.05	1.37 ± 0.06	1.35 ± 0.05	1.31 ± 0.05	1.32 ± 0.05

Table 5: Histopathological analysis of pancreatic tissue.

	Acinar necrosis	Fatty tissue necrosis	Inflammation	Edema	Overall
group 1 (CCK-8)	2.5 (0.0-3.0)	0.7 (0.0-3.0)	1.7 (0.0-3.0)	2.7 (1.7-3.0)	7.1 (2.7-12.0)
group 2 (non-CCK-8)	2.7 (2.0-3.0)	2.0 (1.0-3.0)	2.5 (1.0-3.0)	3.0 (2.0-3.0)	9.7 (8.3-12.0)
P	**p=0.016**	p=0.166	p=0.305	**p=0.019**	p=0.062

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)) for advanced hemodynamic monitoring. Two central venous catheters were surgically introduced into the internal and external jugular vein for volume administration and injection of cold indicator for trans-cardiopulmonary thermodilution 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% 130/0.4 and Ringer's solution at a fixed ratio of 1:2. Macrocirculation was assessed continuously and maintained identically in all animals during the entire procedure according to an established algorithm for goal-directed fluid management[12-14]. Body temperature was kept constant between 38°C to 39°C using forced-air warming and a heating pad. After repositioning of the pigs into supine position, a gastric tube was placed and the abdomen was opened by a transverse upper laparotomy. A urinary catheter was placed directly into the bladder for urinary drainage. The pancreas and duodenum were mobilized and fixed at the laparotomy incision for intraoperative measurements. After dissection and cannulation of the main pancreatic duct (Vasofix 0,8 mm, B. Braun, Melsungen, Germany) between pancreas and duodenal wall a flexible polarographic measuring probe (CCP1, Licox, Kiel, Germany) for continuous measurement of the tissue oxygen tension (tpO_2) was placed in the pancreatic head [15,16]. 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 (tpO_2) and the microcirculation in the pancreatic head with a laser Doppler imager (LDI, Moore, UK) [17]. Afterwards acute necrotizing pancreatitis was introduced by intraductal infusion of glycodeoxycholic acid (GDOC, 10 mmol/l, pH8, Sigma - Aldrich, St. Louis, MO, USA) over a period of 15 min as previously described, using an automated infusion system (Perfusor® fm (MFC), B Braun, Melsungen, Germany) to avoid pancreatic pressure necrosis [17-19]. The cannula was removed and the pancreatic duct was ligated. 60 minutes (M1) and 120 (M2) min after completion of the intraductal infusion measurements were repeated. Directly after M2, the animals of group 1 (CCK treatment) received a bolus of 0,5 µg/kg KG CCK - 8 (CCK8, Sigma - Aldrich, Chemie GmbH, Germany) was applied via the central venous catheter. After start of the therapy a stabilization

Table 6: Histopathological analysis of pancreatic tissue.

Acinar necrosis	Fatty tissue necrosis (in relation to plane)	Inflammation (plasma cells, lymphocytes, granolocyts)	Edema
0 = none	0 = none	0 = none	0 = none
1 = <10 single necrosis/lobule	1 = < 1/3 of plane	1 = loose infiltrates (≤30 cells/HPF)	1 = interlobular edema
2 = ≥10 single necrosis/lobule	2 =≥1/3 to <2/3 of plane	2 = moderate infiltrates (> 30; ≤ 100 cells/HPF)	2 = interlobular edema ≥ 2 lobules
3 =≥1/3 of plane	3 = ≥ 2/3 of plane	3 = dense infiltrates (> 100 cells/HPF)	3 = interlobular edema ≥ 2 lobules

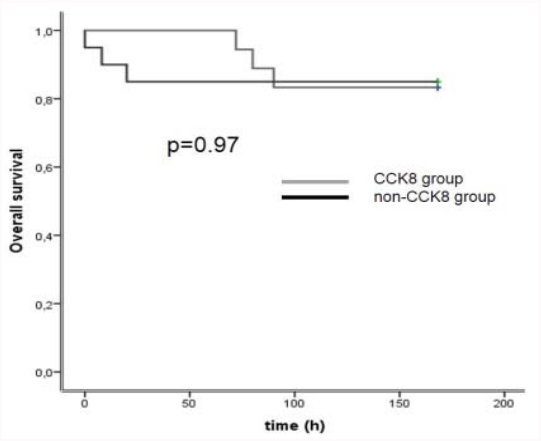

Figure 1: The overall survival of the pigs.

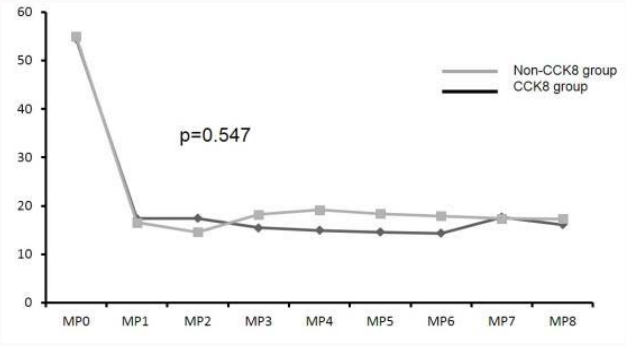

Figure 2: The tissue oxygenation of the pancreas during the operative course of both groups (M0-M8).

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 (piritamide 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.

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 Interleucin - 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.

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