Original Paper

Pancreatology

Pancreatology 2006;6:232–239
DOI: 10.1159/000091962

Received: June 6, 2005
Accepted after revision: October 31, 2005
Published online: March 9, 2006

Improvement of Impaired Microcirculation and Tissue Oxygenation by Hemodilution with Hydroxyethyl Starch plus Cell-Free Hemoglobin in Acute Porcine Pancreatitis

Marc Freitag[a] Thomas G. Standl[a] Helge Kleinhans[b] André Gottschalk[a] Oliver Mann[b] Christian Rempf[a] Kai Bachmann[b] Andreas Gocht[c] Susan Petri[c] Jakob R. Izbicki[b] Tim Strate[b]

Departments of [a]Anesthesiology, [b]Surgery and [c]Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Key Words

Acute pancreatitis · Hemodilution · Bovine hemoglobin · Pancreatic tissue oxygen tension · Pancreatic microcirculation

Abstract

Aims: To avoid the progression from mild edematous acute pancreatitis (AP) to the severe necrotizing form, one therapeutic option is to improve pancreatic microcirculation and tissue oxygenation. The aim of the study was to evaluate the influence of improved rheology (isovolemic hemodilution) plus enhanced oxygen supply (bovine hemoglobin HBOC-301) on pancreatic microcirculation, tissue oxygenation and survival in severe acute experimental pancreatitis. ***Methods:*** Severe AP was induced in 39 pigs (25–30 kg BW) by stimulation with intravenous administration of cerulein plus a pressure- and volume-controlled 10-min intraductal infusion of glycodeoxycholic acid. Seventy-five minutes after induction of AP, animals were randomized and hemodiluted isovolemically (PAOP constant) with either 10% hydroxyethyl starch (HES) 200,000/0.5 plus HBOC-301 (+0.6 g/dl plasmatic hemoglobin; Oxyglobin®, Biopure, Cambridge, Mass., USA), or 10% HES 200,000/0.5, or Ringer's solution to a hematocrit of 15%. Hemodynamics, oxygen transport parameters, pancreatic microcirculation and tissue oxygen tension were evaluated over 6 h. Then the abdomen was closed, animals were extubated and observed for 6 days. After that, the surviving animals were sacrificed and specimens were taken from the pancreas. The histopathologic findings were scored by two blinded pathologists who quantified acinar necrosis, fat necrosis, inflammation and edema. ***Results:*** Isovolemic hemodilution with HES plus HBOC-301 reduced mortality and preserved pancreatic microcirculation compared with Ringer's solution, but was not significantly different from hemodilution with HES alone. Only treatment with HES plus HBOC-301 normalized pancreatic tissue oxygen tension compared with IHD with HES or Ringer's solution alone. ***Conclusions:*** IHD with HES plus HBOC-301 as a combination of rheologic and O_2-delivering therapy may represent a novel therapeutic option for treatment of AP.

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Dr. Marc Freitag
Department of Anesthesiology
University Hospital Hamburg-Eppendorf, Martini Strasse 52
DE–20246 Hamburg (Germany)
Tel. +49 40 42803 2415, Fax +49 40 42803 4963, E-Mail Freitag@uke.uni-hamburg.de

Introduction

Acute pancreatitis (AP) is still a life-threatening disease burdened with high mortality when it progresses from the self-limiting mild edematous to the more severe hemorrhagic necrotizing form [1, 2]. The disturbance of the pancreatic microcirculation, and as a consequence decreased tissue oxygenation, induces progression from mild to severe pancreatitis independent of the early intracellular events, including protease activation. Specific therapy must be directed towards microperfusion failure and impaired tissue oxygenation as a secondary pathogenetic step, since the initial enzyme activation and cytokine release are irreversible at the time of clinical presentation [3, 4]. Preservation or improvement of pancreatic microcirculation and oxygenation could possibly prevent further progression, tissue necrosis and severe or even lethal complications thus decreasing morbidity and mortality [5]. In experimental designs, preservation of the pancreatic microcirculation in AP has been achieved by improving blood rheology using hemodilution with colloids [6]. Our group studied the beneficial effect of improved microcirculation and an increase in pancreatic oxygen tensions using a cell-free oxygen carrier (HBOC-301) in rats [7]. Both therapeutic principles have proven beneficial in the past but have not been combined up to now.

The aim of this study was to evaluate the therapeutic effect of isovolemic hemodilution with hydoxyethyl starch plus HBOC-301 compared with hydroxyethyl starch or Ringer's solution alone on pancreatic microcirculation, tissue oxygen tension, tissue damage and outcome in experimental porcine AP.

Material and Methods

After approval of the Ethics Committee of the Hamburg Federal Board of Veterinary Medicine and Animal Care, 39 pigs (German hybrid program; body weight: 25–30 kg) entered the study. After 36 h of fasting with free access to water and 20% glucose up to 12 h before induction, animals were premedicated with 10 mg/kg Ketamine (Ketanest™, Atarost, Twistingen, Germany), 4 mg/kg Azaperone (Stresnil™, Janssen-Cilag, Neuss, Germany), and 0.015 mg/kg atropine sulfate (Atropin™, B. Braun, Melsungen, Germany) intramusculary (i.m.).

Induction of Anesthesia and Monitoring

The animals were connected with a 6-lead electrocardiograph, pulse oximetry for noninvasive monitoring of heart rate and oxygen saturation (Marquette, Milwaukee, Wisc., USA). Anesthesia was induced with 0.5 mg/kg midazolam (Dormicum™, Janssen-Cilag, Neuss, Germany) and 0.05 mg/kg fentanyl (Fentanyl-Janssen™, Janssen-Cilag), intravenously. The animals were intubated (6.0–7.0 Ch) and mechanically ventilated (FIO_2 0.3–0.35) to keep arterial pO_2 >90 mm Hg (ABD 725 Blood-Gas-Analyser, Radiometer, Copenhagen, Denmark). End-expiratory pCO_2 was continuously controlled (Normocap®, Datex, Helsinki, Finland) and maintained at 35–40 mm Hg. Balanced anesthesia was maintained by 0.05 mg/kg/h fentanyl) and isoflurane (1–1.5 vol%) (Forene™, Abbott, Wiesbaden, Germany). Animals were fixed on their backs, their necks shaved and draped sterilely. After a 10-cm skin incision along the right sternocleidomastoid muscle and separation of the platysma, the internal and external jugular veins and carotid artery were isolated. A Shaldon catheter (12 F Certofix Trio SB 1225, B. Braun, Melsungen, Germany) was introduced into the external jugular vein for isovolemic hemodilution. Thereafter, an 8 F lock was introduced into the internal jugular vein to allow the insertion of a thermodilution pulmonary catheter (7 F, 110 cm, Baxter, Munich, Germany). The catheter was advanced until pulmonary artery occlusion pressure (PAOP), pulmonary arterial pressure (PAP) and central venous pressure (CVP) were monitored correctly at the respective position. Cardiac output, cardiac index (CI), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated as previously described [8].

Core temperature was measured continuously by the pulmonary artery catheter. Afterwards, an arterial catheter was placed into the carotid artery allowing continuous monitoring of systolic, diastolic and mean arterial pressure. The skin was temporarily closed and stable body temperature of 38°C was maintained using a heating lamp, heating pad and warmed Ringer's solution (Boehringer, Ingelheim, Germany) was given continuously over time to maintain a PAOP of 9 ± 1 mm Hg in all animals until randomization.

Laparotomy and Induction of AP

The abdomen was opened though a transverse upper abdominal laparotomy. To avoid blood pooling, splenectomy was performed. A catheter was placed into the bladder for urinary drainage. The pancreas and duodenum were mobilized thereafter to allow the distal cannulation of the pancreatic duct (Vasofix 0.8 mm, B. Braun, Melsungen, Germany). Then a flexible Licox probe (pO_2 microprobe, GMS, Kiel, Germany) was positioned into the tissue of the pancreatic corpus in order to measure tissue oxygen tension (tpO_2) directly. The abdomen was partially closed to minimize fluid loss and 30 min were allowed for equilibration. This was followed by baseline measurements (M0).

Afterwards severe AP was initiated by intraductal infusion of 0.4 ml/kg glycodeoxycholic acid (GDOC) (10 mmol/l, pH 8, Sigma, Steinheim, Germany) at a perfusion pressure of <25 mm Hg over 10 min. This was paralleled by continuous intravenous infusion of cerulein at 5 μg/kg/h (Takus™, Pharmacia & Upjohn, Erlangen, Germany) for 1 h [9]. The end of intraductal injection of GDOC defined the beginning of AP. Fifteen minutes were allowed for equilibration before the first measurement after the initiation of AP was made (M1) followed by the second measurement 60 min later (M2). This model was validated before [10]. In addition, 2 animals were sham operated. The pancreatic duct was cannulated, but no AP was induced.

Pancreatic Tissue Oxygen Tension

Pancreatic tpO_2 was measured using a Licox probe (pO_2 microprobe, GMS, Kiel, Germany). At the distal end of this flexible probe which was placed in the pancreatic tissue there is an opening over

a length of 1.2 cm, with a pO_2-sensitive area, where tpO_2 is measured by the polarographic method. At each time of measurement (M0–8) 120 single measurements were recorded (one measurement every 5 s over 10 min). The mean of the plotted values (histograms) was used for statistical calculation.

Pancreatic Microcirculation

To assess pancreatic microcirculation, a laser-Doppler scanner was used (Laserdoppler Imager 5137, Moore, Millway, UK), which measures microvascular blood flow in a depth of 2–3 mm with reflected Doppler shifted light. Microcirculation was expressed in flux (arbitrary unit, moving particles/surface) [11, 12]. Following completion of all other measurements, the same area including the pancreatic head was scanned in apnea to avoid movement artifacts.

Randomization and Therapy

After M2, the cannula was removed from the pancreatic duct and the defect was closed. Animals were randomly allocated to three groups using a computer-generated randomization list (SPSS Inc., Chicago, Ill., USA). Isovolemic hemodilution (IHD) was started from a hematocrit of 30%, which was the baseline hematocrit of the pigs, aiming at a hematocrit of 15%, which is equal to a 45–50% reduction of the initial hematocrit. This reduction of the initial hematocrit was chosen according to recently published studies from our group [8] and another group [13].

Blood was replaced in increments of 50 ml by hydroxyethyl starch (HES) (10% HAES-steril 200,000/0.5, Fresenius, Bad Homburg, Germany) in groups 1 (HES/HBOC) and 2 (HES) and with Ringer's solution (4 times the removed blood volume) in group 3 (Ringer) to guarantee a constant PAOP of 9 ± 1 mm Hg. Animals in group 1 additionally received a dose of ultrapurified, polymerized, bovine hemoglobin HBOC-301 (Oxyglobin™, Biopure, Cambridge, Mass., USA) to ensure a plasmatic hemoglobin concentration of +0.6 g/dl during the experiment. The amount of HBOC-301 was calculated according to the following formula: HBOC-301 (ml) = body weight × 0.6/ 0.13 [14], which resulted in an amount between 115 and 140 ml of infused HBOC-301. This dose was chosen based on data from a prior study [15] which found that an augmentation of +0.6 g/dl HBOC-301 was highly effective in restoring tissue oxygenation to baseline values after significant reduction by profound hemodilution. HBOC-301 has a hemoglobin concentration of 13 ± 1 $g \cdot dl^{-1}$ (methemoglobin and oxyhemoglobin = 10%) and an oncotic pressure of 17 mm Hg. HBOC-301 was prepared from bovine red cells by lysis, filtration, chromatography and polymerization with glutaraldehyde (65,000 < MW < 500,000). The sterile pyrogen-free solution contains <0.5 $EU \cdot ml^{-1}$ endotoxin and <3 n*M* phospholipids and physiological concentrations of electrolytes.

After 30 min of equilibration, the next measurements followed at 60-min intervals (M3–8).

Survival and Histology

After the last measurement (M8), all catheters were removed, defects were closed and the abdomen was lavaged thoroughly using 6 liters of Ringer's solution. The abdomen and neck were closed and analgesia was ensured with piritramide 22 mg every 6 h if necessary (15 mg piritramide is equivalent to 10 mg of morphine) [16]. Anesthesia was terminated and after spontaneously breathing, animals were extubated and transferred to heated boxes with free access to water. All animals were closely monitored by a veterinarian for the next 6 days and fed with porcine standard diet (UNA-Hakra, Hamburg, Germany) after postoperative day 2.

Table 1. Histology score describing tissue damage of severe porcine AP

Acinar necrosis	
0	nil
1	<10 single necrosis/lobule
2	≥10 single necrosis/lobule
3	>1/3 of plane
Fatty tissue necrosis (in relation to plane)	
0	nil
1	<1/3 of plane
2	≥1/3 of plane
3	≥2/3 of plane
Inflammation (plasma calls, lymphocytes and granulocytes outside parenchymal and fatty tissue necrosis)	
0	nil
1	loose infiltrates (≤ 30 cells/HPF)
2	moderate infiltrates (>30; ≤ 100 cells/HPF)
3	dense infiltrates (>100 cells/HPF)
Edema	
0	nil
1	interlobular edema
2	interacinar edema, ≥ 2 lobules
3	intercellular edema, ≥ 2 lobules

Range (sum): 0 points (no pancreatitis) – 12 points (severe pancreatitis). HPF = High power fields.

After 6 days, the animals were killed by intracardial injection of T61 ad us vet.™ (Intervet, Unterschleissheim, Germany) and after immediate laparotomy, the pancreas was removed and placed in 3.5% buffered formalin, routinely processed, and embedded in paraffin embedded. The histopathologic findings were scored as previously described [17].

Two 5-μm slices were taken from two areas of each pancreas around the median portion. The slices were stained with hematoxylin and eosin. The slides were examined in a blinded fashion by two pathologists using light microscopy and evaluated using a scoring system (table 1). A mean score (two slices of one area) was calculated and the results were added (sum of two different areas: range: 0–24). In case an animal died within the observational period, the abdomen was opened immediately and pancreatic tissue processed accordingly.

Power Calculation and Statistical Analysis

Analysis was performed using SPSS for windows 11.0 (SPSS Inc., Chicago, Ill., USA). The primary endpoint was survival, secondary endpoint parameters were microcirculation, tissue oxygenation and tissue damage (histology score). Since there are no studies on animal survival in severe AP after hemodilution, an educated guess suggested a group size of 13 animals (detectable difference of 20 vs. 80%, 5% alpha error, 80% power) [18].

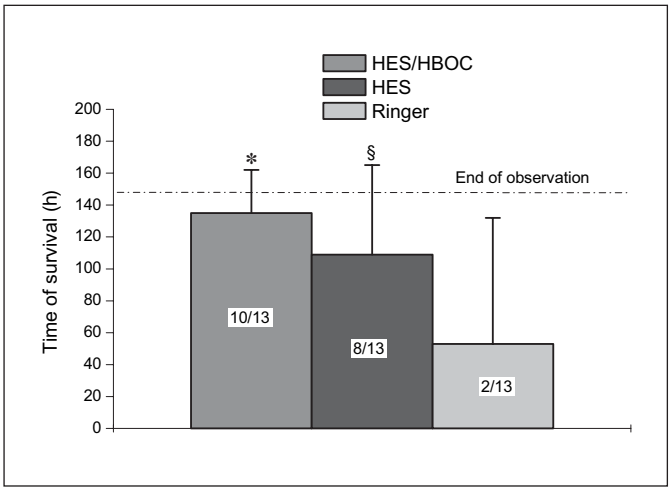

Fig. 1. Mean survival time at the end of the observation period (144 h) in each group. Numbers in the columns indicate the number of animals which survived until postoperative day 6. Mean ± SD; * $p < 0.01$ HES/HBOC vs. Ringer, § $p < 0.05$ HES vs. Ringer.

Descriptive analysis of parametric data is expressed as means and standard deviation (SD). Ordinal data are expressed as medians and range. Normal distribution of data was tested with the Kolmogorov-Smirnov test. Survival rate was compared using the χ^2 test. Statistical significance was evaluated using the Mann-Whitney U test for nonnormally distributed data and the unpaired Student's t test for normally distributed data. The level of significance was set to $p < 0.05$.

Results

The sham-operated animals showed no differences with regard to hemodynamics, oxygen transport and laboratory parameters compared with baseline. Nor did microcirculation, tissue oxygen tension differ compared with baseline. The animals survived for 6 days and the pancreas showed no tissue damage using the previously described score.

Survival

All animals survived the experiment (anesthesia and surgery). The mean survival time at the end of the observation period is given in figure 1. Of the HBOC-301 treated animals, 10 survived the observational period and 3 died on the 3rd postoperative day. In the HES group, 5 animals died (2 on day 0, 1 on the 1st, 3rd, and 5th day) while in the Ringer group only 2 animals survived (8 animals died on day 0, 2 on the 1st and 1 on the 3rd postoperative day). Survival rate was different between groups ($p < 0.001$) with a significant difference between HES/HBOC and Ringer ($p = 0.002$; χ^2 test), and between HES and Ringer ($p = 0.016$), but no difference between HES/HBOC and HES.

Hemodynamics

Hemodynamic variables are given in table 2. PAOP as a parameter for isovolemic conditions did not change over time in any group. Nor were differences in heart rate seen between the groups. After IHD, the MAP decreased in the HES and Ringer groups and was lower compared with the HES/HBOC group ($p < 0.001$). SVR also decreased after IHD in the HES and Ringer groups and was lower than in the HES/HBOC group ($p < 0.001$).

PAP and PVR increased after IHD in the HES/HBOC group compared with baseline from M5 in the Ringer group. PAP and PVR were higher in the HES/HBOC than in the HES group ($p < 0.05$), and after M5 also higher in Ringer than in HES group ($p < 0.05$).

Laboratory Parameters

The activities of amylase and lipase were below 400 U/l respectively 20 U/l before the induction of pancreatitis. Slight nonsignificant differences between groups in the rise of amylase and lipase activities up to 4,000 and 500 U/l following induction were observed between the experimental groups (data not shown).

Oxygen Transport

Initial hemoglobin concentrations at baseline (M0) were not different between groups (range: 9.7–10.4 $g \cdot dl^{-1}$). After isovolemic hemodilution (M2) the total hemoglobin concentrations were also not different between the groups (range: 5.2–5.8 $g \cdot dl^{-1}$; including the plasma hemoglobin concentration provided by the HBOC-301 in the HES/HBOC group). Temperature and arterial blood gases did not change in any group over time. There were no differences among groups with regard the other variables pertaining to the global oxygen transport like arterial and mixed-venous oxygen content and arterio-venous oxygen difference. Oxygen consumption and oxygen extraction ratio were higher in M5 and M6 in the Ringer than in the HES/HBOC group ($p < 0.05$).

Tissue Oxygenation

The changes of pancreatic tissue oxygen tensions (tpO_2) are given in figure 2. After induction of AP the pancreatic tpO_2 decreased in all groups (M0 vs. M2 and M3) and increased significantly after IHD in the HES/

Table 2. Hemodynamic variables

	MAP mm Hg	PAP mm Hg	CI $l \cdot min^{-1} \cdot m^{-2}$	SVR $dyn \cdot s^{-1} \cdot cm^{-5}$	PVR $dyn \cdot s^{-1} \cdot cm^{-5}$
HES/HBOC					
Baseline	76 ± 3	17 ± 2	4.9 ± 0.7	1,551 ± 319	155 ± 32
M1	74 ± 4	18 ± 3	4.7 ± 0.7	1,511 ± 292	189 ± 53
M2	72 ± 4	19 ± 2	5.0 ± 0.8	1,418 ± 326	188 ± 45
M3	81 ± 6*, **	22 ± 4*, **	5.3 ± 0.6*	1,528 ± 232*, **	227 ± 56*, **
M4	78 ± 8*, **	21 ± 4*	5.5 ± 0.5	1,332 ± 216*, **	215 ± 41*
M5	78 ± 10*, **	21 ± 3*	5.3 ± 0.7	1,331 ± 203*, **	210 ± 65*
M6	74 ± 8*, **	21 ± 3*	5.1 ± 0.6*	1,326 ± 241*, **	235 ± 43*
M7	74 ± 7*, **	22 ± 2*	5.1 ± 0.5*	1,277 ± 174*, **	238 ± 54*
M8	71 ± 7*, **	21 ± 2*, **	5.5 ± 1.0	1,201 ± 350*, **	216 ± 55*
HES					
Baseline	72 ± 12	16 ± 3	4.6 ± 0.8	1,345 ± 265	142 ± 54
M1	72 ± 11	17 ± 3	4.5 ± 1.0	1,375 ± 364	155 ± 57
M2	68 ± 11	18 ± 2	4.5 ± 1.0	1,343 ± 243	183 ± 56
M3	64 ± 8	19 ± 3	6.2 ± 1.3	913 ± 192	143 ± 39
M4	62 ± 7	18 ± 3	6.1 ± 1.4	862 ± 240	129 ± 44
M5	59 ± 5	18 ± 3***	5.8 ± 1.3	877 ± 153	134 ± 35***
M6	59 ± 4***	18 ± 2***	6.0 ± 1.1***	819 ± 156	124 ± 40***
M7	59 ± 6***	17 ± 2***	6.1 ± 0.8	839 ± 145	124 ± 31***
M8	58 ± 5	18 ± 2***	5.9 ± 1.0	798 ± 121	131 ± 42***
Ringer					
Baseline	72 ± 12	16 ± 4	4.5 ± 1.4	1,361 ± 213	154 ± 61
M1	70 ± 4	18 ± 2	4.4 ± 1.0	1,439 ± 324	181 ± 48
M2	69 ± 13	19 ± 2	4.7 ± 1.2	1,412 ± 380	197 ± 62
M3	60 ± 13	18 ± 4	5.7 ± 1.5	1,037 ± 296	158 ± 58
M4	58 ± 6	19 ± 5	5.4 ± 1.4	995 ± 443	161 ± 83
M5	54 ± 7	21 ± 3	5.2 ± 1.2	883 ± 98	208 ± 85
M6	50 ± 9	22 ± 4	4.9 ± 0.7	887 ± 166	184 ± 88
M7	50 ± 8	24 ± 5	5.2 ± 1.0	935 ± 440	191 ± 60
M8	52 ± 11	24 ± 2	5.8 ± 1.9	787 ± 217	215 ± 54

MAP = Mean arterial pressure; PAP = mean pulmonary artery pressure; CI = cardiac index; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance.

Mean ± standard deviation; * $p < 0.05$ HES/HBOC vs. HES; ** $p < 0.05$ HES/HBOC vs. Ringer; *** $p < 0.05$ HES vs. Ringer.

HBOC treated group compared with the HES and Ringer groups ($p < 0.001$). TpO_2 remained decreased in the HES and Ringer groups and further decreased at M7 ($p = 0.046$) and M8 ($p = 0.015$) in the Ringer group resulting also in lower values when compared to HES at these times.

Microcirculation

The changes of pancreatic microcirculation are given in figure 3 and show a decreased microcirculation after induction of AP (M0 vs. M2 and M3) in all groups. After therapy, the microcirculation improved in the HES/HBOC and HES groups compared with the Ringer group ($p < 0.001$). During the whole observation period, the flux remained decreased in the Ringer group and was only significantly higher in the HES/HBOC group compared with the HES group at M8 ($p = 0.017$).

Histology

There was a significant difference between the groups regarding acinar necrosis ($p = 0.001$; Kruskal-Wallis test), inflammation ($p = 0.002$) and total score ($p = 0.002$) (table 3). Comparing the groups (Mann-Whitney U test), greater tissue damage in the Ringer group vs. HES/HBOC was detected regarding acinar necrosis (median 5.5 vs. 3; range 4–6 vs. 0–5; $p = 0.001$) and inflammation (4 vs. 4;

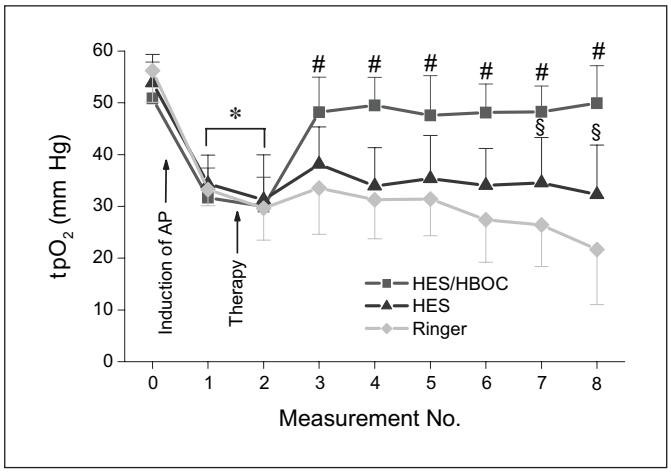

Fig. 2. Changes of pancreatic tissue oxygen tensions (mm Hg) before and after induction of AP. M0 = before induction of AP, M1 and M2 = after induction of AP, M3–8 = 1–5.5 h after therapy. Median ± SD; * $p < 0.001$ M1 and M2 vs. baseline; # $p < 0.001$ HES/HBOC vs. HES and Ringer; § $p < 0.05$ HES vs. Ringer.

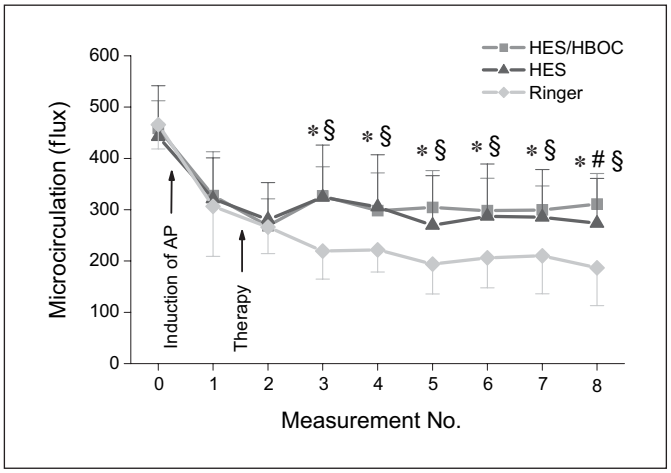

Fig. 3. Changes of pancreatic microcirculation before and after induction of AP. M0 = before induction of AP, M1 and M2 = after induction of AP, M3–8 = 0.5–5.5 h after therapy. Mean ± SD; * $p < 0.05$ HES/HBOC vs. Ringer; # $p < 0.05$ HES/HBOC vs. HES; § $p < 0.05$ HES vs. Ringer.

Table 3. Comparison of groups regarding acinar necrosis, fatty tissue necrosis, inflammation and edema

Group (n = 13)	Acinar necrosis (0–6)*, ***	Fatty tissue necrosis (0–6)	Inflammation (0–6)*, **	Edema (0–6)	Overall score (0–24)*, ***
HES/HBOC	3 (0–5)	1 (0–5)	3 (1–4)	4 (2–5)	11 (4–17)
HES	3 (0–5)	1 (0–3)	4 (3–5)	4 (2–4)	12 (7–15)
Ringer	5.5 (4–6)	3 (1–5)	4 (3–5)	4 (3–5)	16 (14–20)

* $p < 0.05$ HES/HBOC vs. Ringer; ** $p < 0.05$ HES/HBOC vs. HES; *** $p < 0.05$ HES vs. Ringer.

3–5 vs. 1–4; p = 0.006). This resulted in a higher overall score in the Ringer group (16 vs. 11; 14–20 vs. 4–17; p = 0.009). Comparing HES vs. Ringer, less acinar necrosis (3 vs. 5.5; 0–5 vs. 4–6; $p < 0.001$) was detected, which resulted in a lower overall score (12 vs. 16; 7–15 vs. 14–20; p = 0.001). Comparing HES/HBOC vs. HES, the only detectable difference was less inflammation in the HBOC-treated animals (3 vs. 4; 1–4 vs. 3–5; p = 0.002).

Discussion

Our animal model of severe AP very closely mimics the clinical course of severe AP characterized by high mortality, enables evaluation of therapeutic effects on micro- and macrocirculation and allows survival (therefore outcome) studies (6-day mortality ranges from 23 to 84% depending on the therapy). Significant evidence has accumulated from experimental and clinical studies suggesting that multiple organ failure is the cardinal cause of mortality in severe pancreatitis [19], which was also the cause of death in our animals. Many models of AP have limitations for the examination of a novel therapy [20]. Whereas some models produce mild self-limiting pancreatitis, which is not suitable for outcome studies, others result in sudden necrotizing injury. Pros and cons of different models have been extensively discussed [21].

In first reports of hemodilution as treatment option for AP, a canine model was chosen that used dextran to im-

prove pancreatic blood flow and pancreatic microcirculation [22, 23], and effective fluid resuscitation was monitored by rather limited parameters (mean arterial pressure and heart rate). For hemodilution a sufficient blood volume is absolutely necessary and isovolemia during hemodilution is very important [13]. We chose PAOP as a more accurate parameter for isovolemic conditions and therefore a large-animal model was chosen.

A novel idea is the additional use of plasmatic oxygen carriers for therapy of AP, because these components are able to improve the impaired oxygenation of pancreatic tissues.

In our experiment, only hemodilution using HES plus HBOC-301 was able to normalize pancreatic tissue oxygen tensions and reduce tissue damage (inflammation) compared with IHD with HES and Ringer solution alone, although the plasmatic Hb concentration was low and total Hb concentration did not differ among groups. Oxygen delivery, oxygen consumption and oxygen extraction ratio as global oxygen transport parameters did not show any differences either. At the same time, only the HBOC-301-treated group showed vasoconstrictor effects indicated by a higher SVR and MAP compared with the other groups. This is caused by the well-known NO-scavenging effect of HBOC [24]. Thus the higher perfusion pressure as a consequence of the higher MAP might be an explanation for the higher tpO_2 in the HES/HBOC group. However, data from Federspiel [25] and Page et al. [26] suggest that free hemoglobin in the plasma phase may enhance oxygen off-loading to the tissues by functionally reducing the intracapillary space between erythrocytes and endothelium thus facilitating oxygen diffusion. This plasmatic oxygen pathway may overcome potential vasoconstrictor effects which have been demonstrated to be associated with the use of hemoglobin solutions. The intensity of vasoconstriction seems to be dependent on the specific features of each hemoglobin formulation and may be related to the degree of purification and tetrameric stabilization [27, 28]. There is evidence that HBOC has an impact on organ blood flow and distributive oxygen transport in the microcirculation which is different from the vasoconstrictive effects on larger vessels [29]. Sherman et al. [30] reported that systemic vasoconstriction after HBOC administration did not reduce mean regional blood flow in the hamster liver and our group showed that tissue areas with significantly reduced tpO_2 profit the most by the infusion of acellular hemoglobin [31]. Another interesting alternative for further experiments in the future in relation to the adverse vasoconstricting effects of most HBOC could be the use of artificially nitrosylated hemoglobin derivates, which have vasodilator properties [32, 33].

Looking at the data of this experiment, no differences between the animals treated with HES/HBOC and those treated with HES could be detected in terms of survival time and rate but oxygenation of the pancreatic tissue in the HES/HBOC was improved compared with the HES group. This might have two reasons: on the one hand there is the possibility that there are no true differences regarding survival time and rate, on the other hand it can be speculated that further differences were not detected because of a low statistical power. In the HES/HBOC-treated group the mean survival time was 130 h (SD 32; 95% CI 110/150), in the HES group, 110 h (SD 57; 95% CI 75/144), a difference of 20 h. At the same time the HES/HBOC group showed a trend towards lower mortality (23 vs. 38%). It is hard to decide whether a difference in survival time of 20 h has a true clinical significance, but the difference in mortality (23 vs. 38%), if it was a true difference, would have an enormous clinical impact. To verify this difference (23 vs. 38%), 165 animals in each group would have been necessary (5% alpha error, 80% power) [18]. Our study aimed at detecting a much larger difference in mortality, which was indeed detected between HES/HBOC and HES compared with Ringer.

The same argument holds true regarding microcirculation. Figure 3 shows a statistically significantly improved microcirculation in the HES/HBOC-301-reated animals after 6 h of therapy. This tendency is paralleled by improved tissue oxygenation in the HES/HBOC-treated group compared with HES and Ringer.

In essence, treatment of experimental severe AP in pigs with hemodilution using HES and addition of a plasmatic oxygen carrier like cell-free bovine hemoglobin HBOC-301 preserved pancreatic microcirculation, reduced mortality and improved pancreatic tissue oxygenation; these effects were paralleled by decreased tissue damage. However, reduced mortality could also be obtained with hemodilution with HES alone. Nevertheless IHD and additional application of HBOC-301 as combination of rheologic and O_2-delivering therapy may represent a novel therapeutic option for the treatment of AP. In this regard, more experimental data and studies with a larger sample size are needed to study the potential and (patho-) physiology of an additional effect of HBOC-301 in the setting of severe AP.

References

1 Lankisch PG, Burchard-Reckert S, Petersen M, Lehnick D, Schirren CA, Kohler H, Stockmann F, Peiper HJ, Creutzfeldt W: Morbidity and mortality in 602 patients with acute pancreatitis seen between the years 1980–1994. Z Gastroenterol 1996;34:371–377.

2 Runzi M, Layer P: Etiology, pathogenesis and pathophysiology of acute pancreatitis. Schweiz Med Wochenschr 1997;127:849–853.

3 Lankisch PG, Blum T, Maisonneuve P, Lowenfels AB: Severe acute pancreatitis: when to be concerned? Pancreatology 2003;3:102–110.

4 Knoefel WT, Kollias N, Warshaw AL, Waldner H, Nishioka NS, Rattner DW: Pancreatic microcirculatory changes in experimental pancreatitis of graded severity in the rat. Surgery 1994;116:904–913.

5 Kusterer K, Poschmann T, Friedmann A, Enghofer M, Zendler S, Usadel KH: Arterial constriction, ischemia-reperfusion, and leukocyte adherence in acute pancreatitis. Am J Physiol 1993;265:165–171.

6 Klar E, Mall G, Messmer K, Herfarth C, Rattner DW, Warshaw AL: Improvement of impaired pancreatic microcirculation by isovolemic hemodilution protects pancreatic morphology in acute biliary pancreatitis. Surg Gynecol Obstet 1993;176:144–150.

7 Strate T, Mann O, Kleinhans H, Schneider C, Knoefel WT, Standl T, Bloechle C, Izbicki JR: Systemic intravenous infusion of bovine hemoglobin significantly reduces microcirculatory dysfunction in experimentally induced pancreatitis in the rat. Ann Surg 2003;238:765–771.

8 Freitag M, Standl T, Horn EP, Wilhelm S, Schulte am Esch J: Acute normovolaemic haemodilution beyond Hct 25%: The ratio of skeletal muscle tissue oxygen tension and cardiac index is not maintained in healthy dogs. Eur J Anaesth 2002;19:487–496.

9 Bloechle C, Kusterer K, Kuehn R, Schneider C, Knoefel WT, Izbicki JR: Inhibition of bradykinin B2 receptor preserves microcirculation in experimental pancreatitis in rats. Am J Physiol 1998;274:42–51.

10 Strate T, Freitag M, Kleinhans H, Schneider C, Mann O, Yekebas E, Standl T, Knoefel WT, Izbicki JR: A new porcine model of severe acute pancreatitis. Pancreas 2002;25:450.

11 Essex TJ, Byrne PO: A laser Doppler scanner for imaging blood flow in skin. J Biomed Eng 1991;13:189–194.

12 Liu DL, Svanberg K, Wang I, Anderson-Engels S, Svanberg S: Laser Doppler perfusion imaging: New technique for determination of perfusion and reperfusion of splanchnic organs and tumor tissue. Lasers Surg Med 1997;20:473–479.

13 Kreimeier U, Messmer K: Hemodilution in clinical surgery: state of the art 1996. World J Surg 1996;20:1208–1217.

14 Liard JF, Kunert MP: Hemodynamic changes induced by low blood oxygen affinity in dogs. Am J Physiol 1993;264:396–401.

15 Standl T, Horn EP, Wilhelm S, Greim C, Freitag M, Freitag U, Sputtek A, Jacobs EE, Schulte am Esch J: Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs. Can J Anesth 1996;43:714–723.

16 Kumar N, Rowbotham DJ: Piritramide. Br J Anaesth 1999;82:3–5.

17 Strate T, Mann O, Kleinhans H, Rusani S, Schneider C, Yekebas E, Freitag M, Standl T, Bloechle C, Izbicki JR: Microcirculatory function and tissue damage is improved after therapeutic injection of bovine hemoglobin in severe acute rodent pancreatitis. Pancreas 2005;30:254–259.

18 Fleiss JL: Statistical Methods for Rates and Proportions, ed 2. New York, Wiley, 1981.

19 Yekebas EF, Strate T, Zolmajd S, Eisenberger CF, Erbersdobler A, Saalmuller A, Steffani K, Busch C, Elsner HA, Engelhardt M, Gillesen A, Meins J, The M, Knoefel WT, Izbicki JR: Impact of different modalities of continuous veno-venous hemofiltration on sepsis-induced alterations in experimental pancreatitis. Kidney Int 2002;62:1806–1818.

20 Schmidt J, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL: A better model of acute pancreatitis for evaluating therapy. Ann Surg 1992;215:44–56.

21 Schneider A, Whitcomb DC, Singer MV: Animal models in alcoholic pancreatitis – what can we learn? Pancreatology 2002;2:189–203.

22 Donaldson LA, Williams RW, Schenk WG Jr: Experimental pancreatitis: Effect of plasma and dextran on pancreatic blood flow. Surgery 1978;84:313–321.

23 Klar E, Herfarth C, Messmer K: Therapeutic effect of isovolemic hemodilution with dextran 60 on the impairment of pancreatic microcirculation in acute biliary pancreatitis. Ann Surg 1990;211:346–353.

24 Hughes GS, Antal EJ, Locker PK, Francom SF, Adams WJ, Jacobs EE: Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996;24:756–764.

25 Federspiel WJ: Pulmonary diffusing capacity: implications of two-phase blood flow in capillaries. Respir Physiol 1989;77:119–134.

26 Page TC, Light WR, McKay CB, Hellums JD: Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 1998;55:54–64.

27 Biro GP, Taichman GC, Lada B, Keon WJ, Rosen AL, Sehgal LR: Coronary vascular actions of stroma-free hemoglobin preparations. Artif Organs 1988;12:40–50.

28 Gilroy D, Shaw C, Parry E, Olding-Smee W: Detection of a vasocontrictor factor in stroma-free haemoglobin solutions. J Trauma 1988;28:1312–1316.

29 Gulati A, Sharma AC, Burhop KE: Effect of stroma-free hemoglobin and diaspirin cross-linked hemoglobin on the regional circulation and systemic hemodynamics. Life Sci 1994;55:827–837.

30 Sherman IA, Dlugosz JA, Perelman V, Hsia CJ, Wong LT, Condie RM: Systemic hemodynamic and hepatic microvascular responses to a 33% blood volume exchange with whole blood, stroma-free hemoglobin, and oxypolyhemoglobin solutions. Biomater Artif Cells Immobilization Biotechnol 1993;21:537–551.

31 Standl T, Freitag M, Burmeister MA, Horn EP, Wilhelm S, am Esch JS: Hemoglobin-based oxygen carrier HBOC-201 provides higher and faster increase in oxygen tension in skeletal muscle of anemic dogs than do stored red blood cells. J Vasc Surg 2003;37:859–865.

32 Gladwin MT, Lancaster JR Jr, Freeman BA, Schechter AN: Nitric oxide's reactions with hemoglobin: a view through the SNO-storm. Nat Med 2003;9:496–500.

33 Deem S, Hedges RG, McKinney S, Polissar NL, Alberts MK, Swenson ER: Mechanisms of improvement in pulmonary gas exchange during isovolemic hemodilution. J Appl Physiol 1999;87:132–141.