ANNALS OF SURGERY
Vol. 229, No. 4, 514-522
© 1999 Lippincott Williams & Wilkins, Inc.

Influence of Zero-Balanced Hemofiltration on the Course of Severe Experimental Pancreatitis in Pigs

Emre F. Yekebas, MD,* Hendrik Treede, MD,* Wolfram T. Knoefel, MD,* Christian Bloechle, MD,* Edwin Fink, MD,† and Jakob R. Izbicki, MD*

*From the *Department of Surgery, University Hospital Eppendorf, Hamburg, Germany, and the †Division of Clinical Chemistry, Department of Surgery, University of Munich, Germany*

Objective
To examine the impact of continuous venovenous hemofiltration (CVVH) on the course of experimental pancreatitis in pigs.

Summary Background Data
The activation of different mediator cascades is assumed to trigger multiple organ dysfunction or failure during necrotizing pancreatitis. CVVH has been suggested to be beneficial in those instances by eliminating several inflammatory mediators released in the circulation.

Methods
Pancreatitis was induced by a combined intraductal injection of sodium taurocholate and enterokinase. Control group animals received no treatment after induction. A second group underwent "therapeutic" CVVH after a 20% decline of mean arterial pressure. In the third group, "prophylactic" CVVH was started simultaneously with the induction of pancreatitis.

The concentrations of tumor necrosis factor-alpha, transforming growth factor-beta$_1$, kinin, and phospholipase A_2 were measured at different time points in blood (pre- and postfilter) and in the hemofiltrate to calculate the respective sieving coefficients that reflect most accurately the plasma clearance of mediators by CVVH.

Results
Survival time was significantly prolonged both by therapeutic and prophylactic CVVH; it was more pronounced in the latter. CVVH did not influence the increase in transforming growth factor concentrations. However, 6 hours after induction, the increases of plasma concentrations of tumor necrosis factor, phospholipase, and kinin were significantly weakened by CVVH compared with controls. In the treatment groups, the plasma concentrations of tumor necrosis factor and phospholipase showed a significant negative correlation with the respective sieving coefficients, which decreased in the later course of the experiments.

Conclusions
Experimental necrotizing pancreatitis was associated with a tremendous increase of plasma concentrations of tumor necrosis factor, phospholipase, and kinin. The effective removal of these mediators by CVVH resulted in significantly improved survival time. Animals that received prophylactic CVVH had a longer survival period than those in which CVVH was started after clinical impairment. The decreasing efficiency of CVVH in eliminating inflammatory mediators in the later course of the experiments suggested that the filter membranes were compromised by long-term application. These findings provide further evidence that CVVH offers therapeutic options even in the absence of conventional indications for blood-purifying treatments.

Because appropriate fluid resuscitation has been accepted for early therapy in acute pancreatitis, hypovolemic shock is no longer responsible for deaths from this disease. However, the development of extensive necrosis, resulting in multiple organ dysfunction and septic complications, still remains a devastating event with a poor prognosis. Death occurs in approximately 20% to 25% of patients with severe necrotizing pancreatitis.[1] The mortality rate increases to approximately 95% in patients with three or more organ failures.[2]

Metabolic waste products and diverse mediators that are liberated into the circulation are assumed to play a predominant role in the pathophysiology of multiple organ dysfunction or failure in the course of severe pancreatitis.

Supported by a grant of the "Forschungs-und Studienstiftung der Vereinigung nordwestdeutscher Chirurgen."
Correspondence: Jakob R. Izbicki, MD, Abteilung für Allgemeinchirurgie, Martinistr. 52, D-20246 Hamburg, Germany.
Accepted for publication October 12, 1998.

Because of the complex interplay between those mediators, treatments have been suggested with the goal of their nonselective elimination to prevent devastating systemic complications after the onset of pancreatitis. Clinical and experimental data suggest that hemofiltration might be of considerable benefit for treatment of multiple organ dysfunction secondary to sepsis[3–7] as well as to severe pancreatitis.[8,9]

The aim of the present study was therefore to evaluate the effect of continuous venovenous hemofiltration (CVVH) on the plasma concentrations and activities of inflammatory mediators in a porcine model of severe necrotizing pancreatitis. Moreover, we investigated whether the removal of pancreatitis-related mediators by CVVH would result in an improvement of the early multiorgan dysfunction that occurs in the course of the disease.

METHODS

Animal Experiments

The study followed the criteria of German legislation on the protection of laboratory animals and was approved by the Animal Care Committee of the University of Hamburg.

Anesthesia and Surgical Preparation

Thirty-five fasted domestic pigs with a body weight of 37 ± 7 kg were premedicated with ketamine (10 mg/kg), flunitrazepam (0.1 mg/kg), and atropine (0.06 mg/kg) given intramuscularly. After endotracheal intubation, the animals were ventilated mechanically with a mixture of O_2 and air to maintain arterial PO_2 (PaO_2) at 120 to 140 mmHg. The ventilation rate was 12 breaths/minute, and the respiratory tidal volume was set to adjust end-expiratory CO_2 to 4.5 vol%. Adequate depth of anesthesia and relaxation was achieved by continuous intravenous application of piritramid (0.8 to 1.0 mg/kg/hr), midazolam (0.3 to 0.5 mg/kg/hr), and pancuronium (0.2 mg/kg/hr). For the duration of the experiments, all animals received a 0.9% NaCl infusion at a rate of 5 ml/kg/hr.

A 7F flow-directed thermodilution triple-lumen catheter was inserted into the pulmonary artery through the right jugular vein. An arterial catheter (Leader Catheter 115.11, Vygon Ecouen, France) was advanced into the abdominal aorta through the right femoral artery. Urine output was measured using a suprapubic catheter.

After median laparotomy, the duodenum was mobilized and the pancreatic duct was identified at its duodenal junction. The pancreatic duct was cannulated with a 5 Ch umbilical vein catheter (Argyle, Sherwood Medical, Sulzbach, Germany). The bladder was catheterized to provide information about urinary output.

Monitoring

Hemodynamic measurements included continuous monitoring of mean arterial blood pressure (MAP), central venous pressure, and heart rate. Cardiac output was measured in triplicate and calculated as the arithmetic mean of at least three consecutive individual values at baseline and every full hour after induction of pancreatitis. Because the O_2 content in the ventilatory gas mixture had to be increased during the course of experiments to maintain a PaO_2 of 120 to 140 mmHg, pulmonary gas exchange was calculated by the oxygenation index (ratio PaO_2 [mmHg]/inspiratory oxygen fraction [FIO_2, %]).

Hemofiltration

Zero-balanced CVVH in hemofiltered animals was performed using a roller pump (NFG 05, Dialysetechnik, Karlsruhe, Germany) and a polysulfone hollow-fiber filter with a cutoff point of 30 kD (Ultraflux AV 600 S, Fresenius, Bad Homburg, Germany). Vascular access was obtained by two Sheldon catheters inserted into each femoral vein in animals undergoing CVVH. Heparin (8 U/kg/hr) was added continuously into the "arterial" port (right femoral vein). Extracorporeal blood flow ranged from 70 to 130 ml/min to achieve standardized ultrafiltrate volumes of 20 ml/hr/kg. Ultrafiltrate was replaced by isotonic hemofiltration fluid (HF 03, Fresenius).

Baseline recordings and blood samples were obtained from all animals 30 minutes after surgical preparation with stable hemodynamic conditions. Immediately after the induction of pancreatitis, and every full hour after the induction, hemodynamics were recorded. Blood samples were collected before and every 2 hours after the induction of pancreatitis.

Calculation of the Sieving Coefficient

To provide information about the elimination of pancreatitis-related cytokines and mediators (tumor necrosis factor-alpha [TNF-α], transforming growth factor-beta1 [TGF-β_1], phospholipase A_2, and kinin), the sieving coefficient (SC) was calculated by:

$$SC = 2C_f/(C_i + C_o)$$

where C_f, C_i, and C_o, respectively, represent concentration in the hemofiltrate, concentration in the "inflow" (prefilter) line, and concentration in the "outflow" (postfilter) line.

Induction of Pancreatitis and Biometric Design

Before the beginning of the experiments, animals were randomly allocated to the control or one of two different treatment groups. Pancreatitis was induced by intraductal injection of sodium taurocholate (5%, 1 ml/kg; Sigma, Deisenhofen, Germany) and enterokinase (10 U/kg; Sigma).

In control animals (n = 11; group 1), the spontaneous course of necrotizing pancreatitis was observed without any further treatment. Group 2 animals (n = 12) underwent CVVH after MAP decreased 20% below baseline values ("therapeutic" CVVH). In group 3 (n = 12), "prophylactic"

CVVH was started simultaneously with the induction of pancreatitis.

Biochemical Measurements

Serum lipase levels were measured turbidimetrically as described previously[10] using an assay kit from Sigma (Delta Test Assay for pancreatic lipase). Hematocrit was measured using a Coulter Counter (S-Plus IV, Coulter Electronics, Krefeld, Germany). Total protein concentrations were measured using an assay kit from Boehringer (SYS 3, Boehringer, Mannheim, Germany). C-reactive protein (CRP) plasma concentrations were determined using an enzyme immunoassay, as recently described by Schroedl.[11] Intra- and interassay variations were <9% and <12%, respectively. Normal values of CRP (0 to 6 mg/L) were defined as mean ± 2 standard deviations of baseline values.

Trypsinogen activation peptide (TAP) samples (2 ml urine) were collected in 20 μl ethylenediaminetetraacetic acid (0.5 M, pH 7.0) and frozen at −20°C until assayed. Quantification of TAP was performed with a competitive enzyme-linked immunosorbent assay (ELISA) as previously described[12–14] using the anti-TAP antiserum R18-74.

The concentration of TNF-α in serum and hemofiltrate was measured using a commercially available specific pig TNF-α ELISA (Endogen, Inc., Eching, Germany). After being collected, serum samples were frozen at −70°C until assayed in triplicate.

TGF-β_1 is known to show high homology between the human and porcine protein.[15] Therefore, a human ELISA (Quantikine, R&D Systems, Wiesbaden, Germany) detecting both human and porcine TGF-β_1 was used for quantification of porcine TGF-β_1 in plasma. Cross-reactivity between human and recombinant porcine TGF-β_1 assessed in preliminary experiments was 92%. After being collected, serum samples were frozen at −70°C until assayed in triplicate.

Concentrations of phospholipase A_2 were measured using a photometric method described previously.[16] One unit was defined as the liberation of 1 μmol fatty acid per minute.

Kinin concentrations in plasma were determined using a radioimmunoassay, as described previously.[17] All samples and standards were assayed in duplicate. Minimal kinin concentrations that caused a reliable signal (defined as 90% of the radioactivity found in the absence of unlabeled kinin) were 50 fmol/ml. In hemofiltered animals (groups 2 and 3), efforts to measure kinin in hemofiltrate were futile; therefore, SCs of kinin could not be calculated.

Statistics

Data are reported as mean ± standard deviation. Normal distribution of data was tested using the Kolmogorov–Smirnov test. Survival times were calculated and compared using Kaplan–Meier analysis (log-rank test). Statistical differences of baseline values *versus* changes of parameters after pancreatitis were evaluated by one-way analysis of variance for repeated measures. Differences between the treatment groups were analyzed by analysis of variance, followed by the Scheffé test when significant differences were found. Correlation analysis was performed using the Pearson correlation method. P < 0.05 was considered to be of statistical significance.

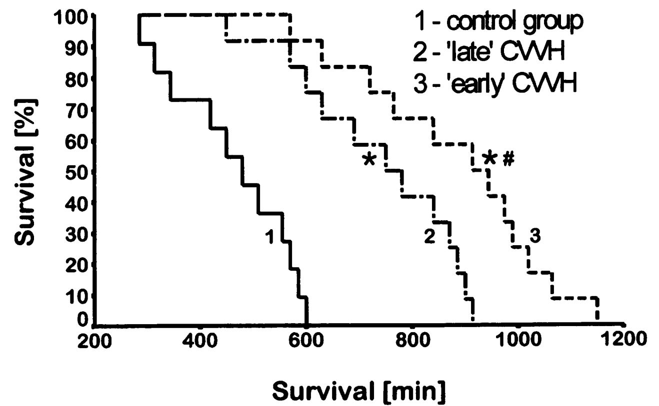

Figure 1. Survival times after pancreatitis. Log-rank test of survival times (Kaplan–Meyer analysis) revealed statistical prolongation of survival in treatment groups compared with controls (*p < 0.001 group 1 *vs.* groups 2 and 3). Also, group 3 showed significant prolongation compared with group 2 (# p < 0.05 group 2 *vs.* group 3).

RESULTS

Survival

Compared with control animals, those that received CVVH had significantly improved survival times. In addition, early CVVH prolonged survival significantly compared with late CVVH (Fig. 1). The respective mean survival times were control group (group 1), 7.0 ± 1.2 hours (p < 0.001 *vs.* groups 2 and 3); "therapeutic" (late) CVVH (group 2), 12.3 ± 1.4 hours (p = 0.017 *vs.* group 3); and "prophylactic" (early) CVVH (group 3), 14.7 ± 1.3 hours.

Hemodynamic Parameters

Control animals showed a rapid decline of MAP and cardiac output after induction of pancreatitis. Two hours after induction, the decrease of MAP was 30% compared with baseline values. The significant decrease in cardiac output and MAP was paralleled by a significant increase in heart rate. Concomitantly, a significant decrease in central venous pressure occurred (Table 1).

In group 2, a 20% decrease of MAP below baseline values before the beginning of therapeutic CVVH occurred a median of 105 minutes (70 to 135 minutes) after the induction of pancreatitis. Changes in hemodynamic parameters 2 hours after induction therefore resembled those in control animals. After CVVH had been started, the deteri-

Table 1. HEMODYNAMIC AND PULMONARY PARAMETERS AFTER PANCREATITIS

Parameter	Group	Baseline (100%)	Postinduction 2 hours	6 hours	10 hours
MAP (mmHg)	1	98 ± 9	−30%*	−65%*	—
	2	103 ± 8	−29%*	−38%*‡	−70%*
	3	95 ± 10	−5%‡§	−21%†‡§	−44%*§
CO (L/min)	1	6.1 ± 1.1	−20%†	−66%*	—
	2	6.0 ± 0.6	−19%†	−35%*‡	−72%*
	3	5.8 ± 0.7	−4%‡§	−21%*†	−53%*§
HR (beats/min)	1	94 ± 7	+26%*	+81%*	—
	2	99 ± 6	+28%†	+47%*‡	+82%*
	3	99 ± 5	+6%‡§	+30%*‡	+68%*
CVP (mmHg)	1	5.6 ± 1.1	−44%*	−98%*	—
	2	5.7 ± 1.2	−34%†	−50%*‡	−76%*
	3	5.6 ± 1.0	−10%‡§	−35%*‡	−56%*§
PaO_2/FIO_2 ratio	1	5.2 ± 1.1	−28%†	−64%*	—
	2	5.0 ± 0.8	−25%†	−32%†‡	−74%*
	3	5.4 ± 1.0	−7%‡§	−30%†‡	−66%*

Percentages of decrease of mean arterial pressure (MAP), cardiac output (CO), central venous pressure (CVP), oxygenation index (PaO_2/FIO_2 ratio), and increase of heart rate (HR) after pancreatitis referred to baseline values (means ± SD). Group 1 = controls; group 2 = late CVVH; group 3 = prophylactic CVVH.
* $p < 0.01$ *vs.* baseline values.
† $p < 0.05$ *vs.* baseline values.
‡ $p < 0.05$ *vs.* the respective difference in controls.
§ $p < 0.05$ *vs.* the respective difference in group 2.

oration of systemic hemodynamic parameters was significantly weakened compared with controls (see Table 1).

The effectiveness of CVVH was more pronounced in group 3 (see Table 1). Compared with control animals, early CVVH resulted in a significant attenuation of hemodynamic deterioration in all parameters and at all time points. Significant differences were also found with regard to group 2.

Gas Exchange

The impairment of hemodynamic parameters was accompanied by a progressive fall in PaO_2 that had to be counteracted by a gradual increase in FIO_2 during the experiments (see Table 1). Before induction, FIO_2 was about 30% in all groups to maintain a PaO_2 of 120 to 140 mmHg. Pancreatitis resulted in a disturbance of transpulmonary O_2 transport, requiring an increase of FIO_2 up to 100% at the end of the experiments. As a result, the PaO_2/FIO_2 ratio, which ranged from 5.0 to 5.4 before induction, decreased to 1.3 to 1.6 before death. The deterioration of the PaO_2/FIO_2 ratio 2 hours after induction was significantly less severe in group 3 (prophylactic CVVH) than in the control group and group 2. Six hours after induction, the decrease of the PaO_2/FIO_2 ratio in both treatment groups was significantly weakened compared with controls.

Fluid Sequestration

Pancreatitis resulted in a significant increase in hematocrit in all groups (Fig. 2). In control animals, pancreatitis led to a significant increase of hematocrit from 31% (baseline) to 46% 6 hours after induction (+48%). Compared with baseline values, this increase was significantly attenuated by both late (+26%) and early (+19%) CVVH. Moreover, the decrease of total protein concentration in plasma (see Fig. 2) was distinctly weakened by CVVH. Six hours after induction, protein loss was 52% in controls *versus* 33% in group 2 ($p < 0.05$ *vs.* group 1) and 14% in group 3 ($p < 0.05$ *vs.* groups 1 and 2).

Biochemical Measurements

The activities of lipase in blood serum ranged from 67 to 95 U/L before the induction of pancreatitis. Pancreatitis resulted in a progressive rise in lipase activities in all groups. Slight differences between groups did not reach statistical significance (Table 2).

Urinary TAP concentrations before the induction of pancreatitis were <1 nmol/L in all groups (see Table 2). Pancreatitis produced a significant increase of TAP concentrations, up to almost 300 nmol/L. There were no statistically significant differences between the control and either of the CVVH-treated groups.

Before the induction of pancreatitis, CRP concentrations were <10 mg/L. Pancreatitis resulted in an increase of more than 10-fold. No significant differences between the experimental groups were found (see Table 2).

Baseline concentrations of TNF-α were in all experimental groups <15 pg/ml. The induction of pancreatitis resulted in a tremendous increase; however, it was significantly delayed by CVVH (Table 3). In the early phase of the experiments, CVVH resulted in TNF-α concentrations in

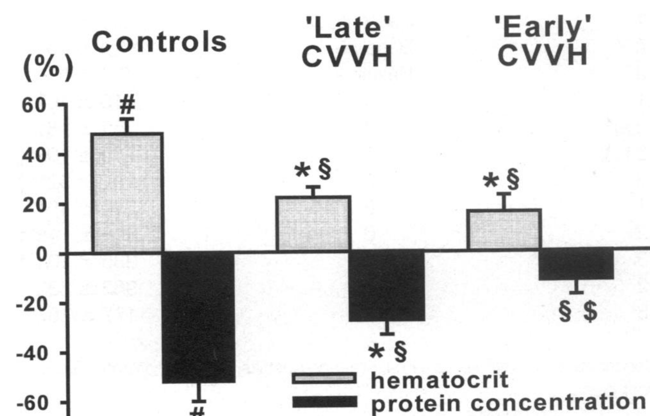

Figure 2. Increase in hematocrit and decrease in total protein concentration 6 hours after pancreatitis induction. The changes are illustrated as percentages of baseline values (0%). Means ± standard deviation. * $p < 0.05$ *vs.* baseline values; # $p < 0.01$ *vs.* baseline values; § $p < 0.05$ *vs.* corresponding values in controls; $ $p < 0.05$ *vs.* group 2 (late CVVH).

Table 2. CHANGES IN TAP, LIPASE, AND CRP AFTER PANCREATITIS

Parameter	Group	Baseline	Postinduction 2 hours	Postinduction 6 hours	Postinduction 10 hours
TAP (nmol/L)	1	0.6 ± 0.4	192 ± 46*	280 ± 92†	—
	2	0.9 ± 0.7	246 ± 75*	276 ± 87†	292 ± 65†
	3	0.7 ± 0.3	228 ± 64*	298 ± 75*	254 ± 58*
lipase (U/L)	1	72 ± 21	570 ± 212†	1180 ± 210†	—
	2	67 ± 17	520 ± 98†	845 ± 160†	1340 ± 380†
	3	75 ± 22	580 ± 258†	1060 ± 325†	1540 ± 371†
CRP (mg/l)	1	5.6 ± 4.6	10.4 ± 6.7	45.0 ± 12.5†	—
	2	6.2 ± 5.7	12.5 ± 7.1	51.3 ± 17.7†	77.2 ± 19.6†
	3	6.8 ± 3.0	13.8 ± 7.4	53.3 ± 14.8†	66.8 ± 23.8†

Concentrations of trypsinogen activation peptides (TAP) in urine, C-reactive protein (CRP) in plasma, and activity of lipase in serum. Means ± SD.
Group 1 = controls; group 2 = late CVVH; group 3 = prophylactic CVVH.
* $p < 0.05$ *vs.* baseline values.
† $p < 0.01$ *vs.* baseline values.

hemofiltrate that were greater than those measured in the postfilter (outflow) line. This evident efficiency of CVVH in eliminating TNF-α was also indicated by the considerable gradient of pre- and postfilter concentrations of TNF-α (see Table 3). The decrease of SCs of TNF-α in the later course of the experiments was paralleled by increasing plasma (prefilter) concentrations (Table 3, Fig. 3). Pearson's correlation analysis revealed a significant negative association of SCs with the effective circulating plasma concentrations of TNF-α ($R = -0.81$, $p = 0.009$; see Fig. 3).

The induction of pancreatitis lead to an enormous increase in TGF-β_1 concentrations. Before induction, they were <100 pg/ml (controls, 75 ± 36 pg/ml; group 2, 99 ± 41 pg/ml; group 3, 57 ± 41 pg/ml). Six hours after induction, they were 430 ± 162 pg/ml in controls, 392 ± 141 pg/ml in group 2, and 381 ± 146 pg/ml in group 3. CVVH did not influence TGF-β_1 concentrations. The SCs of TGF-β_1, in contrast to those of TNF-α, decreased only slightly during CVVH.

The induction of pancreatitis resulted in a significant increase in phospholipase A_2 concentrations (Table 4). The significant attenuation of this increase 2 and 6 hours after

Table 3. CONCENTRATION OF TNF-α IN PLASMA AND HEMOFILTRATE (pg/ml)

Group	Hours After Induction	Plasma Prefilter‖	Plasma Postfilter‖	Hemofiltrate	SC
1	Baseline	6.8 ± 5.1	—	—	—
2	Baseline	11.2 ± 6.7	—	—	—
3	Baseline	13.3 ± 5.2	—	—	—
1	2	410 ± 167*	—	—	—
2(a)¶	2	352 ± 181*			
2(b)¶	2	219 ± 103*	141 ± 66	161 ± 44	0.87 ± 0.23
3	2	151 ± 92*†‡	78 ± 43	93 ± 38	0.82 ± 0.29
1	6	1210 ± 491*	—	—	—
2	6	418 ± 148*†	167 ± 98	210 ± 81	0.72 ± 0.14
3	6	385 ± 104*†	207 ± 54	163 ± 79	0.55 ± 0.24
2	10	962 ± 332*	731 ± 211	296 ± 97	0.35 ± 0.16§
3	10	1177 ± 287*	845 ± 213	283 ± 97	0.28 ± 0.11§

Concentration of TNF-α in plasma (pre- and postfilter) and hemofiltrate. Group 1 = controls; group 2 = therapeutic CVVH; group 3 = prophylactic CVVH; SC = sieving coefficient.
* $p < 0.001$ *vs.* baseline.
† $p < 0.01$ *vs.* group 1.
‡ $p < 0.05$ *vs.* group 2.
§ $p < 0.05$ *vs.* the respective SC 2 hours postinduction.
‖ In hemofiltered animals.
¶ 2(a): All group 2 animals (n = 12), including both hemofiltered (n = 7) and nonhemofiltered animals (n = 5) at that time point; 2(b): subgroup consisting only of those animals treated by CVVH already 2 hours postinduction (n = 7).

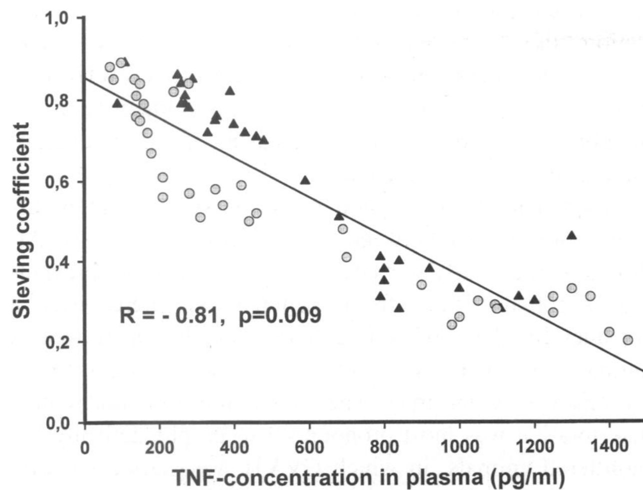

Figure 3. Correlation analysis between the circulating plasma ("prefilter") concentration and the SC of TNF-α. The SC was calculated by relating the concentrations in hemofiltrate to those in prefilter and postfilter blood. Filled triangles, group 2 animals; open circles, group 3 animals. Because no significant difference between group 2 and 3 animals was seen concerning the correlation of SCs with the corresponding plasma TNF-α concentration, the entire population of hemofiltered animals (n = 24) was entered into the analysis.

induction by CVVH was accompanied by considerable amounts of phospholipase A_2 in hemofiltrate. Consistent with the results obtained from the TNF-α measurements, the circulating (prefilter) plasma concentrations of phospholipase A_2 in hemofiltered animals showed a significant negative correlation with the SCs ($R = -0.79$, $p = 0.016$). Ten hours after induction, concentrations of phospholipase A_2 in both treatment groups reached values comparable with those obtained 6 hours after induction in controls.

The kinin concentrations in controls peaked 2 and 6 hours after induction (Table 5). In hemofiltered animals, the kinin response was significantly attenuated at these time points. Moreover, 2 and 6 hours after induction, there was a significant difference between prefilter and postfilter concentrations of kinin in treatment groups, although no kinin was detectable in hemofiltrate. Ten hours after induction, the kinin concentrations in group 2 and 3 animals were comparable to those detected 6 hours after induction in controls.

DISCUSSION

Soon after the introduction of hemofiltration for the treatment of acute renal failure, several clinical and experimental studies were presented suggesting that patients with severe septic diseases might benefit from blood purification, in particular hemofiltration. Several mechanisms were discussed that might explain this hypothetical benefit, such as the removal of sepsis mediators by convective filtration through the filters or by adsorption of mediators to the filter membrane.

Hemofiltration is assumed to improve the clinical course of sepsis and multiple organ failure.[3,4,18] In a 1995 clinical study, hemofiltration was reported to improve survival significantly, even in devastating sepsis-related diseases.[5]

Table 4. CONCENTRATION OF PHOSPHOLIPASE A_2 IN PLASMA AND HEMOFILTRATE (U/L)

Group	Hours After Induction	Plasma: Prefilter‖	Plasma: Postfilter‖	Hemofiltrate	SC
1	Baseline	8 ± 6	—	—	—
2	Baseline	7.6 ± 11	—	—	—
3	Baseline	14 ± 10	—	—	—
1	2	144 ± 42*	—	—	—
2(a)¶	2	129 ± 33*			
2(b)¶	2	107 ± 38*	108 ± 31	84 ± 42	0.78 ± 0.14
3	2	86 ± 21*†‡	82 ± 22	63 ± 38	0.75 ± 0.19
1	6	201 ± 64*	—	—	—
2	6	135 ± 68*†	127 ± 58	99 ± 42	0.76 ± 0.21
3	6	138 ± 23*†	85 ± 41	75 ± 30	0.67 ± 0.24
2	10	186 ± 61*	174 ± 66	81 ± 47	0.45 ± 0.19§
3	10	200 ± 73*	192 ± 79	92 ± 51	0.47 ± 0.20§

Concentration of phospholipase in plasma (pre- and postfilter) and hemofiltrate. Group 1 = controls; group 2 = therapeutic CVVH; group 3 = prophylactic CVVH; SC = sieving coefficient.
* $p < 0.001$ *vs.* baseline.
† $p < 0.05$ *vs.* group 1.
‡ $p < 0.05$ *vs.* group 2.
§ $p < 0.05$ *vs.* the respective SC 2 hours postinduction.
‖ In hemofiltered animals.
¶ 2(a): all group 2 animals (n = 12), including both hemofiltered (n = 7) and nonhemofiltered animals (n = 5) at that time point; 2(b): subgroup consisting only of those animals treated by CVVH already 2 hours postinduction (n = 7).

Table 5. CONCENTRATION OF KININ IN PLASMA (fmol/ml)

Group	Hours After Induction	Plasma Prefilter§	Plasma Postfilter§
1	Baseline	55 ± 14	—
2	Baseline	67 ± 28	—
3	Baseline	57 ± 16	—
1	2	244 ± 72*	—
2(a)‖	2	229 ± 83*	
2(b)‖	2	113 ± 38*†	82 ± 38‡
3	2	117 ± 41*†	62 ± 34‡
1	6	238 ± 56*	—
2	6	155 ± 41*†	99 ± 58‡
3	6	144 ± 39*†	125 ± 42
2	10	198 ± 60*	173 ± 65
3	10	244 ± 83*	249 ± 70

Concentration of kinin in plasma (pre- and postfilter) and hemofiltrate. Group 1 = controls; group 2 = therapeutic CVVH; group 3 = prophylactic CVVH; SC = sieving coefficient.
* $p < 0.001$ *vs.* baseline.
† $p < 0.05$ *vs.* group 1.
‡ $p < 0.05$ *vs.* the respective prefilter value.
§ In hemofiltered animals.
‖ 2(a): all group 2 animals (n = 12) including both hemofiltered (n = 7) and nonhemofiltered animals (n = 5) at that time point; 2(b): subgroup consisting only of those animals treated by CVVH already 2 hours postinduction (n = 7).

Nearly three quarters of the patients who had an APACHE II score >30—usually associated with a mortality rate >95%[19]—survived. Others suggested that CVVH would result in stabilizing hemodynamic and metabolic parameters and pulmonary gas exchange during sepsis or cardiogenic shock.[4,7,20–22] Storck et al,[23] in a discussion about the better results of pump-driven hemofiltration compared with spontaneous arteriovenous hemofiltration, reported a direct correlation between the cumulative volume of ultrafiltrate and the survival rate in patients with acute renal failure. The observation that high-volume hemofiltration was of greater benefit than a low turnover of plasma, which was consistent with other experimental studies,[20,24] may be ascribed to better control of uremia, more effective removal of septic mediators, or both.

Mediators considered able to be filtered, or those that indeed have been assessed in hemofiltrate, are for example cytokines,[5,8,24,25] activated factors of the complement system,[4,5,8] arachidonic acid metabolites,[4,5,26] platelet-activating factor,[5,27] myocardial depressant factor,[7] kinins,[3,5] and phospholipase A_2.[8]

In our study, CVVH was performed in a "zero-balanced" fashion to rule out additional benefit of fluid replacement. The hemofilter used permitted the removal of all plasma solutes up to a molecular weight of approximately 30 kD. The prefilter concentrations of TNF-α, TGF-β_1, kinin, and phospholipase A_2 were measured to obtain information about the effectiveness of CVVH in decreasing the circulating plasma concentrations of inflammatory mediators. By relating the prefilter to postfilter and hemofiltrate concentrations, the SCs were assessed. SCs were calculated several times after animals had been connected to CVVH. This provided information about changes in the integrity of the membrane surface in terms of decreasing efficiency in eliminating inflammatory mediators.

Major hemodynamic parameters and survival times differed considerably between the experimental groups. Although neither early nor late CVVH succeeded in preventing eventual death from pancreatitis, there was a considerable delay of hemodynamic impairment in treated animals compared with controls, resulting in significantly prolonged survival times. The attenuation of hemodynamic deterioration was most pronounced in prophylactically hemofiltered animals, in which CVVH was started simultaneously with the induction of pancreatitis. In therapeutically hemofiltered animals, CVVH was started after hemodynamic impairment (defined as a 20% decrease in MAP below baseline values); this was the case approximately 2 hours after induction.

The dramatic decline of the oxygenation index (PaO_2/FiO_2 ratio) in control animals, indicating progressive respiratory failure, was significantly improved by CVVH. This finding might have diverse causes: both in control and treated animals, the decrease in protein concentrations in plasma was paralleled by a concomitant fall in central venous pressure, indicating a fluid shift to the extravascular compartment. Protein and fluid extravasation by capillary leakage was possibly the reason for progressive edema of the pulmonary parenchyma, finally resulting in respiratory insufficiency. Other mechanisms that might have contributed to progressive respiratory dysfunction are the damaging effects of pancreatitis-derived mediators (*e.g.*, phospholipase A_2); this has been suggested to play an important role in pancreatogenic disturbance of gas exchange by hydrolysis of lecithin of pulmonary surfactant.[28–32] These results were consistent with previous studies suggesting an improvement in pulmonary gas exchange during sepsis or cardiogenic shock with the use of CVVH.[7,22–23]

Urinary TAP concentrations were assayed as a parameter for pancreatic necrosis. Several studies have demonstrated that elevated TAP concentrations are a sensitive and specific marker for pancreatic necrosis formation and therefore are helpful in predicting the severity of acute pancreatitis.[12,13] The presence of TAP in a sample indicates that trypsinogens are being activated. The tremendous increase in urinary TAP concentrations immediately after the induction of acute necrotizing pancreatitis in the present study demonstrates the substantial trypsinogen "burn" that accompanies this disease. The absence of any significant difference in urinary TAP concentrations between the untreated control group and either of the treated groups therefore shows that CVVH treatment did not affect the rate of ongoing TAP activation in the pancreas. Therefore, the prolongation of survival by CVVH most likely resulted from the elimina-

tion of toxic metabolites released as a consequence of the acute disease process.

In accord with these results, CVVH had no impact on lipase activities in serum, measured as a parameter of pancreatic enzyme release into the bloodstream, nor was the elevation of CRP concentrations in plasma suppressed after the onset of the disease. This indicated that CVVH in this setting attenuated a systemic inflammatory response not reflected by the increase in CRP.

Protein loss, with subsequent fluid shift to the extravascular compartment and hemoconcentration, occurred in all groups. These changes, indicating capillary leakage, have been ascribed in several studies to vasoactive inflammatory mediators (*e.g.*, kinins). In agreement with our results, Griesbacher et al[33] reported a considerable decrease in blood volume (28%) resulting from a loss in plasma volume (48%), with concomitant hemoconcentration, in experimental pancreatitis.

The analysis of TNF-α, TGF-β_1, kinin, and phospholipase A_2 concentrations in plasma and hemofiltrate strongly suggested that CVVH was able, at least partly, to remove some inflammatory mediators released in the course of the disease. CVVH resulted in a significant delay in the increase of plasma concentrations of TNF-α, phospholipase A_2, and kinin. In contrast, CVVH did not influence the tremendous increase in TGF-β_1 concentrations. The SCs of TNF-α and phospholipase A_2, calculated as a parameter of CVVH-induced plasma clearance, showed a significant negative correlation with the circulating plasma (prefilter) concentrations. The longer CVVH lasted, the more ineffective it became in removing TNF-α and phospholipase A_2. This was indicated by decreasing SCs associated with increasing plasma concentrations of TNF-α and phospholipase A_2 in the course of the experiments. CVVH in treatment groups resulted also in an attenuated plasma kinin response compared with control animals. Moreover, CVVH resulted in a considerable gradient of prefilter to postfilter concentrations of kinin. Attempts to detect kinin in hemofiltrate were unsuccessful. We assume that during the passage through the filter, free bradykinin was transformed to des-Arg-bradykinin, undetectable in the kinin radioimmunoassay used in this study.

In contrast to the close relation between the SCs and the circulating plasma concentrations of TNF-α and phospholipase A_2, CVVH did not influence the increase of TGF-β_1 when treatment groups were compared with control animals. Although TGF-β_1 was detected in considerable concentrations in hemofiltrate, CVVH did not produce a decrease in circulating plasma concentrations. In agreement with these findings, the SCs of TGF-β_1 were considerably lower than those of TNF-α and phospholipase A_2.

The concentrations of TNF-α, phospholipase A_2, and kinin, measured 10 hours after induction in treatment groups, resembled those obtained 6 hours after pancreatitis induction in controls. This finding may have resulted from the fact that albumin or other plasma solutes progressively fouled the membrane sites of the filters. No studies have been performed to date investigating the question of whether SCs may be compromised by long-term application of the same hemofilter, with gradual saturation of convective and adsorptive capacities. If so, this might be a conclusive rationale for our finding that CVVH in this experimental setting was able to provide only transient, but not definitive, plasma clearance of TNF-α, phospholipase A_2, and kinin. In this regard, Ronco et al[27] hypothesized that there may be a need for more frequent changes of hemofilters to prevent a progressive loss of efficiency of CVVH, but further studies are needed in this area.

In summary, we conclude that interventional blood-purifying treatments such as CVVH represent promising therapeutic options if applied early in acute pancreatitis. Further experimental efforts are required to investigate whether hemofiltration might have a major impact on the survival rate, as well as the survival time, in patients with severe pancreatitis

Acknowledgments

The authors gratefully acknowledge the kind support of Fresenius AG, Blaubeuren, Germany. The antibody to kinins used in the radioimmunoassay was generously provided by Professor K. Shimamoto, Saporo, Japan. Anti-TAP antiserum R18-74 was kindly provided by Prof. J. Hermon-Taylor, Department of Surgery, St. George's Hospital, London, United Kingdom. The authors thank Mrs. G. Godec for excellent technical assistance.

References

1. Fernandez-Cruz L, Navarro S, Valderrama R, et al. Acute necrotizing pancreatitis: a multicenter study. Hepato-Gastroenterol 1994; 41:185–189.
2. Knaus WA, Draper EA, Waner DP, Zimmerman JE. Prognosis in acute organ-system failure. Ann Surg 1985; 202:685–692.
3. Gotloib L, Barzilay E, Shustak A, Lev A. Sequential hemofiltration in nonoliguric high capillary permeability pulmonary edema of severe sepsis: preliminary report. Crit Care Med 1984; 12:997–1000.
4. Gotloib L, Barzilay E, Shustak A, et al. Hemofiltration in septic ARDS. The artificial kidney as an artificial endocrine lung. Resuscitation 1986; 13:123–132.
5. Gotloib L, Shostak A, Lev A, et al. Treatment of surgical and nonsurgical septic multiple organ failure with bicarbonate hemodialysis and sequential hemofiltration. Intensive Care Med 1995; 21:104–111.
6. Barzilay E, Kessler D, Berlot G, et al. Use of extracorporeal supportive techniques as additional treatment for septic-induced multiple organ failure patients. Crit Care Med 1989; 17:634–637.
7. Gomez A, Wang R, Unruh H, et al. Hemofiltration reverses left ventricular dysfunction during sepsis in dogs. Anesthesiology 1990; 73:671–685.
8. Blinzler L, Haußer J, Bödeker H, et al. Conservative treatment of severe necrotizing pancreatitis using early continuous venovenous hemofiltration. In: Sieberth HG, Mann H, Stummvoll HK, eds. Continuous hemofiltration. Contrib Nephrol 1991; 93:234–236.
9. Gebhardt C, Bödeker H, Blinzler L, et al. Wandel in der Therapie der schweren akuten Pankreatitis. Chirurgerie 1994; 65:33–41.
10. Vogel WC, Zieve L. A rapid and sensitive turbidimetric method for serum lipase estimation based upon differences between the lipase of normal and pancreatitis serum. Clin Chem 1963; 9:168–175.

11. Schroedl W. Diagnostische Verwendbarkeit ausgewählter immunologischer Parameter in Seren von Schweinen und Pferden. Doctoral thesis, University of Leipzig, Germany, 1994.
12. Karanjia ND, Widdison AL, Jehanli A, et al. Assay of trypsinogen activation in the cat experimental model of acute pancreatitis. Pancreas 1993; 8:189–195.
13. Gudgeon AM, Heath DI, Hurley P, et al. Trypsinogen activation peptides assay in the early prediction of severity of acute pancreatitis. Lancet 1990; 335:4–8.
14. Hurley PR, Cook A, Jehanli A, et al. Development of radioimmunoassays for free tetra-L-aspartyl-L-lysine trypsinogen activation peptides (TAP). J Immunol Methods 1988; 111:195–203.
15. Murtaugh MP. Porcine cytokines. Vet Immunol Immunopathol 1994; 43:37–44.
16. Hoffmann GE, Neumann U. Modified photometric method for the determination of phospholipase A activities. Klin Wochenschr 1989; 67:106–109.
17. Shimamoto K, Ando T, Tanaka S, Iimura O. The determination of kinin and glandular kallikrein in human biological fluids. Atemweg- und Lungenkrkh 1988; 14(Supp 1):29–36.
18. Groeneveld ABJ. Septic shock and multiple organ failure: treatment with hemofiltration? Intensive Care Med 1990; 16:489–490.
19. Chang RWS, Jacobs S, Lee B. Predicting outcome among intensive care unit patients using computerized trend analysis of daily APACHE II scores corrected for organ system failure. Intensive Care Med 1988; 14:558–566.
20. Grootendorst AF, van Bommel EF, van der Hoven B, et al. High-volume hemofiltration improves right ventricular function in endotoxin-induced shock in the pig. Intensive Care Med 1992; 18:235–240.
21. Coraim FJ, Coraim HP, Ebermann R, Stellwag FM. Acute respiratory failure after cardiac surgery: clinical experience with the application of continuous arteriovenous hemofiltration. Crit Care Med 1986; 14:714–718.
22. Bellomo R, Martin H, Parkin G, et al. Continuous arteriovenous haemodiafiltration in the critically ill: influence on major nutrient balances. Intensive Care Med 1991; 17:399–402.
23. Storck M, Hartl WH, Zimmerer E, Inthorn D. Comparison of pump-driven and spontaneous continuous haemofiltration in postoperative acute renal failure. Lancet 1991; 337:452–455.
24. Lee PA, Matson JR, Pryor RW, Hinshaw LB. Continuous arteriovenous hemofiltration therapy for *Staphylococcus aureus*-induced septicemia in immature swine. Crit Care Med 1993; 21:914–924.
25. Bellomo R, Tipping P, Boyce N. Continuous veno-venous hemofiltration with dialysis removes cytokines from the circulation of septic patients. Crit Care Med 1993; 21:522–526.
26. Staubach KH, Rau HG, Kooistra A, et al. Can hemofiltration increase survival time in acute endotoxemia—a porcine shock model. Second Vienna Shock Forum 1989:821–826.
27. Ronco C, Tetta C, Lupi A, et al. Removal of platelet-activating factor in experimental continuous arteriovenous hemofiltration. Crit Care Med 1995; 23:99–107.
28. Aho HJ, Ahola RA, Tolvanen AM, Nevalainen TJ. Experimental pancreatitis in the rat. Changes in pulmonary phospholipids during sodium taurocholate-induced acute pancreatitis. Res Exp Med (Berl) 1983; 182:79–84.
29. Guice KS, Oldham KT, Wolfe RR, Simmons RH. Lung injury in acute pancreatitis: primary inhibition of pulmonary phospholipid synthesis. Am J Surg 1987; 153:54–61.
30. Lankisch PG, Koop H, Woltjen HH, Rahlf G. Post-mortem findings in the lungs in severe acute pancreatitis. Gastroenterol Clin Biol 1979; 3:299A.
31. Maciver AG, Metcalfe IL, Possmayer F, et al. Alteration of surfactant chemistry in experimental hemorrhagic pancreatitis. J Surg Res 1977; 23:311–314.
32. Morgan AP, Jenny ME, Haessler H. Phospholipids, acute pancreatitis and the lungs: effect of lecithinase infusion on pulmonary surface activity in dogs. Ann Surg 1968; 167:329–335.
33. Griesbacher T, Tiran T, Lembeck F. Pathological events in experimental acute pancreatitis prevented by the bradykinin antagonist, HOE 140. Br J Pharmacol 1993; 108:405–411.