Complement C5A Antagonist Treatment Improves the Acute Circulatory and Inflammatory Consequences of Experimental Cardiac Tamponade

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Objective: Cardiogenic shock often leads to splanchnic macro- and microcirculatory complications, and these events are linked to local and systemic inflammatory activation. Our aim was to investigate the consequences of complement C5a antagonist treatment on the early circulatory and inflammatory changes in a clinically relevant large animal model of cardiac tamponade.

Design and Setting: A randomized, controlled in vivo animal study in a university research laboratory.

Subjects: Anesthetized, ventilated, and thoracotomized Vietnamese mini pigs (24 ± 3 kg).

Interventions: Group 1 ($n = 6$) served as sham-operated control. In group 2 ($n = 7$), cardiac tamponade was induced for 60 minutes by the administration of intrapericardial fluid, while the mean arterial pressure was kept in the interval 40 to 45 mm Hg. Group 3 ($n = 6$) was treated with a complement C5a antagonist compound (the peptide acetyl-peptide-A, 4 mg/kg) after 45 minutes of tamponade.

Measurements and Main Results: The macrohemodynamics, including the superior mesenteric artery flow, was monitored; the average red blood cell velocity in the small intestinal mucosa was determined by an intravital orthogonal polarization imaging technique. The whole blood superoxide production, the plasma level of high-mobility group box protein-1 and big-endothelin and the small intestinal MPO activity were measured. One hundred eighty minutes after the relief of tamponade, the mean arterial pressure was decreased, while the plasma levels of superoxide, high-mobility group box protein-1, and big-endothelin, and the intestinal MPO activity were increased. The administration of acetyl-peptide-A normalized the mean arterial pressure and preserved the cardiac output, while the superior mesenteric artery flow and mucosal average red blood cell velocity were increased significantly, and the plasma superoxide, high-mobility group box protein-1, big-endothelin, and intestinal MPO levels were reduced.

Conclusions: These results provide evidence that blockade of the C5a effects significantly influences the acute splanchnic macro- and microhemodynamic complications and decreases the potentially harmful inflammatory consequences of experimental cardiogenic shock. (*Crit Care Med* 2013; 41:00–00)

Key Words: big-endothelin; cardiac tamponade; complement C5a antagonist; high-mobility group box protein-1; histamine; intestinal microcirculation

Cardiogenic shock is common in patients with cardiac or extracardiac filling disorders, such as cardiac tamponade (1). The pericardial tamponade caused by effusions of blood, pus, fluids, or gases is a life-threatening medical emergency that demands immediate active intervention (2). Drainage of the pericardial sac through pericardiocentesis or surgical pericardiotomy is the first choice, but supportive medical therapy should be considered following or parallel to the relief of the intrapericardial pressure with a view to avoid low flow–induced peripheral complications. The administration of inotropic agents is controversial as they may cause tachycardia

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (http://journals.lww.com/ccmjournal).

Supported, in part, by research grants OTKA K75161; OTKA K104656; TÁMOP4.2.1; TÁMOP4.2.2.A; and TÉT JP 16/09.

The authors have not disclosed any potential conflicts of interest.

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DOI: 10.1097/CCM.0b013e31828a6768

<DOI:10.1097/CCM.0b013e31828a6768>

that can lead to a deterioration of the coronary perfusion (3). Indeed, it has been shown that cardiac tamponade is accompanied by the release of different types of vasopressors (4), with a consequent elevation of peripheral resistance. The increased afterload may subsequently worsen the hypoperfusion of the peripheral organs and result in an increased oxygen demand of the myocardium.

The generalized hemodynamic impairment is characterized by a circulatory redistribution leading to regional hypoperfusion of the splanchnic area with gastrointestinal (GI) mucosal damage (5, 6). As a consequence of hypoxia and reoxygenation, the complement system is activated, and a significant amount of anaphylatoxin C5a may be produced (7). The C5a fragment, a biologically active side-product of the complement cascade, can induce smooth muscle contraction, chemotaxis, and the activation of neutrophils (8), with the release of reactive oxygen species (ROS) and cytokines (9–12). Complement activation is further enhanced by the bacterial translocation through the hypoperfused intestinal mucosa (13), a process accompanied by the excessive release of endothelin-1 (ET-1) (14, 15), which might contribute to a further impairment of the microcirculation through its vasoconstrictor and proadhesive effects (16). Furthermore, the release of high-mobility group box protein-1 (HMGB-1) is also directly mediated by the C5a through C5a-like receptor 2 binding (C5L2) (17). HMGB-1 is an important factor of leukocyte recruitment, inducing the release of other proinflammatory cytokines (18).

Acute failure of the myocardial pump is accompanied by a high rate of complications, even after otherwise adequate treatment. It is widely accepted that inflammatory activation plays a decisive role in these conditions, although the potential of "anti-inflammatory" compounds to prevent or cure low perfusion–induced in vivo processes is very limited. Our primary aim was to investigate the effects of a cardiac tamponade on the overall hemodynamics and the intestinal microcirculatory alterations in a clinically relevant large animal model. Next, we hypothesized that the early inhibition of C5a might well reduce the adverse hemodynamic and inflammatory consequences after the relief of the cardiac tamponade through modulation of the release of other, potentially vasoconstrictive and proadhesive mediators. For this purpose, we decided to use acetyl-peptide-A (AcPepA), a synthetic, antisense peptide, which is capable of binding directly to C5a (in its 37–53 amino acid region) and has proved to be effective in pilot endotoxin shock studies (19–21). The results furnish evidence that the administration of AcPepA significantly limits the extent of intestinal microcirculatory damage in this scenario, in association with reduced HMGB-1 and big-endothelin (big-ET) levels, and neutrophil activation.

MATERIALS AND METHODS

The experiments were carried out in strict adherence to the National Institutes of Health guidelines for the use of experimental animals, and the study was approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged.

Animals and Instrumentation

Inbred Vietnamese mini pigs of both sexes (n = 19, weighing 24 ± 3 kg) were fasted for 12 hours preoperatively, but received water ad libitum. Anesthesia was induced with a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) IM and maintained with a continuous infusion of propofol (50 μL/min/kg IV; 6 mg/kg/hr). After endotracheal intubation, the animals were mechanically ventilated with the tidal volume set at 9 ± 2 mL/kg, and the respiratory rate was adjusted to maintain the end-tidal pressure of Co_2 and $Paco_2$ in the range 35 to 45 mm Hg. Positive end-expiratory pressure was not applied during the cardiac tamponade.

The animals were placed in a supine position on a heating pad for maintenance of the body temperature between 36°C and 37°C and received an infusion of Ringer's lactate at a rate of 10 mL/kg/hr during the experiments. The right femoral artery and jugular vein were cannulated for the measurement of mean arterial pressure (MAP) and cardiac output (CO) by thermodilution (PICCO Catheters; PULSION Medical Systems, Munich, Germany) and for fluid or drug administration, respectively. After a midline abdominal incision, the root of the superior mesenteric artery (SMA) was dissected free. An ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the exposed SMA to measure the mesenteric blood flow. In all protocols, the animals were monitored continuously, arterial blood gases were regularly checked (Cobas b121; Roche, Vienna, Austria), and a period of 30 minutes was allowed for recovery from surgery. AQ3

The animals were randomly allocated into one or other of three experimental groups. Group 1 (n = 6) served as sham-operated control, with the same time frame and sampling as in groups 2 (n = 7), and 3 (n = 6), but without the induction of a cardiac tamponade. Left lateral thoracotomy was performed in all groups, and in the cardiac tamponade groups, a cannula was fixed into the pericardial cavity. A pericardial tamponade was induced for 60 minutes by the intrapericardial administration of colloid solution, while the MAP was kept in the interval 40 to 45 mm Hg. After this period, the fluid was removed from the pericardial sac and the animals were monitored for 180-minute post-tamponade. Group 3 was treated with AcPepA (a single administration of 4 mg/kg in 5 mL saline IV into the jugular vein in a 5-minute infusion) after 45th minute of cardiac tamponade. The beginning of tamponade is denoting 0 minute. Vehicle (saline) administration was applied in groups 1 and 2 by the same protocol.

Peripheral blood samples were taken at baseline, after 75 and 150 minutes, and at the end of the observation period (240 minutes) to detect the levels of HMGB-1, big-ET, and whole blood superoxide production. Small intestinal tissue biopsies were taken at the end of the experiments for MPO activity measurements.

Hemodynamic Measurements

Central venous pressure (CVP) and blood flow signals were monitored continuously and registered with a computerized data acquisition system (SPELL Haemosys; Experimetria, Budapest, Hungary). The MAP, CO, and heart rate (HR) were

measured with the PICCO Plus monitoring system (PULSION Medical Systems) and the global end-diastolic volume index (GEDI) was calculated with the PICCO system.

C5a Antagonist Treatment

PepA (ASGAPAPGPAGPLRPMF) containing an acetylated N-terminal alanine (AcPepA) was synthesized and purified (>95% purity) by Biologica (Nagoya, Japan). The peptide was dissolved in saline and used in a concentration of 2 mg/mL, as reported previously (21).

Intravital Videomicroscopy of the Microcirculation

An intravital orthogonal polarization spectral imaging technique (Cytoscan A/R; Cytometrics, Philadelphia, PA) was used for noninvasive visualization of the mucosal microcirculation of the small intestine. This technique uses reflected polarized light at the wavelength of the isobestic point of oxy- and deoxyhemoglobin (548 nm). As polarization is preserved in reflection, only photons scattered from a depth of 200 to 300 μm contribute to image formation. A ×10 objective was placed onto the serosal surface of the ascending colon, and microscopic images were recorded with an S-VHS video recorder 1 (Panasonic AG-TL 700; Matsushita Electric Ind, Osaka, Japan). Quantitative assessment of the microcirculatory parameters was performed off-line by frame-to-frame analysis of the videotaped images. Red blood cell velocity (RBCV, μm/s) changes in the postcapillary venules were determined in three separate fields by means of a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary). All microcirculatory evaluations were performed by the same investigator.

MPO Activity

The activity of MPO, a marker of neutrophil activation, was determined in ileal biopsy samples according to the method of Kuebler et al (22). Briefly, a reaction mixture containing 50-mM K_3PO_4 buffer (pH 6.0), 2 mM 3,3′,5,5′-tetramethylbenzidine (dissolved in DMSO) and 100 μL of undiluted plasma sample was incubated for 5 minutes at 37°C. The reaction was started with 0.6 mM hydrogen peroxide (dissolved in 0.75 mL K_3PO_4 buffer) and was stopped after 5 minutes with 0.2 mL of H_2SO_4 (2 M), and the hydrogen peroxide–dependent oxidation of tetramethylbenzidine was detected spectrophotometrically at 450 nm (UV-1601 Spectrophotometer; Shimadzu, Kyoto, Japan). MPO levels were calculated via a calibration curve prepared with a MPO standard (Sigma-Aldrich GmbH, Munich, Germany). To detect the tissue MPO activity, ileal samples were homogenized with Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 mM phenylmethanesulfonyl fluoride to block tissue proteases and then centrifuged at 4°C for 20 minutes at 24,000*g*. The MPO activities of the samples were measured as described above, and the data were referred to the protein content.

Whole Blood Superoxide Production

For the whole blood superoxide production measurements, the chemiluminometric method of Zimmermann et al (23) was used. During the measurements, 10 μL of whole blood was added to 1-mL Hank's solution (PAA Cell Culture) and the mixture was kept at 37°C until assay. The chemiluminometric response was measured with a Lumat LB9507 luminometer (Berthold, Wildbad, Germany) during a 30-minute period after the addition of 100 μL of lucigenin.

High-Mobility Group Box Protein-1, Big-ET-1, and Histamine Measurements in Plasma

Four-milliliter blood samples were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg/mL) at baseline, after 75 and 150 minutes, and at the end of the observation period (240 minutes). The blood samples were centrifuged at 1,200*g* for 10 minutes at 4°C. The plasma samples were next collected and stored at –70°C until assay.

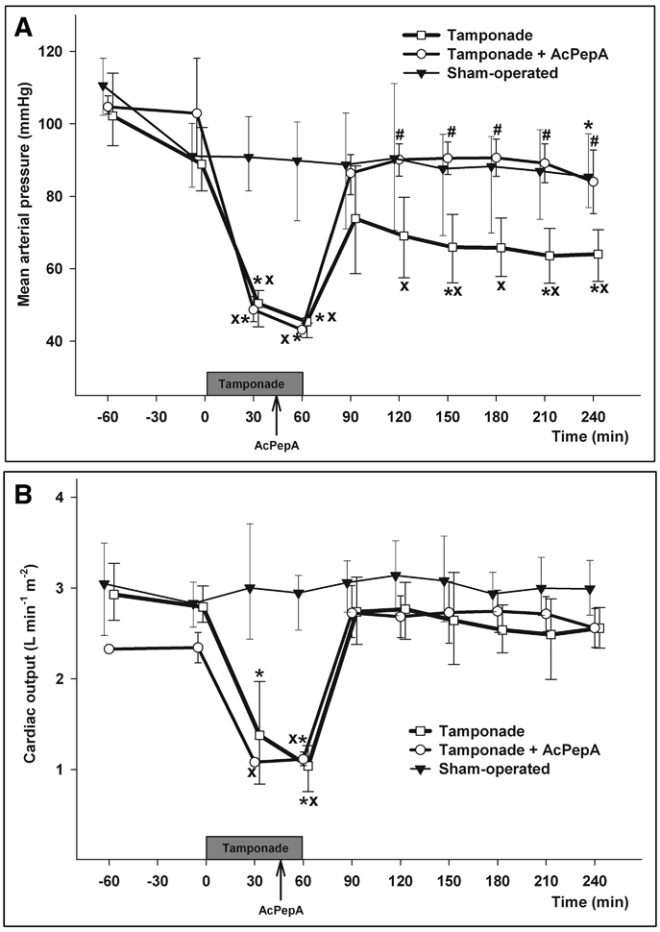

Figure 1. Changes in mean arterial pressure (**A**) and cardiac output (**B**) in the sham-operated ($n = 6$; *solid triangles with continuous line*), cardiac tamponade ($n = 7$; *empty squares with solid line*), and acetyl-peptide-A (AcPepA)-treated ($n = 6$; *empty circles with solid line*) groups. The *box* indicates the duration of the cardiac tamponade; the *arrow* shows the treatment with AcPepA. The plots demonstrate the median values and the 25th (*lower whisker*) and 75th (*upper whisker*) percentiles; $^{*}p < 0.05$ within groups versus baseline values (Friedman repeated-measures analysis of variance on ranks followed by Dunn's method), $^{x}p < 0.05$ between groups versus sham-operated group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method), and $^{\#}p < 0.05$ between AcPepA-treated group versus cardiac tamponade group (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method).

The plasma concentration of HMGB-1 was measured with a commercially available HMGB-1 ELISA kit (Shino-Test, Kanagawa, Japan). Plasma levels of big-ET, a 38-amino acid precursor protein of ET-1, were measured with a commercially available kit (Biochemica Hungaria Kft., Budapest, Hungary). Plasma histamine concentrations were determined by commercially available enzyme-linked immunoassay (Quantikine ultrasensitive EIA kit for histamine; Biomedica Hungaria Kft).

Rodent Experiments

We have designed an additional validation study to examine the long-term effects of AcPepA administration in rats. We have used a partial aorta occlusion model, which evokes standardized low flow in the splanchnic area and may correlate well with the effects of cardiac tamponade–induced hypoperfusion in this circulatory bed (see the detailed description of the protocol and results in the **supplemental data**, Supplemental Digital Content 1, http://links.lww.com/CCM/A660).

Statistical Analysis

Data analysis was performed with a statistical software package (SigmaStat for Windows; Jandel Scientific, Erkrath, Germany). Friedman repeated-measures analysis of variance on ranks was applied within groups. Time-dependent differences from the baseline for each group were assessed by Dunn's method, and differences between groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method for pairwise multiple comparison. In the figures, median values and 25th and 75th percentiles are given; p values of less than 0.05 were considered significant.

RESULTS

Macrohemodynamics

In sham-operated group 1, there were no significant hemodynamic changes when compared with the baseline values, and the mediator levels did not change significantly during the observation period. The MAP was kept in the interval 40 to 45 mm Hg during cardiac tamponade for 60 minutes (**Fig. 1*A***) by the infusion of colloid fluid into the pericardial sac, and this resulted in a significant 65% decline in CO in both groups undergoing cardiac tamponade (**Fig. 1*B***) and a significant increase in HR (**Fig. 2*C***). After relief of the tamponade, the MAP was significantly lower in the nontreated cardiac tamponade group when compared with the control group, while the CO and HR returned to the baseline values, despite the reduced MAP.

The decline in the venous return during the tamponade was evidenced by the increased CVP (**Fig. 2*A***). This process was accompanied by a decrease in GEDI (**Fig. 2*B***). The CVP was significantly decreased and GEDI did not reach the baseline values in the nontreated cardiac tamponade group (Fig. 2*A* and 2*B*) after relief of the tamponade. These changes demonstrate to the long-lasting impairment of the venous return flow following the cardiac tamponade.

The significant decrease in SMA blood flow during the tamponade indicated that redistribution deteriorated the mesenteric circulation. After the removal of the pericardiac fluid, the SMA flow returned to the control values (**Fig. 3*A***).

Administration of AcPepA after 45 minutes of the cardiac tamponade resulted in an elevation of the MAP, did not influence the CO, and caused a significant decrease in the HR when compared with the untreated cardiac tamponade group in the post-tamponade period (Figs. 1*A*, 1*B*, and 2*C*). Most importantly, we observed significant increases in the preload parameters and SMA flow in the post-tamponade period. Furthermore, the CVP was set to a significantly higher level

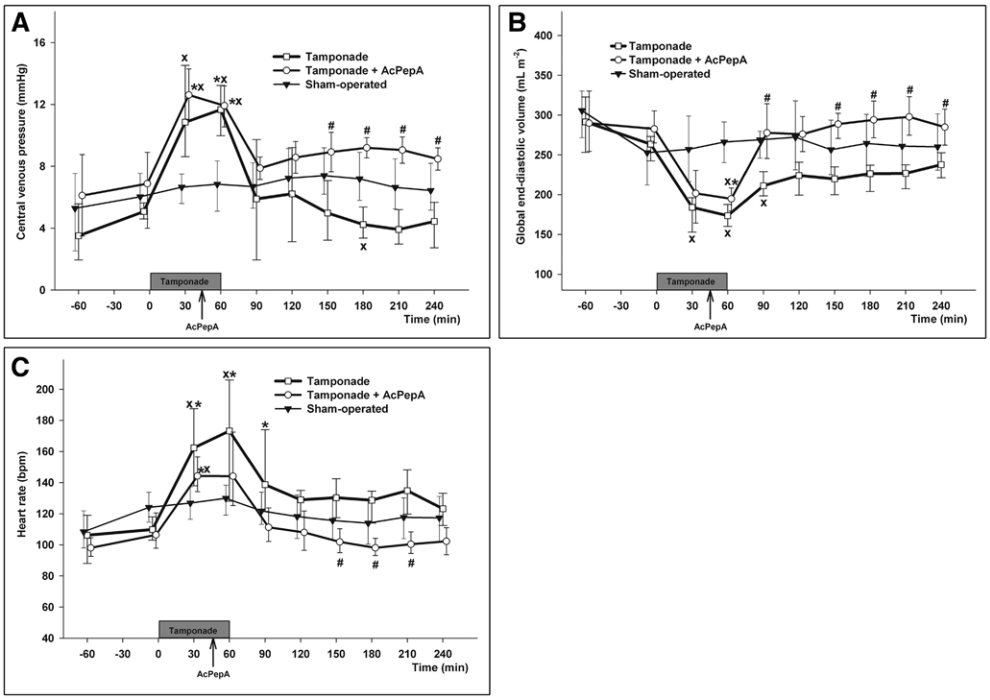

Figure 2. Changes in central venous pressure (**A**), global end-diastolic volume (**B**), and heart rate (**C**) in the sham-operated ($n = 6$; *solid triangles with continuous line*), cardiac tamponade ($n = 7$; *empty squares with solid line*), and acetyl-peptide-A (AcPepA)-treated ($n = 6$; *empty circles with solid line*) groups. The *box* indicates the duration of the cardiac tamponade; the *arrow* shows the treatment with AcPepA. The plots demonstrate the median values and the 25th (*lower whisker*) and 75th (*upper whisker*) percentiles; $^{*}p < 0.05$ within groups versus baseline values (Friedman repeated-measures analysis of variance on ranks followed by Dunn's method), $^{x}p < 0.05$ between groups versus sham-operated group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method), and $^{\#}p < 0.05$ between AcPepA-treated group versus cardiac tamponade group (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method).

and the GEDI demonstrated the increased returning blood flow (Figs. 2*A*, 2*B*, and 3*A*).

Microcirculation

A heterogeneous oscillating microcirculation was found in the small intestinal mucosa in all groups, and therefore, the weighted average of RBCV was calculated. This was based on the duration of the fast and slow flow periods and the RBCV during the respective phases, as reported previously (24). The RBCV during the fast flow periods showed no change in the sham-operated group, but at the end of the cardiac tamponade a significant decrease from the baseline was observed in the untreated cardiac tamponade group (**Fig. 3*B***). By the end of the post-tamponade phase, in the AcPepA-treated group, it was significantly increased from the baseline level and also relative to the nontreated group (cardiac tamponade group: M = 641.3; p25 = 584.5; p75 = 684.7 μm/s vs AcPepA group: M = 1,031.3; p25 = 935.3; p75 = 1108.3 μm/s).

The duration of the slow flow period in the cardiac tamponade group was increased significantly by 240 minutes, but it was reduced in the treated group (cardiac tamponade group: M = 15.01; p25 = 12.56; p75 = 17.56 s vs AcPepA group: M = 7.65; p25 = 7.33; p75 = 8.67 s).

By the end of the experiments, the characteristic variable of the mucosal microcirculation, the average RBCV of the flow pattern, was significantly reduced in the cardiac tamponade group when compared with the AcPepA group (Fig. 3*B*).

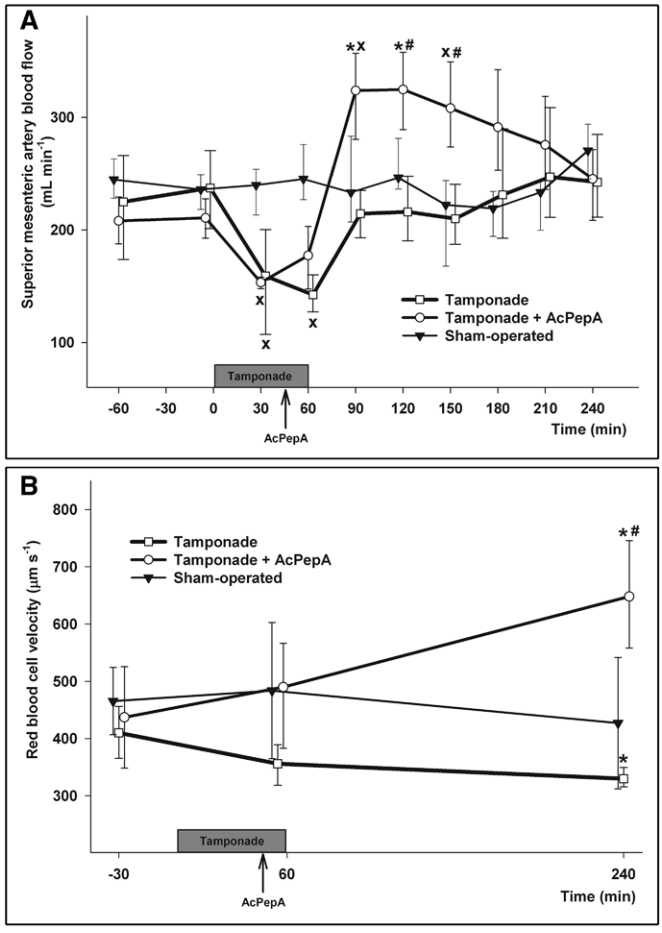

Figure 3. Changes in superior mesenteric artery blood flow (**A**) and average red blood cell velocity (**B**) in the sham-operated ($n = 6$; *solid triangles with continuous line*), cardiac tamponade ($n = 7$; *empty squares with solid line*), and acetyl-peptide-A (AcPepA)-treated ($n = 6$; *empty circles with solid line*) groups. The *box* indicates the duration of the cardiac tamponade; the *arrow* shows the treatment with AcPepA. The plots demonstrate the median values and the 25th (*lower whisker*) and 75th (*upper whisker*) percentiles; $^{*}p < 0.05$ within groups versus baseline values (Friedman repeated-measures analysis of variance on ranks followed by Dunn's method), $^{x}p < 0.05$ between groups versus sham-operated group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method), and $^{\#}p < 0.05$ between AcPepA-treated group versus cardiac tamponade group (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method).

Biochemical Parameters

Peripheral blood samples were taken at baseline, after 75 and 150 minutes, and at the end of the observation period (240 minutes). Changes in histamine concentration are presented in **Figure 4*A***. As a result of the cardiac tamponade, the histamine level was increased significantly by 15 minutes of the post-tamponade phase. F4

Big-ET is a stable precursor of ET-1 with a longer half-life; it is released from different types of cells. The plasma big-ET levels increased significantly four- to five-fold in the nontreated group after cardiac tamponade (**Fig. 4*B***).

HMGB-1 is a very effective signal for neutrophil activation, which causes an escalation of the inflammatory process. The plasma level of HMGB-1 was elevated significantly after the compression of the heart (**Fig. 4*C***). The rate of neutrophil accumulation was determined through the measurement of the MPO activity of the intestinal tissue samples taken at the end of the experiments. The level of MPO activity was significantly higher in the small intestine tissue samples of the cardiac tamponade group, indicating the increased accumulation of neutrophils (**Fig. 5*A***). In the cardiac tamponade group, increased superoxide production was observed in the blood at the beginning of the post-tamponade phase (**Fig. 5*B***). F5

After AcPepA administration, the characteristic biochemical changes following the cardiac tamponade were significantly different. The AcPepA treatment reduced the concentrations of histamine, big-ET, and HMGB-1 in the plasma. The amount of oxygen free radicals formed was also reduced by the treatment, and the MPO activity too was decreased (Figs. 4 and 5).

DISCUSSION

Wide-ranging investigations were carried out to characterize the acute hemodynamic effects of temporary mechanical compression of the heart and to outline the consequences of C5a antagonism on the early splanchnic circulatory changes. In this porcine model, the post-tamponade period was characterized by decreased levels of MAP and CVP, whereas the HR was significantly elevated. These acute hemodynamic alterations were accompanied by definite signs of inflammatory activation, with the release of vasoactive and proinflammatory mediators,

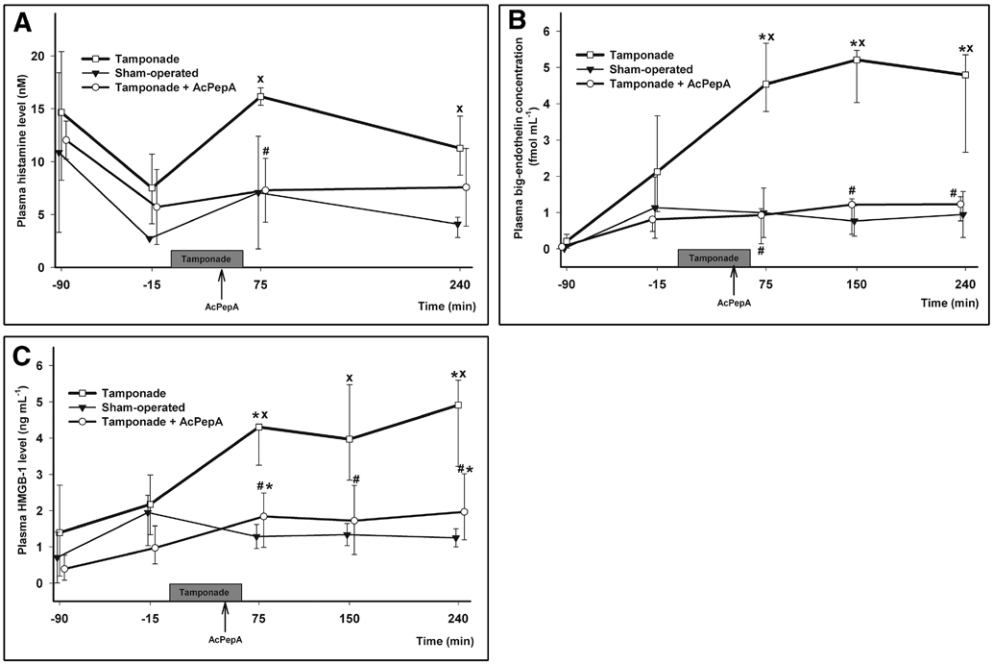

Figure 4. Changes in plasma histamine level (**A**), plasma big-endothelin concentration (**B**), and plasma high-mobility group box protein-1 (HMGB-1) level (**C**) in the sham-operated ($n = 6$; *solid triangles with continuous line*), cardiac tamponade ($n = 7$; *empty squares with solid line*), and acetyl-peptide-A (AcPepA)-treated ($n = 6$; *empty circles with solid line*) groups. The *box* indicates the duration of the cardiac tamponade; the *arrow* shows the treatment with AcPepA. The plots demonstrate the median values and the 25th (*lower whisker*) and 75th (*upper whisker*) percentiles; $^{*}p < 0.05$ within groups versus baseline values (Friedman repeated-measures analysis of variance on ranks followed by Dunn's method), $^{x}p < 0.05$ between groups versus sham-operated group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method), and $^{\#}p < 0.05$ between AcPepA-treated group versus cardiac tamponade group (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method).

including histamine, HMGB-1, and big-ET. The deterioration of the systemic circulation led to the parallel impairment of the microcirculation in the splanchnic area, and these responses were accompanied by an increase in local MPO activity, a quantitative marker of ROS-producing neutrophils in the plasma and various tissues (25, 26).

After the relief of the cardiac tamponade, the MAP was decreased, whereas the CO was kept compensated, and there were no significant differences when compared with the control group. This was achieved by the elevated HR, which shows the increased strain of the heart muscle. Following AcPepA treatment, the MAP was elevated to the control level and the CO was also maintained. Thus, the most pronounced differences between the compensating mechanisms of treated and nontreated tamponade groups were the restored CO and the lower HR. This seems to be especially crucial if we consider the fact that the circulation of the heart muscle is provided during the diastolic phase, and nearly stops during the systoles. If the HR is increased, the length of the systole does not change, while the diastole is shortened. A higher frequency then results in less time for oxygen delivery to the cardiac muscle cells, and therefore, a lower HR and maintained CO provide better oxygenation for the heart. The elevated CVP and GEDI in the treated group also indicate that the main compensatory mechanism in this early phase is switched from an increased afterload to an elevation of the preload. These changes might be the consequences of reduced ET and histamine release after AcPepA treatment. Indeed, it has been shown that nonselective ET-receptor antagonism increases the CO and decreases the peripheral resistance in patients with congestive heart failure (27). On the contrary, C5a causes histamine release from mast cells, which may lead to enhanced vascular permeability and a relative blood loss into the dilated vessels. The elevation in plasma histamine level in the early post-tamponade phase, together with previous data, shows that this level is higher in the portal venous blood than that in the arterial blood (4). In the presence of AcPepA, the histamine release was reduced, and this effect too can contribute to the increased venous return.

In this porcine model, the pericardial tamponade triggered characteristic macro- and microcirculatory changes in the intestines. The share of the splanchnic area from the reduced CO was diminished during the tamponade, but the SMA flow, which reflects the blood supply of the small intestine and colon, was restored thereafter, and no differences were observed relative to the sham-operated group. Nevertheless, in the AcPepA-treated group, the SMA flow was significantly elevated at the beginning of the post-tamponade phase, and gradually returned to the control level by the end of the experiments. This early flow elevation may be attributable to the relative lack of vasoconstrictor ET-1 effects because it has been demonstrated that ET-A receptor inhibition is able to improve the splanchnic circulation in similar scenarios (28).

In shock conditions, an important feature of the splanchnic microcirculatory disturbances is heterogeneity, which worsens the oxygen supply and metabolism of the cells (29, 30). A heterogeneously oscillating microcirculation can be characterized well by the average RBCV (24, 31). It has been demonstrated that duration of the high-flow component of a heterogeneous microcirculation is increased significantly following ET-A receptor inhibition (24). From this point of view, the excessive release of ET-1 can be an important part of the worsening inflammatory responses, when intensive complement activation is present. ET-1 release can contribute to the impairment of the microcirculation and, through its vasoconstrictor effects, to the changes in the macrohemodynamics throughout such conditions, and it can even influence the pattern of cytokine release (32).

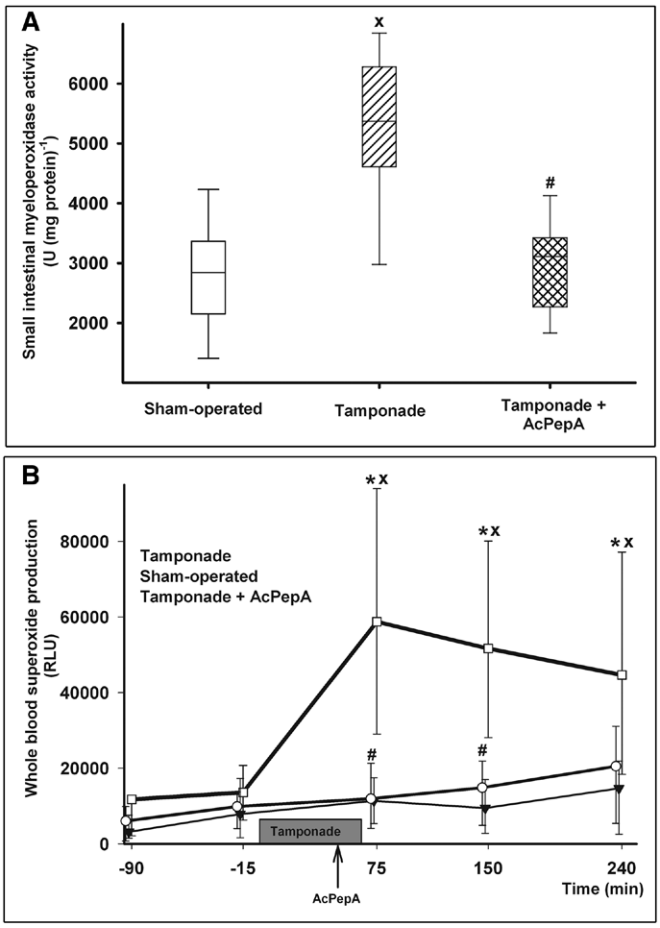

Figure 5. Changes in small intestinal MPO activity (**A**) in the sham-operated ($n = 6$; *empty box*), cardiac tamponade ($n = 7$; *striped box*), and acetyl-peptide-A (AcPepA)-treated ($n = 6$; *checked box*) groups. Changes in whole blood superoxide production (**B**) in the sham-operated ($n = 6$; *solid triangles with continuous line*), cardiac tamponade ($n = 7$; *empty squares with solid line*) and AcPepA-treated ($n = 6$; *empty circles with solid line*) groups. The *box* indicates the duration of the cardiac tamponade; the *arrow* shows the treatment with AcPepA. The plots demonstrate the median (*horizontal line in the box*) and the 25th and 75th percentiles. $^{*}p < 0.05$ within groups versus baseline values (Friedman repeated-measures analysis of variance on ranks followed by Dunn's method), $^{x}p < 0.05$ between groups versus sham-operated group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method), and $^{\#}p < 0.05$ between AcPepA-treated group versus cardiac tamponade group values (Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method).

The cause of the improved mucosal RBCV in the AcPepA-treated group may be multifactorial. The decreased levels of ET-1 formation and leukocyte activation can each effectively reduce the extent of reactive oxygen intermediate formation. By the reduction of these sources of tissue damage, the endothelial function can be preserved, which subsequently results in the improvement of microcirculation.

We found increased HMGB-1 levels after tamponade, which were significantly reduced by AcPepA treatment. These findings correlate to the similar results of continuous AcPepA treatment in a primate endotoxin shock model (21). Furthermore, additional data support the notion of the long-term effectiveness of C5a antagonist treatment in a similar model of a low-flow–mediated splanchnic dysfunction (supplemental data, Supplemental Digital Content 1, http://links.lww.com/CCM/A660). These results can be explained by the involvement of the C5L2 receptor, a high-affinity C5a receptor, which was earlier thought to be a nonsignaling, scavenger receptor of C5a as it is incapable of G-protein coupling. Recent findings, however, have proved that C5L2 has a more important role in mediating inflammatory responses of the innate immune system. The survival rate in C5L2 knockout mice was increased after cecal ligation puncture–induced sepsis relative to the survival rate in wild-type mice. Other data have revealed that the release of the potent proinflammatory cytokine HMGB-1 after endotoxin + C5a or only endotoxin administration was diminished in C5L2 knockout macrophages (17).

To determine the effects that can be linked to C5a antagonist treatment, we had to consider and rule out artificial influences that may originate from the experimental design. We, therefore, applied standard fluid replacement therapy in all groups to exclude the effects of the volume status on the microcirculation. The clinical limitations are evident, but it is highly likely that a preserved microcirculatory function in the GI tract is mediated directly or indirectly by C5a pathways in this experimental model of anesthetized, ventilated, and thoracotomized mini pigs.

In conclusion, these results demonstrate the relative significance of complement activation in the acute circulatory complications of cardiac tamponade, and the potential role of C5a antagonism to reduce signals that are important components in the development of a secondary splanchnic microcirculatory disturbance.

ACKNOWLEDGMENTS

We are grateful to Ms. Ágnes Fekete, Csilla Mester, and Lilla Kovács for skillful assistance.

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