Original Paper

Inflammatory Activation after Experimental Cardiac Tamponade

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Abstract

Background/Purpose: Cardiac tamponade is a medical emergency situation associated with a high rate of life-threatening complications, even after immediate interventions. Our aim was to characterize the acute inflammatory consequences of this event in a clinically relevant large animal model. ***Methods:*** Cardiac tamponade was induced for 60 min in anesthetized, ventilated and thoracotomized minipigs by intrapericardial fluid administration, the mean arterial pressure (MAP) being maintained in the interval of 40–45 mm Hg (n = 8). A further group (n = 7) served as sham-operated control. The global macrohemodynamics, including the right- and left-heart end-diastolic volumes (RHEDV and LHEDV), the pulmonary vascular resistance index (PVRI) and the superior mesenteric artery (SMA) flow, were monitored for 240 min, and the intestinal microcirculatory changes (pCO_2 gap) were evaluated by indirect tonometry. Blood samples were taken for the determination of cardiac troponin T and vasoactive inflammatory mediators, including histamine, nitrite/nitrate, big-endothelin, superoxide and high-mobility group box protein-1 levels in association with intestinal leukocyte and complement activation. ***Results:*** The cardiac tamponade induced significant decreases in MAP, cardiac output, LHEDV and SMA flow, while the PVRI and the pCO_2 gap increased significantly. After the removal of fluid from the pericardial sac, the MAP and the LHEDV were decreased, while

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European Surgical Research

Eur Surg Res 2013;51:1–13

DOI: 10.1159/000352089

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the PVRI and the pCO_2 gap remained elevated when compared with those in the sham-operated group. In the posttamponade period, the abrupt release of inflammatory mediators was accompanied by a significant splanchnic leukocyte accumulation and complement activation. **Conclusions:** The macrocirculatory and splanchnic microcirculatory disturbances were accompanied by a significant proinflammatory reaction; endothelin and the complement system may be significant components of the inflammatory cascade that is activated in this porcine model of pericardial tamponade.

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Introduction

Cardiogenic shock may occur in patients with cardiac or extracardiac filling disorders such as cardiac tamponade [1, 2]. The acute rise in pericardial pressure compresses the atria and the right ventricle and hinders the diastolic filling of the heart [3], thereby leading to a decreased cardiac output (CO). Vasoconstriction is a general compensatory reaction in the forms of shock that involve low CO, occurring as an appropriate response that serves to restore the declining arterial flow and pressure towards former values. The vasoconstriction is nonuniform, and the redistributed blood flow can maintain the blood supply for the heart and brain at a relatively normal level in the short run. This process is mediated through activation of the sympathetic nervous system and various humoral mediators [4], leading to systemic vasoconstriction, tachycardia and fluid retention [2]. However, the price for the potentially beneficial effects of selective vasoconstriction is severe hypoxia in the underperfused organs [5–7]. Moreover, the increased afterload may subsequently worsen the peripheral hypoperfusion and also increases the oxygen demand of the myocardium.

Drainage of the pericardial sac through pericardiocentesis or surgical pericardiotomy is the first choice for therapy [8], but removal of the pericardial fluid can lead to a reperfusion phenomenon with further peripheral reactions. As a consequence of hypoxia and reoxygenation, reactive oxygen species are generated and polymorphonuclear neutrophil (PMN) leukocytes are activated. The formation of these radicals may lead to disintegration of the cell membranes, structural damage and decreased cellular functions [9, 10]. High-mobility group box protein-1 (HMGB1), released passively by necrotic and damaged cells, was recently identified as an important signal for leukocyte recruitment [11]. Further factors identified in the background of PMN leukocyte accumulation are an increased level of endothelin-1 (ET-1) formation and a decreased level of nitric oxide (NO) formation, which coexist in ischemia-reperfusion syndromes [12]. ET-1, one of the most powerful endogenous vasoactive mediators, may contribute to the impairment of the microcirculation through its vasoconstrictor and proadhesive effects [13]. Moreover, it results in histamine release from resident mast cells [14], and influences the activation of the complement cascade [15].

With regard to this background, we hypothesized that acute failure of the myocardial pump function is accompanied by significant inflammatory activation, which can play important roles in further clinical complications. Our primary aim was to investigate the immediate effects of a cardiac tamponade on the systemic and peripheral circulations in a clinically relevant, large animal model. We also aimed to characterize the major components of the proinflammatory profile of the posttamponade phase, in association with the changes in overall hemodynamics. We assumed that a better understanding of the elements of this mechanism may lead to new prospects for interventions designed to dampen or reverse the secondary detrimental consequences of acute heart failure.

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Eur Surg Res 2013;51:1–13

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Materials and Methods

The experiments were carried out in strict adherence to the NIH 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 (V./148/2013).

Animals and Instrumentation

Inbred Vietnamese minipigs of both sexes (n = 15, weighing 24 ± 3 kg) were fasted for 12 h preoperatively, but received water ad libitum. Anesthesia was induced with ketamine (20 $mg \cdot kg^{-1}$) and xylazine (2 $mg \cdot kg^{-1}$) i.m. and maintained with a continuous infusion of propofol (50 $\mu l\ min^{-1} \cdot kg^{-1}$ i.v.; 6 $mg \cdot kg^{-1} \cdot h^{-1}$) supplemented with ketamine (5 $mg \cdot kg^{-1}$) – xylazine (0.5 $mg \cdot kg^{-1}$) mixture i.m. before thoracotomy and after 90 min of the observation period. After endotracheal intubation, the animals were mechanically ventilated with the tidal volume set at 9 ± 2 $ml \cdot kg^{-1}$, and the respiratory rate was adjusted to maintain the end-tidal pressure of CO_2 and arterial pCO_2 ($PaCO_2$) in the range 35–45 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 and 37°C, and received an infusion of Ringer's lactate at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ during the experiments. The right jugular vein was cannulated (7 F; Edwards Lifesciences LLC, Irvine, Calif., USA) for the measurement of central venous pressure (CVP) and for fluid administration. The right femoral artery and vein were dissected and a thermodilution catheter (PULSION Medical Systems SE, Munich, Germany) was placed in the femoral artery for the measurement of mean arterial pressure (MAP) and CO by a transpulmonary thermodilution method (PiCCO; PULSION Medical Systems SE). A pulmonary artery catheter (PV2057 VoLEF Catheter; PULSION Medical Systems SE) was inserted via the right femoral vein into the pulmonary artery by tracing the pressure signals.

After a midline abdominal incision, the root of the superior mesenteric artery (SMA) was dissected free. An ultrasonic flow probe (Transonic Systems Inc., Ithaca, N.Y., USA) was placed around the exposed SMA to measure the mesenteric blood flow. In all protocols, the animals were monitored continuously and a period of 30 min was allowed for recovery from surgery.

The animals were randomly divided into 2 experimental groups. Group 1 (n = 7) served as a sham-operated control, with the same time-frame and sampling as in group 2 (n = 8) with the induction of a cardiac tamponade. Left lateral thoracotomy was performed and a cannula was fixed into the pericardial cavity in both groups. A pericardial tamponade was induced for 60 min by the intrapericardial administration of colloid solution (60–90 ml hydroxyethyl starch 6%; Fresenius Kabi Deutschland GmbH, Homburg, Germany), while the MAP was kept in the interval 40–45 mm Hg. After this period, the fluid was removed from the pericardial sac and the animals were monitored for 180 min posttamponade.

Peripheral blood samples were taken at baseline, after 75 min, after 150 min and at the end of the observation period (240 min) to detect the levels of vasoactive and inflammatory mediators (big-ET, histamine, nitrite/nitrate, HMGB1 and whole-blood superoxide production). Small intestinal tissue biopsies were taken at the end of the experiments for myeloperoxidase (MPO) activity measurements and immunohistochemical analysis of the complement C3 deposit.

Hemodynamic Measurements

CVP and blood flow signals were monitored continuously and registered with a computerized data-acquisition system (SPELL Haemosys; Experimetria Ltd., Budapest, Hungary). For further hemodynamic monitoring, we used a combination of PiCCO Plus V 5.2.2 and VoLEF V 1.0 (PULSION Medical Systems SE) monitors [16]. The MAP, CO, heart rate (HR) and extravascular lung water index (EVLWI) were measured with the PICCO Plus monitoring system, while the PiCCO VoLEF monitor system was applied to measure the pulmonary arterial pressure (PAP), and calculate the right- and left-heart end-diastolic volumes (RHEDV and LHEDV), systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI). All hemodynamic parameters were indexed for body surface area or body weight. A detailed description of the transpulmonary thermodilution and volumetric analysis is provided elsewhere [17].

Evaluation of Intestinal Microcirculation: pCO$_2$ Gap Measurements

A difference between local tissue and $PaCO_2$ levels is a sensitive parameter with which to evaluate the effectiveness of therapy aimed at counteracting a microcirculatory dysfunction in the gastrointestinal tract [18]. A silastic balloon-free tonometric probe (Tonosoft Medical Technical and R&D Ltd., Hungary) was intro-

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duced into the intestinal lumen through a small enterotomy to monitor intramucosal pCO_2 levels by capnometry [19]. For calculation of the pCO_2 gap values, the simultaneously taken $PaCO_2$ levels were subtracted from the tonometric pCO_2 levels. Arterial and venous blood samples were taken at the baseline and once an hour, and blood-gas parameters were measured with a blood-gas analyzer (Cobas b121; Roche, Austria).

Biochemical Measurements
MPO Activity
The activity of MPO, a marker of PMN leukocyte activation, was determined in ileal biopsy samples by the method of Kuebler et al. [20]. Briefly, the sample was homogenized with Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride to block tissue proteases, and then centrifuged at 4°C for 20 min at 2,000 g. The MPO activities of the samples were measured at 450 nm (UV-1601 spectrophotometer; Shimadzu, Japan) 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. [21] was used. During the measurements, 10 μl of whole blood was added to 1 ml of Hank's solution and the mixture was kept at 37°C until assay. The chemiluminometric response was measured with a Lumat LB9507 luminometer (Berthold, Vienna, Austria) over 30 min after the addition of 100 μl of lucigenin.

Plasma Nitrite/Nitrate Level Measurements
The levels of plasma nitrite/nitrate (NO_x), stable end-products of NO, were measured by means of the Griess reaction. The assay depends on the enzymatic reduction of nitrate to nitrite, which is then converted into a colored azo compound detected spectrophotometrically at 540 nm [22].

HMBG1, Big-ET and Histamine Measurements in Plasma
Blood samples of 4 ml were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg·ml^{-1}) and then centrifuged at 1,200 g for 10 min at 4°C. The plasma samples were next collected and stored at –70°C until assay.
The plasma concentration of HMGB1 was measured with a commercially available HMGB1 ELISA kit (Shino-Test Corporation, Kanagawa, Japan). Plasma levels of big-ET, a 38-amino-acid precursor protein of ET-1, were measured with a commercially available kit (Biomedica Hungaria Kft., Budapest, Hungary). Plasma histamine concentrations were determined by commercially available enzyme-linked immunoassay (Quantikine ultrasensitive EIA kit for histamine; Biomedica Hungaria Kft.).

Plasma Troponin T Level Measurements
Cardiac troponin T levels were measured from plasma samples by highly sensitive electrochemilumi-nescent immunoassay (ECLIA; Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany). The analytical sensitivity was 5 ng/l, the intra- and interassay variations at the measured concentration range were 3.2 and 6.2 CV%, respectively.

Immunohistochemistry
The presence of complement C3 deposit in the small intestinal mucosa was detected by immunohisto-chemistry (IHC) [23] on formalin-fixed, paraffin-embedded small intestine sections, using rabbit polyclonal anticomplement fragment C3c primary antibody (Bioss Inc., Woburn, Mass., USA). The sections were deparaffinized for 5 min, which was followed by antigen retrieval with citrate buffer for 20 min. The activity of endogenous peroxidases was blocked with 5% H_2O_2 for 10 min. The nonspecific interactions were inhibited during the next 30 min of incubation. The primary antibody was diluted 1:500 in antibody diluent for IHC (BD Pharmingen, San Diego, Calif., USA). After washing of the sections, the secondary antibody was diluted 1:500 and the samples were incubated for 8 min. Sections were counterstained with 3,3′-diaminobenzidine and hematoxylin. The entire IHC investigation was carried out with an automatic Leica Bond-max IHC machine (Leica Microsystems, Tokyo, Japan). For quantitative analysis, immunostained sections were examined under a light microscope. The coded sections were analyzed by an independent specialist in histo-pathology (M.F.). The numbers of capillaries positive for complement fragment C3c were assessed at a magni-fication of ×400.
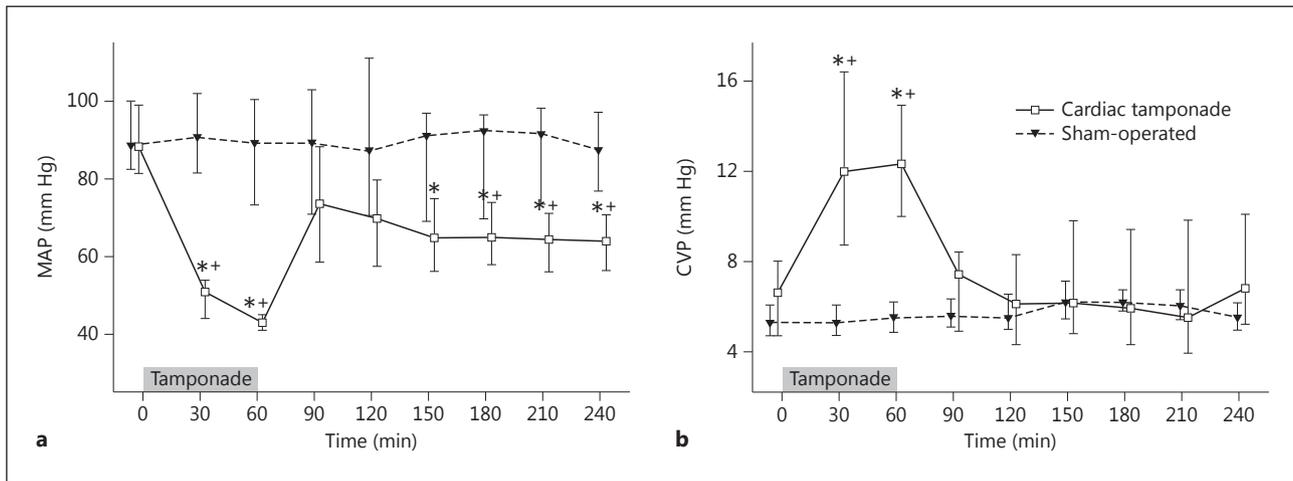
European Surgical Research

Eur Surg Res 2013;51:1–13

DOI: 10.1159/000352089

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Fig. 1. Changes in MAP (**a**) and CVP (**b**) in the sham-operated and cardiac-tamponade groups. The grey boxes indicate the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.

Statistical Analysis

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). The distribution of our experimental data was analyzed by the Kolmogorov-Smirnov normality test. Failure of the normality test indicated nonparametric distribution of the data. The Friedman repeated-measures analysis of variance on ranks was applied within groups. Time-dependent differences from the baseline for each group were assessed by the Dunn method, and differences between groups were analyzed with the Mann-Whitney test. In the figures, median values and 25th and 75th percentiles are given; p values <0.05 are considered statistically significant.

Results

Hemodynamics

In the sham-operated group, there were no significant hemodynamic changes compared to the baseline values, and the mediator levels did not change significantly during the observation period.

The MAP was maintained in the interval 40–45 mm Hg during the tamponade for 60 min (fig. 1a) by the infusion of colloid fluid into the pericardial sac, and this resulted in a significant decline of approximately 60% in CO in the group undergoing cardiac tamponade. The SVRI and HR were increased significantly (by 32 and 66%, respectively; table 1). After relief of the tamponade, the MAP was significantly lower in the cardiac tamponade group compared with the control group, while the CO and HR returned to the baseline, despite the reduced MAP.

The decline in the venous return during the tamponade was evidenced by the increased CVP (fig. 1b). This process was accompanied by decreases in RHEDV and LHEDV (fig. 2a, b). After relief of the tamponade, the CVP and RHEDV were normalized, but the LHEDV did not reach the baseline value in the tamponade group; it remained significantly lower compared with the sham-operated group (fig. 2a, b). These changes demonstrate the long-lasting impairment of the left ventricular function following the cardiac tamponade.

The cardiac tamponade resulted in a significant, transient decrease in PAP and a 2-fold elevation of the PVRI (table 2). In the posttamponade period, further long-lasting significant

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Table 1. Effects of cardiac tamponade on CO, HR and SVRI

	–5 min	30 min	60 min	90 min	120 min	180 min	240 min
CO, l·min^{-1}·m^{-2}							
Sham-operated	2.60	2.73	2.74	2.85	2.94	2.85	2.82
	(2.39; 2.97)	(2.25; 3.34)	(2.30; 3.14)	(2.60; 3.28)	(2.65; 3.30)	(2.51; 3.35)	(2.62; 3.33)
Cardiac tamponade	2.82	1.36*, **	1.19*, **	2.44	2.48	2.40	2.40
	(2.46; 3.01)	(0.95; 1.89)	(1.03; 1.30)	(2.29; 3.01)	(2.16; 3.03)	(2.13; 2.74)	(2.07; 2.79)
HR, beat·min^{-1}							
Sham-operated	124	128	125	126	127	120	122
	(120; 137)	(118; 134)	(119; 134)	(115; 137)	(104; 135)	(104; 128)	(106; 126)
Cardiac tamponade	113	175*, **	188*, **	125	118	114	113
	(103; 118)	(136; 203)	(173; 206)	(122; 143)	(113; 120)	(105; 116)	(98; 122)
SVRI, mm Hg·ml^{-1}·min^{-1}·kg^{-1}							
Sham-operated	0.79	0.81	0.74	0.68	0.64	0.66	0.67
	(0.73; 0.83)	(0.75; 0.85)	(0.69; 0.79)	(0.66; 0.77)	(0.58; 0.68)	(0.57; 0.71)	(0.61; 0.75)
Cardiac tamponade	0.78	0.91	1.03*	0.83	0.83**	0.82**	0.79
	(0.71; 0.92)	(0.80; 0.94)	(0.84; 1.06)	(0.72; 0.85)	(0.69; 0.93)	(0.73; 0.91)	(0.71; 0.91)

Values are medians (25th percentile; 75th percentile).
* $p < 0.05$ within group; ** $p < 0.05$ between groups vs. control group.

Table 2. Effects of cardiac tamponade on PAP, PVRI and EVLWI

	–5 min	30 min	60 min	90 min	120 min	180 min	240 min
PAP, mm Hg							
Sham-operated	30.6	31.1	28.9	31.2	30.3	30.3	30.8
	(27.0; 35.0)	(27.3; 35.4)	(27.5; 35.6)	(26.0; 36.0)	(27.4; 34.8)	(26.8; 32.5)	(28.3; 33.6)
Cardiac tamponade	30.4	24.3*, **	30.0	38.4	36.5	41.2*, **	37.9
	(27.3; 34.2)	(21.1; 27.8)	(19.2; 35.6)	(30.0; 41.3)	(34.4; 42.7)	(35.3; 43.2)	(31.9; 42.8)
PVRI, mm Hg·ml^{-1}·min^{-1}·kg^{-1}							
Sham-operated	0.37	0.35	0.31	0.29	0.29	0.32	0.31
	(0.31; 0.39)	(0.30; 0.43)	(0.29; 0.35)	(0.25; 0.35)	(0.25; 0.32)	(0.24; 0.36)	(0.23; 0.38)
Cardiac tamponade	0.41	0.75*, **	0.88*, **	0.48**	0.51**	0.54**	0.49**
	(0.26; 0.50)	(0.66; 0.98)	(0.81; 0.95)	(0.42; 0.53)	(0.44; 0.63)	(0.49; 0.60)	(0.47; 0.54)
EVLWI, ml·kg^{-1}							
Sham-operated	8.0	8.2	8.5	8.5	8.5	8.4	8.4
	(7.8; 9.0)	(8.0; 9.0)	(8.0; 9.0)	(8.0; 9.1)	(8.0; 9.1)	(8.0; 9.0)	(8.0; 9.0)
Cardiac tamponade	8.0	8.0	8.0	8.5	9.0	9.5*	10.0*, **
	(8.0; 9.0)	(7.5; 8.8)	(7.3; 8.9)	(8.0; 10.8)	(8.3; 10.8)	(8.5; 10.0)	(9.0; 11.0)

Values are medians (25th percentile; 75th percentile).
* $p < 0.05$ within group; ** $p < 0.05$ between groups vs. control group.

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Eur Surg Res 2013;51:1–13

DOI: 10.1159/000352089

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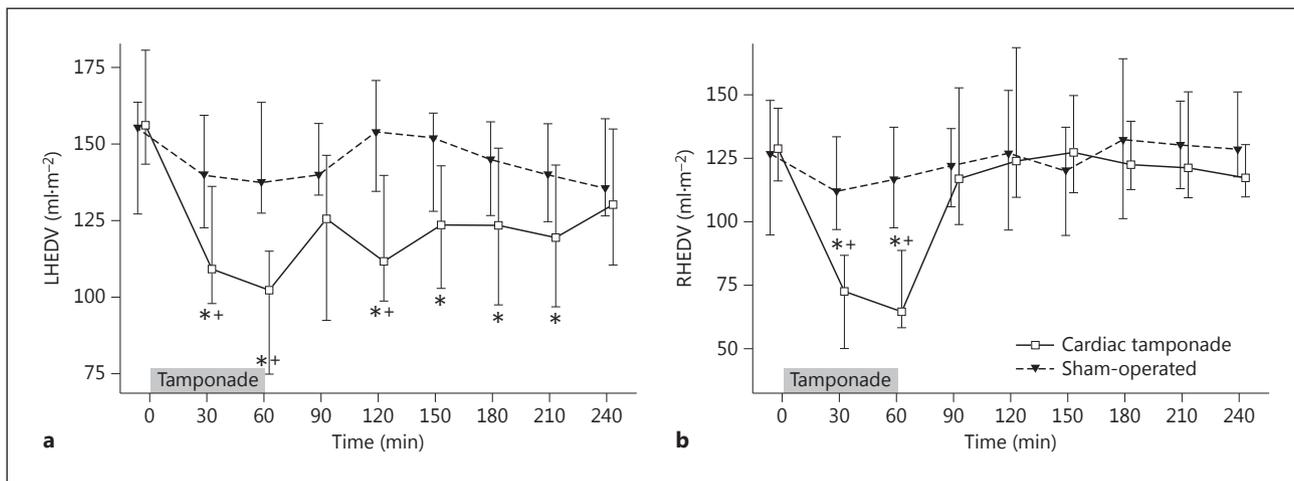Vass et al.: Inflammatory Activation after Experimental Cardiac Tamponade

Fig. 2. Changes in LHEDV (**a**) and RHEDV (**b**) in the sham-operated and cardiac-tamponade groups. The grey boxes indicate the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.

increases in PVRI and PAP occurred when compared with the sham-operated group, while the EVLWI was significantly elevated at the end of the observation period (table 2).

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

The pCO_2 gap, the difference between the local tissue and the arterial pCO_2, is a reliable index of local tissue perfusion. The pCO_2 gap increased significantly during the tamponade, while relief of the tamponade resulted in a significantly lower gap, though the values remained significantly higher than that for the sham-operated control group throughout the posttamponade period (fig. 3b; online suppl. table 1, www.karger.com/doi/10.1159/000352089).

Biochemical Parameters

Peripheral blood samples were taken at baseline, after 75 min, after 150 min and at the end of the observation period (240 min). In the cardiac tamponade group, increased superoxide radical production was observed at the beginning of the posttamponade phase (fig. 4a). In parallel, the plasma histamine levels were increased significantly at 75 and 240 min of the observation period: M = 16.2 nM (p25 = 15.5; p75 = 16.6) and M = 10.3 nM (p25 = 9.1; p75 = 12.8), respectively, versus the baseline (M = 7.5 nM; p25 = 6.4; p75 = 8.8) or versus the corresponding value for the sham-operated group (M = 7.4 nM; p25 = 5.7; p75 = 9.4).

The NO_x concentration in the plasma allows an estimate of the changes in NO production. The consequence of the cardiac tamponade was a slight, but statistically significant increase in NO_x level at the end of the posttamponade period when compared with the baseline level and with that for the sham-operated group (fig. 4b).

Big-ET is a stable precursor of ET-1 with a longer half-life. The plasma big-ET level increased significantly, 4- to 5-fold, in response to the cardiac tamponade (fig. 5a).

HMGB1 is an effective signal for leukocyte activation, which causes an escalation of the inflammatory process. The plasma level of HMGB1 was elevated significantly after the compression of the heart (fig. 5b).
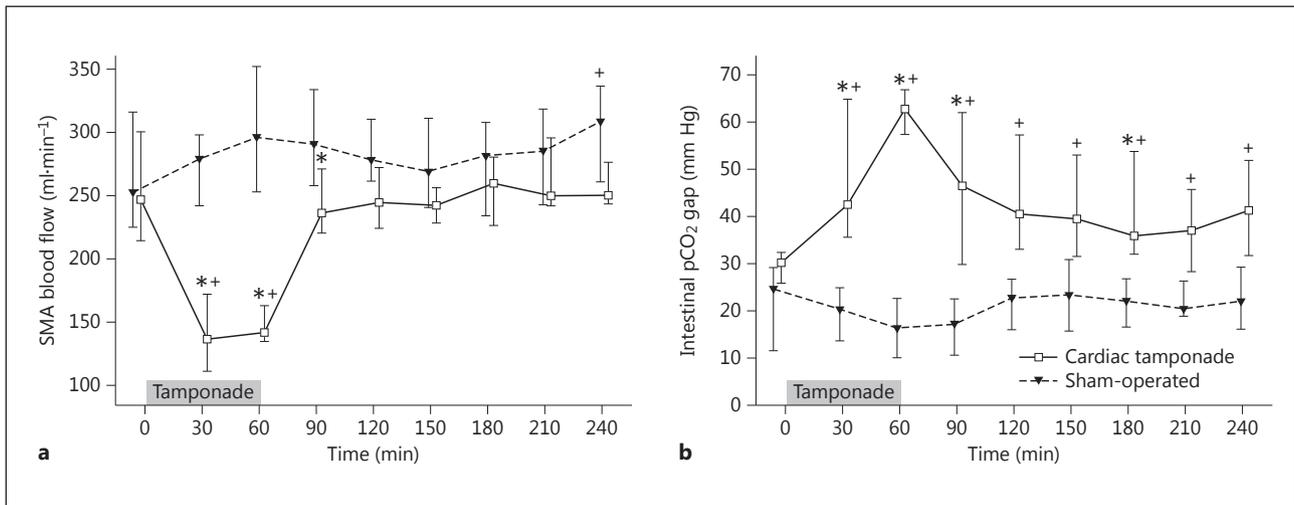
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DOI: 10.1159/000352089

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Fig. 3. Changes in SMA blood flow (**a**) and intestinal pCO_2 gap (**b**) in the sham-operated and cardiac-tamponade groups. The grey boxes indicate the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.
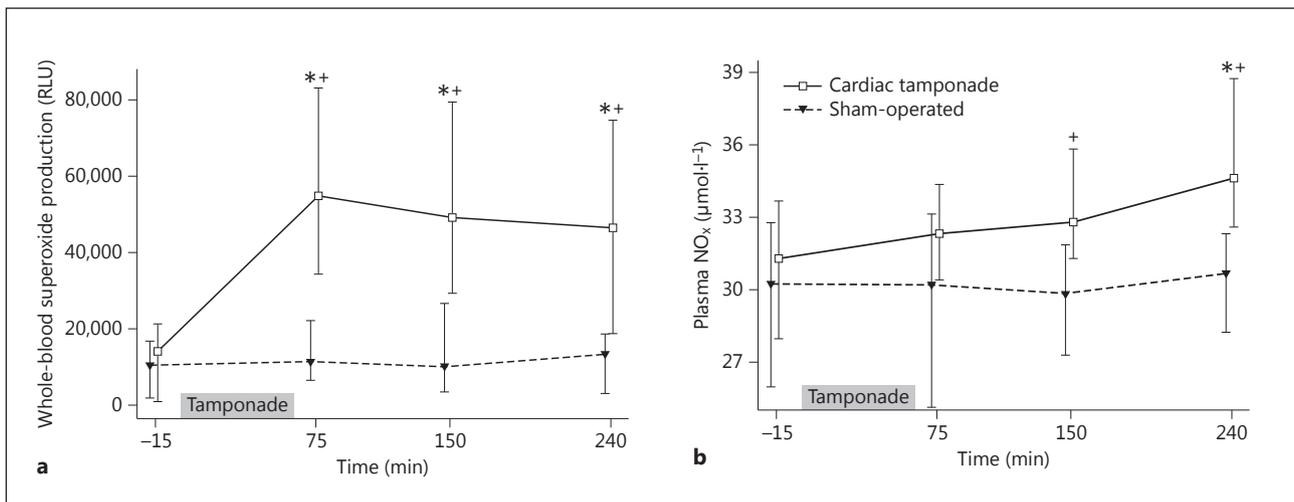

Fig. 4. Changes in whole-blood superoxide production (**a**) and plasma NO_x level (**b**) in the sham-operated and cardiac-tamponade groups. The grey boxes indicate the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.

The troponin T level in the plasma allows an estimate of the cardiomyocyte damage. The mean concentrations of troponin T were significantly increased after the compression of the heart and at the end of the posttamponade period when compared with the baseline and with those for the sham-operated group (fig. 6).

The rate of PMN leukocyte accumulation was determined through measurement of the MPO activity of the intestinal tissue samples taken at the end of the experiments. The level of
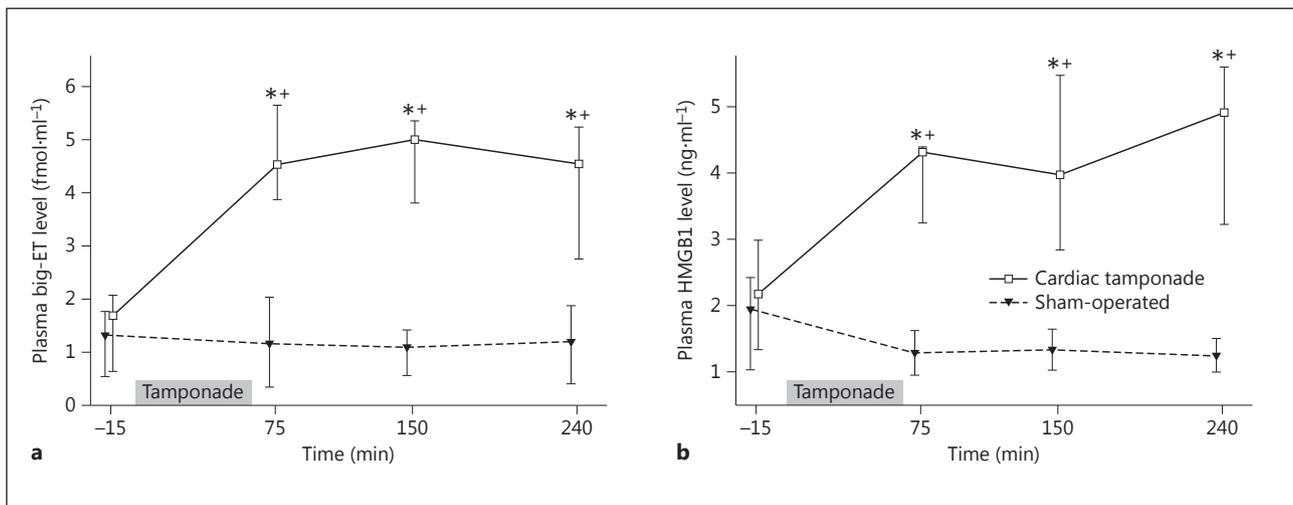
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Fig. 5. Changes in plasma big-ET concentration (**a**) and HMGB1 level (**b**) in the sham-operated and cardiac-tamponade groups. The grey boxes indicate the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.

Fig. 6. Changes in plasma troponin T level in the sham-operated and cardiac-tamponade groups. The grey box indicates the duration of the cardiac tamponade. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles. * $p < 0.05$ within groups vs. baseline values, + $p < 0.05$ between groups vs. sham-operated group values.
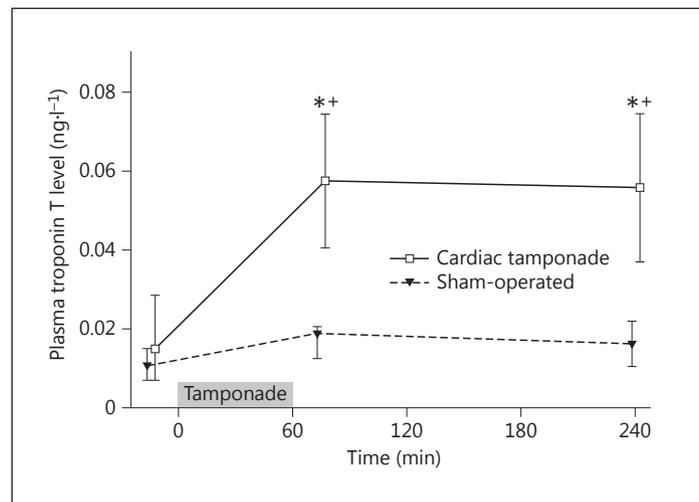
MPO activity was significantly higher in the small intestine tissue samples of the cardiac tamponade group, indicating the increased accumulation of PMN leukocytes (cardiac tamponade: M = 5.37 (p25 = 4.8; p75 = 6.22; U·mg protein^{-1}) versus the sham-operated group: M = 2.84 (p25 = 2.19; p75 = 3.22).

Activation of the complement cascade was evaluated by the presence of a complement C3 deposit in the small intestinal mucosa with the IHC method (fig. 7). In each field of view of the slides, the number of capillaries showing C3 deposit positivity was counted. The number of C3 deposits was significantly higher in the tamponade group than in the sham-operated group (M = 3; p25 = 1; p75 = 4.5 vs. M = 0; p25 = 0; p75 = 1 deposits/field of view).

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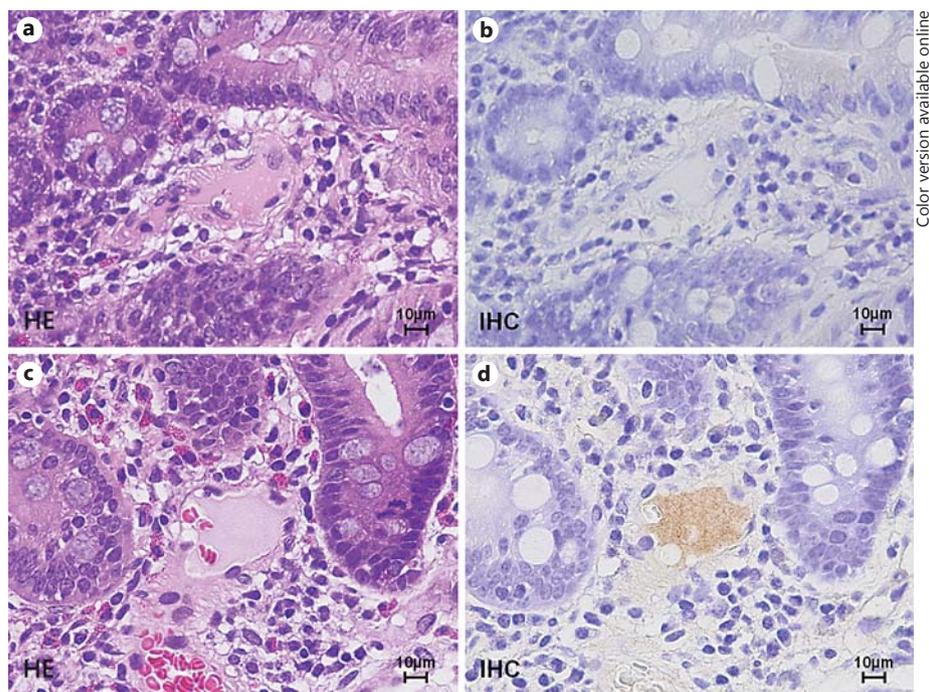
Fig. 7. Changes in complement C3 deposition demonstrated by IHC analyses in the small intestinal mucosal biopsies taken at the end of the experiments: HE staining in the sham-operated (**a**) and cardiac-tamponade (**c**) groups; extensive C3 deposition with IHC staining in the cardiac-tamponade group (**d**) compared to the sham-operated group (**b**).

Discussion

Pericardial tamponade is accompanied by high mortality and postoperative complication rates, even in the event of adequate treatment. In the case of blunt chest trauma, the mortality might exceed 80% [24], while a 22% mortality rate was found after elective open-heart surgery with clinically proven cases of localized cardiac tamponade [25].

Our goal was to characterize the hemodynamic effects of temporary mechanical compression of the heart and to outline the proinflammatory elements which may play a role in the cascade of events induced by relief of the tamponade. Our major finding is that the post-tamponade period is characterized by a decreased level of systemic perfusion and by an impaired pulmonary circulation, as evidenced by the MAP, LHEDV, PAP, PVRI and EVLWI data. The pCO_2 gap changes suggested that, in parallel, a significant intestinal microcirculatory dysfunction evolved in this porcine model. More importantly, these responses were associated with abrupt increases in superoxide radical production, big-ET, troponin T, HMGB1, histamine, intestinal MPO activity and complement activation during the posttamponade phase.

The cardiac filling disorder induced different vasoconstrictive compensatory reactions in the systemic and pulmonary circulations: the increase in PVRI was much higher (112%) than that in SVRI (32%). Previous experimental and clinical data suggest that the increasing pericardial pressure causes a continuous decline in coronary blood flow due to an increase in coronary vascular resistance [26, 27]. Skalidis et al. [27] observed a decreased hyperemic flow under increased pericardial pressure, which implies an augmented susceptibility to myocardial ischemia. The significantly elevated troponin T during the acute phase of the

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Eur Surg Res 2013;51:1–13

DOI: 10.1159/000352089

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tamponade also demonstrates the deterioration of oxygenation and damage of the cardiac muscle cells.

After the relief of the tamponade, the MAP was decreased, while the CO was kept compensated, and there were no significant differences compared to the control group. This may be explained by the normalized preload, as evidenced by the normalized CVP and RHEDV and the moderate elevation of the afterload. However, the significant decrease in LHEDV indicates a left ventricular dysfunction during the posttamponade phase. The persistent elevations in PAP and PVRI could contribute to this process, together with the lung edema as revealed by the elevated EVLWI. These conclusions are supported by clinical observations on early cardiac failure and pulmonary edema after removal of the pericardial effusion [28, 29].

In cardiogenic shock, prompt treatment of hypotension is needed in order to avoid the generation of inflammatory mediators [30]. Indeed, the pericardial tamponade triggered characteristic macro- and microcirculatory changes in the intestines. While the SMA flow, which reflects the blood supply of the small intestine and colon, was diminished during the tamponade, a prolonged impairment of the mucosal microcirculation was detected. This is in accordance with previous assumptions that the macro- and microhemodynamics may change relatively independently, or may be dissociated in stress conditions [31].

Against the background of these hemodynamic alterations, a multi-faceted role of humoral mediators, including ET-1, is proposed. Hypoxia is considered to be one of the basic stimuli for ET-1 synthesis. This peptide is produced predominantly by the endothelium, but in pathophysiological states, other cell types such as leukocytes, macrophages, smooth muscle cells, cardiomyocytes and mesangial cells can also serve as sources of its release [12]. The increased plasma level of ET-1 could be responsible for the decreased coronary perfusion [32] and pulmonary hypertension. The activation of vasoconstrictor ET receptors can further play a decisive role in acute microcirculatory disorders of the peripheral cardiovascular system. It has been shown that selective ET-A receptor antagonism increases the CO, decreases the peripheral resistance [14, 33] and reduces intestinal microvascular injury and PMN leukocyte accumulation during ischemia-reperfusion [34].

In addition to its independent role as a dominant vasoconstrictor, the peptide may also influence the functions of other cell types in the circulatory system. ET-1 has been reported to induce leukocyte rolling and adherence through a predominantly ET-A receptor-mediated mechanism [13]. There is a close relationship between a compromised mucosal blood flow and the magnitude of PMN leukocyte-endothelial cell interactions in the intestines [34]. On the other hand, ET-1 also causes histamine release from mast cells [14], which may lead to enhanced vascular permeability and a relative blood loss into the dilated vessels. Histamine release in the pulmonary circulation contributes to the increase in EVLWI [35], while in the splanchnic area histamine release probably plays a role in the counterregulation of the excessive, prolonged vasoconstriction that contributes to the lethal outcome [4]. Furthermore, ET-1 activates NADPH oxidase, resulting in an increased superoxide radical production [36], which can simultaneously reduce NO production, leading to the formation of the highly cytotoxic peroxynitrite [37]. From this point of view, the excessive release of ET-1 can be the key player as concerns the spreading inflammatory responses, when intensive complement activation is also ignited. The presence of complement C3 deposits was verified in this tamponade model and we found increased plasma levels of HMGB1 too, the release of which is additionally directly mediated by the complement cascade. In this scenario, HMGB1 release is a further danger signal to responsive cells; it amplifies the production and secretion of other proinflammatory mediators and finally induces excessive inflammation [38, 39].

This study has some limitations. Firstly, thoracotomy causes severe surgical trauma. Diaphragmatic window through laparotomy could be a possible alternative to reach the peri-

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cardial cavity [30]. Nevertheless, safe catheterization of the pericardial sac and quick guidance of the diagnostic instrumentation into correct positions into the heart cavities are advantages of the open-chest model. Secondly, the inflammatory reaction may be nonspecific because cardiogenic and hypovolemic shock components are not mutually exclusive. Decreases in MAP, CO, splanchnic perfusion and microcirculatory damage can occur in nearly all forms of circulatory shock (e.g. hypovolemic, cardiogenic or distributive). Nevertheless, there are tamponade-specific consequences as well, such as elevation of the CVP, decrease in LHEDV and increased plasma troponin T level, which could all be direct signs and consequences of tamponade-induced cardiac ischemia.

In conclusion, we have demonstrated characteristic macrohemodynamic changes, together with apparent signs of a splanchnic inflammatory reaction after the relief of tamponade. The evidence further suggests that ET-1 and the complement system may be significant components of the inflammatory cascade that is activated in this porcine model of pericardial tamponade.

Acknowledgements

Disclosure Statement

The authors declare no conflicts of interest.

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