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Original Article

Cardiac damage by intra-abdominal hypertension in a canine model

Dong Liu[1*], Jian Yu[1*], Xiaojuan Fu[3], Mingtao Chang[1], Yang Li[1], Wenhua Du[2], Lianyang Zhang[1]

[1]Trauma Center, State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, [2]Department of Ultrasound, Daping Hospital, Third Military Medical University, Chongqing, China; [3]Chongqing Medical and Pharmaceutical College, Chongqing, China. [*]Equal contributors.

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Abstract: To explore the mechanism of myocardial injury by intra-abdominal hypertension. A total of 54 healthy hybrid dogs were randomized into three groups, Group I: sham group (n = 6), the abdomen puncture was applied without gas injection; Group II: IAH group (n = 24), with subgroups of 6 animals each that were subjected to an IAP of 15, 20, 25, or 30 mmHg for 4 h; Group III: IAH decompression group (n = 24), decompressed for 2 h on the basis of Group II. Blood samples were obtained to detect markers of myocardial damage before IAH, at 0.5, 2, and 4 h post-IAH and at 1 and 2 h after decompression. The hearts were isolated, and myocardium was sectioned for pathological examination, ICAM-1 immunohistochemistry and for the detection of NO/NOS content. During the early stage of IAH, the markers of myocardial injury were not significantly different. Visible pathological damage was difficult to observe, but myocardial edema, exudation, Z line breakage, dissolution of myonemes and endothelial cell swelling were observed under both the light microscope and the electron microscope. IAH caused a decrease in the concentrations of cardiac NO and TNOS, and an increase in the content of iNOS. The expression of ICAM-1 increased after IAH. The mechanism of IAH-induced myocardial injury may involve the damage of myocardial microvascular endothelial cells caused by IAH.

Keywords: Intra-abdominal hypertension, myocardium, vascular endothelial cell

Introduction

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) have been shown to occur with high prevalence in patients in intensive care units in the present day and are causes of significant mortality [1, 2]. Research has shown that the adverse effects of elevated intra-abdominal pressure (IAP) can occur at lower levels than previously thought and even before the presentation of clinically overt ACS [3]. A correlation has been observed between elevated IAP and reduced cardiac output, which is primarily because of the progressive compression of the inferior vena cava and portal vein as well as the reduction in venous return to the heart [4]. On the contrary, increased IAP also causes progressive hypoperfusion and ischemia of the intra-abdominal organs and even remote organs, and thus intra-abdominal hypertension (IAH) may silently harm multiple organ systems [5]. However, the mechanisms that link IAH and organ dysfunction are not yet completely understood.

Endothelial cell integrity and the capillary network are critical for the maintenance of a constant supply of oxygen and nutrients to cardiomyocytes. The interactions between endothelial cells and cardiomyocytes play a key role in the regulation of cardiac function by the modulation of vascular tone and by the stimulation of proliferation of neighboring cells [6]. However, this arrangement of endothelial cells and cardiomyocytes in the heart also allows for cell-to-cell signaling between cardiomyocytes and endothelial cells which may be of significance during cellular stresses such as ischemia and reperfusion. Several substances that are released or metabolized by cardiac endothelial cells have direct effects on the function of cardiac myocytes, including nitric oxide (NO), endothelin-1 and natriuretic peptides. Likewise, agents produced by cardiac myocytes such as

growth factors, can also affect endothelial cell function. Over the last several decades, the interaction between endothelial cells and cardiac myocytes was recognized to be important both physiologically and in disease states. Nevertheless, little is known about the pathological or pathophysiological changes of the coronary vascular endothelium during the early stage of intra-abdominal hypertension. In the present study, we investigated the ultrastructural manifestations of coronary vascular endothelial cells in the early stage of gradient intra-abdominal hypertension.

Materials and methods

Animal protocol

All animal experiments were carried out in compliance with the animal protection guidelines of the Third Military Medical University, China. The Internal Animal Care and Use Committee of the Third Military Medical University reviewed the protocol and granted permission. The principles of laboratory animal care (NIH Publication No. 85-23, revised 1985) were followed.

The average animal weight was (mean ± SEM) 11.39±1.7 kg. Animals were fasted for at least 12 hours before surgery and were anaesthetized with an intravenous bolus injection of 3% sodium pentobarbital at a dose of 30 mg/kg and then a dose of 10 mg/hour/kg for maintenance. The animals were fastened to the experimental platform, and the skin around the surgical area was disinfected with iodophor. The right femoral artery and vein were each isolated for catheterization which was performed with a single lumen catheter. The catheters were connected, via a triplet, to a transducer and multifunctional monitor for the continuous detection of hemodynamics. A core needle (with diameter of 1.6 mm) was selected for the puncture. Gas was insufflated into the peritoneal cavity, where one end was connected to a sphygmomanometer via a triplet through a plastic tube to monitor the intra-abdominal pressure. The puncture location was set at the point 2 cm immediately below the xiphoid process, and the needle was perpendicularly inserted without any injury to blood vessels and was then fastened.

Instrument protocol and study groups

Canines were divided into the following three groups: the sham-operated group (n = 6), the IAH group (IAH Group, n = 24) and the intra-abdominal hypertension decompression group (IAH-Decom Group, n = 24). Moreover, the IAH group and IAH-Decom group were each divided further into the following four sub-groups: Grade I, Grade II, Grade III and Grade IV, according to criteria of the World Society on Abdominal Compartment Syndrome consensus definitions. Grade I, Grade II, Grade III and Grade IV signified an intra abdominal hypertension as high as 15 mmHg, 20 mmHg, 25 mmHg and 30 mmHg, respectively. Canines in the sham-operated group were anaesthetized and underwent the same surgical procedure as the canines in the other two groups but without gas insufflation into the peritoneal cavity. For the canines in each of the IAH sub groups, ambient air was slowly injected into the abdomen at a rate of 5 ml/s using a 50 ml syringe until the intra-abdominal pressure reached the level of graded IAH, which was maintained for 4 hrs. For the IAH-Decom subgroups, canines were first insufflated with ambient air to the level of graded IAH which was maintained for 4 hrs, and then, the IAH was decompressed through the opening of the triplet; these dogs were observed for an additional 2 hrs. Blood samples were obtained from a vein to detect markers of myocardial damage before the initiation of IAH, at 0.5, 2, and 4 h post-IAH as well as at 1 and 2 h after decompression. At the end of the experiment, the canines were sacrificed with an intravenous injection of 10 ml 10% KCL. The hearts were then isolated and washed with saline. The hearts were stored in the refrigerator for 20 mins, then in the freezer at -20°C, and were then sliced transversely as 2-mm thick sections from the apex to base; these slices were stained with 1% triphenyltetrazolium chloride (TTC) solution.

Cardiac tissue specimens were fixed in 10% formaldehyde, embedded with paraffin wax, stained with hematoxylin and observed microscopically. Additionally, some of these specimens were prepared for immunohistochemical observation of the expression of intercellular adhesion molecule-1 (ICAM-1). A portion of these heart specimens was fixed with 2.5% glutaraldehyde, washed twice with 2-methyl arsenate buffer solution and fixed again with osmium tetroxide. The specimens were again washed with buffer solution, dehydrated with acetone, embedded with epoxy resin, stained with uranyl acetate-lead citrate, and were finally

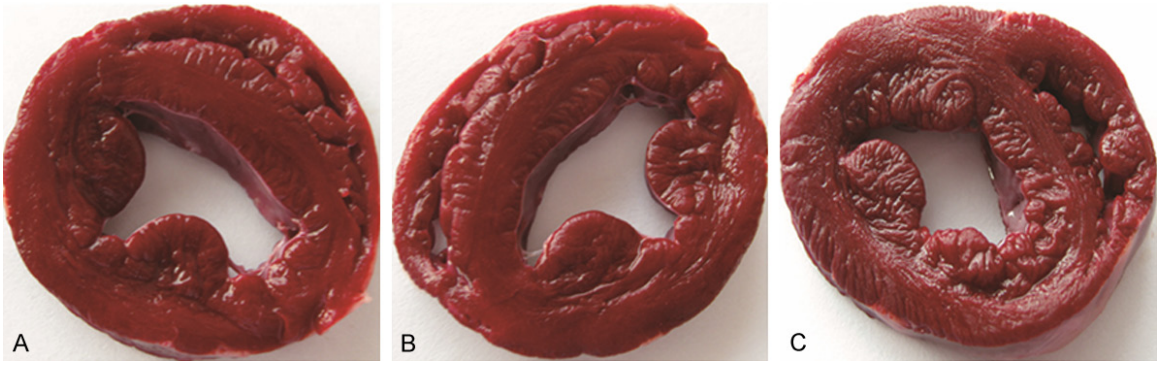

Figure 1. A. Normal myocardial tissues of the sham-operated group; B. Myocardial tissues after maintenance of IAH at 30 mmHg for 4 h; C. Myocardial tissues after maintenance of IAH at 30 mmHg for 4 h and decompression for 2 hours.

examined by transmission electron microscopy (Hitachi H-7500). Homogenated cardiac tissues were centrifuged, and the supernatant was sampled for the determination of nitric oxide (NO) and nitric oxide synthase (NOS) through chemical colorimetry.

Statistical analysis

All data collected from this experiment were analyzed using the SPSS 18.0 statistical package. All data are expressed as the mean ± standard deviation (SD). ANOVA and the Kruskal-Wallis variance tests were used for parametric data and nonparametric data, respectively, to compare the differences among the groups. The p values <0.05 were considered statistically significant.

Results

Effect of IAH on the markers of myocardial injury

In the sham group, the concentrations of plasma creatine kinase MB (CK-MB), troponin T (cTnT), and myoglobin were difficult to measure, and thus the results were negative. Compared with the sham group, the values of the above indicators were negative at each time point in the subgroups of the IAH group (i.e., 15, 20, 25 mmHg). However, in the 30 mmHg IAH group, the levels of CK-MB and myoglobin were also negative, while the concentration value of cTnT was positive at 4 h after IAH with a positive rate of 100%, which remained positive at 1 h and 2 h after decompression.

Manifestations of triphenyltetrazolium chloride (TTC) staining

Triphenyltetrazolium chloride (TTC) stains viable myocardium a brick red color, while the ischemic or infarct zone does not stain and appears white or yellow in color. In this experiment, anatomical and observable hemorrhage were not found in either the epicardium or the endocardium, as TTC staining revealed that all cardiac specimens from each of the experimental groups were a brick red color, which indicates viable myocardium (**Figure 1A-C**).

Morphological manifestations of cardiac tissues using microscopy

Cardiac tissues from the sham-operated group showed polygonal, round or striated fibers with peripheral nuclei. The fibers were grouped into a normal pattern of fascicles, while microvessels were distributed regularly and clearly in the tissues (**Figure 2A**). In the IAH group (15 mmHg and 20 mmHg), after 4 hrs of persistent intra abdominal hypertension, severe rouleaux of erythrocytes and even focal leakage of erythrocytes outside of the microvessels were observed in the myocardial tissues (**Figure 2B**). In the other two IAH groups (25 mmHg and 30 mmHg), after 4 hrs of persistent intra abdominal hypertension, the exudation of neutrophils and the adhesion of neutrophils to the surface of endothelial cells were observed (**Figure 2C**). Interestingly, in the IAH decompression sub groups at 2 hrs post decompression, cardiac tissue injuries, such as rouleaux of erythrocytes, exudation of neutrophils, congestion and

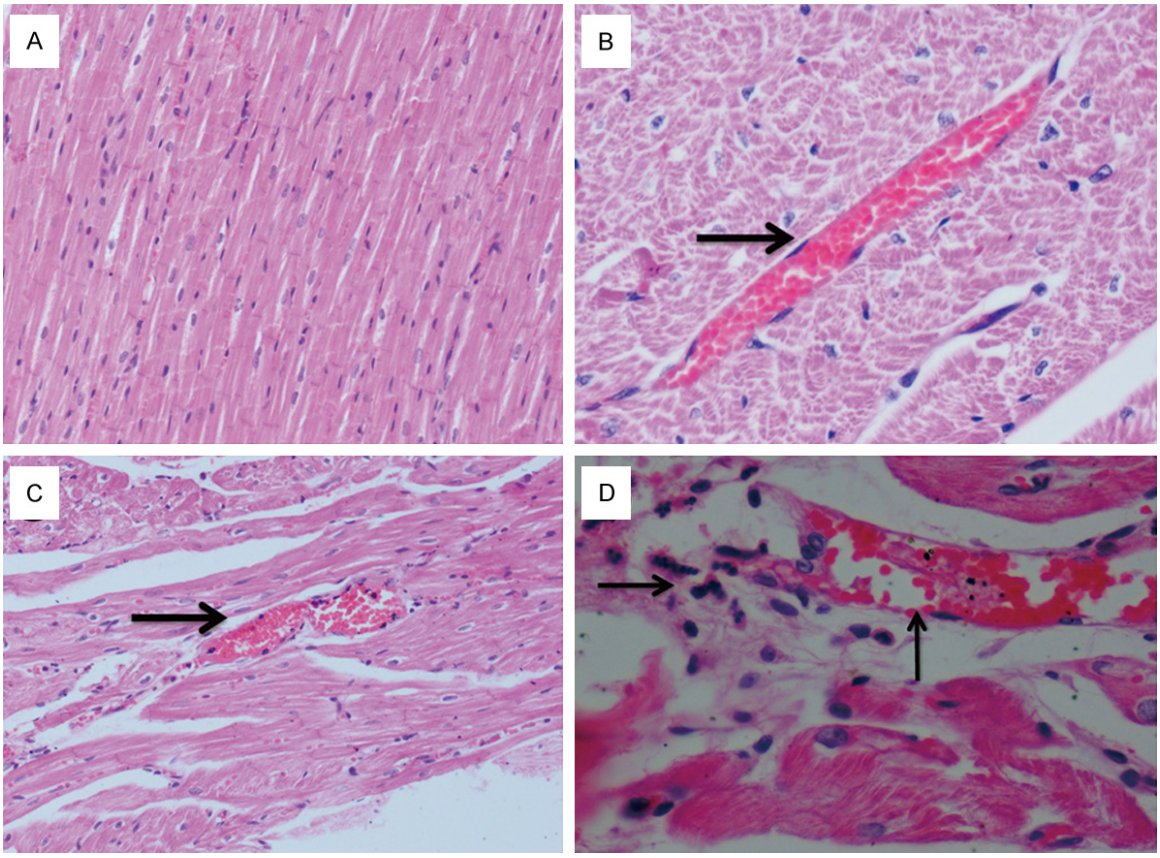

Figure 2. Histopathology of cardiac tissues (H&E staining). A. Normal cardiac tissue from the sham-operated group (×400). B. Cardiac tissue after 4 h of IAH at 15 mmHg IAP (×400). C. Cardiac tissue after 4 h of IAH at 25 mmHg IAP (×200). D. Cardiac tissue after 30 mmHg IAP for 4 h and 2 h of decompression (×400).

even discontinuity of the endothelium, appeared much more obvious (**Figure 2D**).

Ultrastructural manifestations of cardiac tissues

Ultrastructural analysis of cardiac tissues from the sham-operated group showed muscle fibers with normal aspects (**Figure 3A**). After 4 hrs of IAH in the 15 mmHg and 20 mmHg subgroups, muscle fibers presented as normal myofibrils, but mitochondrial swelling, a decreased number of cristae and disorganized vague cristae were observed (**Figure 3B**). Meanwhile, in the other two IAH sub groups (25 mmHg and 30 mmHg), after 4 hrs IAH, morphological changes were observed such as intracellular and intermyofibrillar edema, the presence of amorphous material, swollen mitochondria with disruption of cristae or electron-dense aggregates of cristae, and obvious swelling of endothelial cells (**Figure 3C**, **3D**). In contrast to the manifestations described above, myocardial tissues in the IAH decompression sub-groups demonstrated many more serious morphological abnormalities such as: swollen endothelium with intraluminal blebs, numerous endoplasmic pinocytotic vesicles, endothelial disruption with extravascular erythrocytes, obvious disruption of sarcomeres and subsarcolemmal swelling, and finally, swollen mitochondria with no cristae (**Figure 3E**, **3F**).

Immunohistochemical analysis of ICAM-1 expression in cardiac tissue

Under normal conditions, cardiomyocytes and endothelial cells expressed little to no levels of intercellular adhesion molecule-1 (ICAM-1) (**Figure 4A**). After 4 hrs of IAH, the expression of ICAM-1 was slightly visible in the sarcoplasma of cardiomyocytes, but was expressed to a greater degree in the sarcolemma of cardiomyocytes and on the surface of the endothelium. Expression of ICAM-1 seemed to increase as the intra-abdominal pressure increased; how-

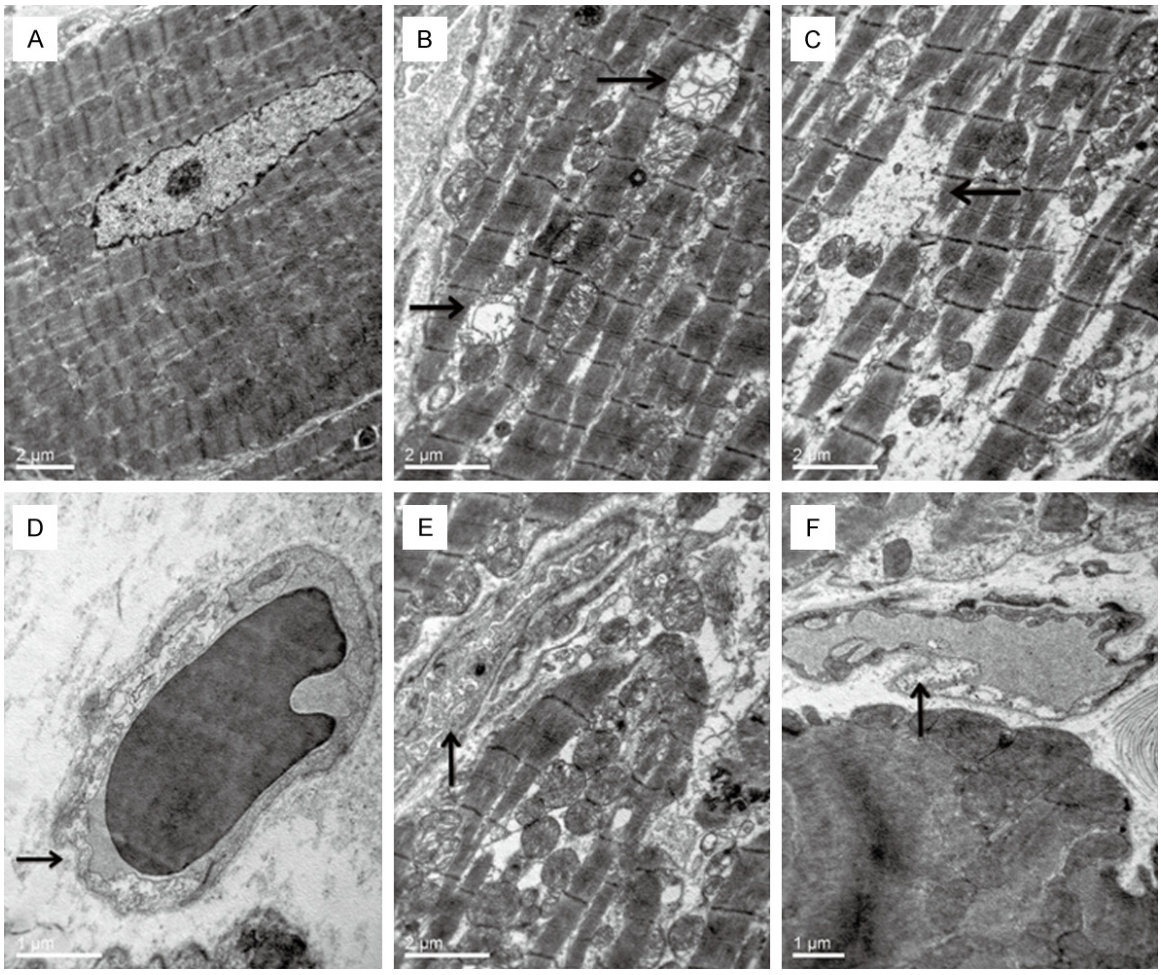

Figure 3. Ultrastructural manifestations in cardiac tissues. A. Normal cardiac tissue from the sham-operated group (×7000). B. Cardiac tissue after 4 h of IAH at 15 mmHg IAP (×10,000). C, D. Cardiac tissue after 4 h of IAH at 25 (Bx7000) or 30 (Cx20,000) mmHg IAP. E. Cardiac tissue after 25 mmHg IAP for 4 h and 2 h of decompression (×10,000). F. Cardiac tissue after 30 mmHg IAP for 4 h and 2 h of decompression (×12,000).

ever, remarkable changes in ICAM-1 expression were not found between the IAH groups and the IAH decompression groups (**Figure 4B-D**).

Changes in the levels of NO and NOS in canine cardiac tissues

Compared with the sham-operated group, the levels of nitric oxide (NO) in the cardiac tissues of each IAH sub group decreased remarkably. Significant differences were observed in the following groups: the IAH 15 mmHg sub group and the sham-operated group (33.715±3.743 μmol/g vs 49.303±5.235 μmol/g, P<0.01); between the IAH 20 mmHg sub group and the sham-operated group (21.682±2.926 μmol/g VS 49.303±5.235 μmol/g, P<0.01); between the IAH 25 mmHg sub group and the sham-operated group (11.434±3.570 μmol/g VS 49.303±5.235 μmol/g, P<0.01); between the IAH 30 mmHg sub group and the sham-operated group (5.990±5.178 μmol/g VS 49.303± 5.235 μmol/g, P<0.01). Furthermore, the levels of NO in the cardiac tissues of each IAH decompression subgroup recovered at 2 hrs after IAH decompression, and these levels were significantly different when compared with each IAH subgroup. However, the levels of NO in the cardiac tissues from each IAH decompression subgroup at 2 hrs after IAH decompression were not significantly different compared with the levels in cardiac tissues from the sham-operated group (**Figure 5A**).

The levels of total nitric oxide synthase (TNOS) decreased in each IAH subgroup (the concen-

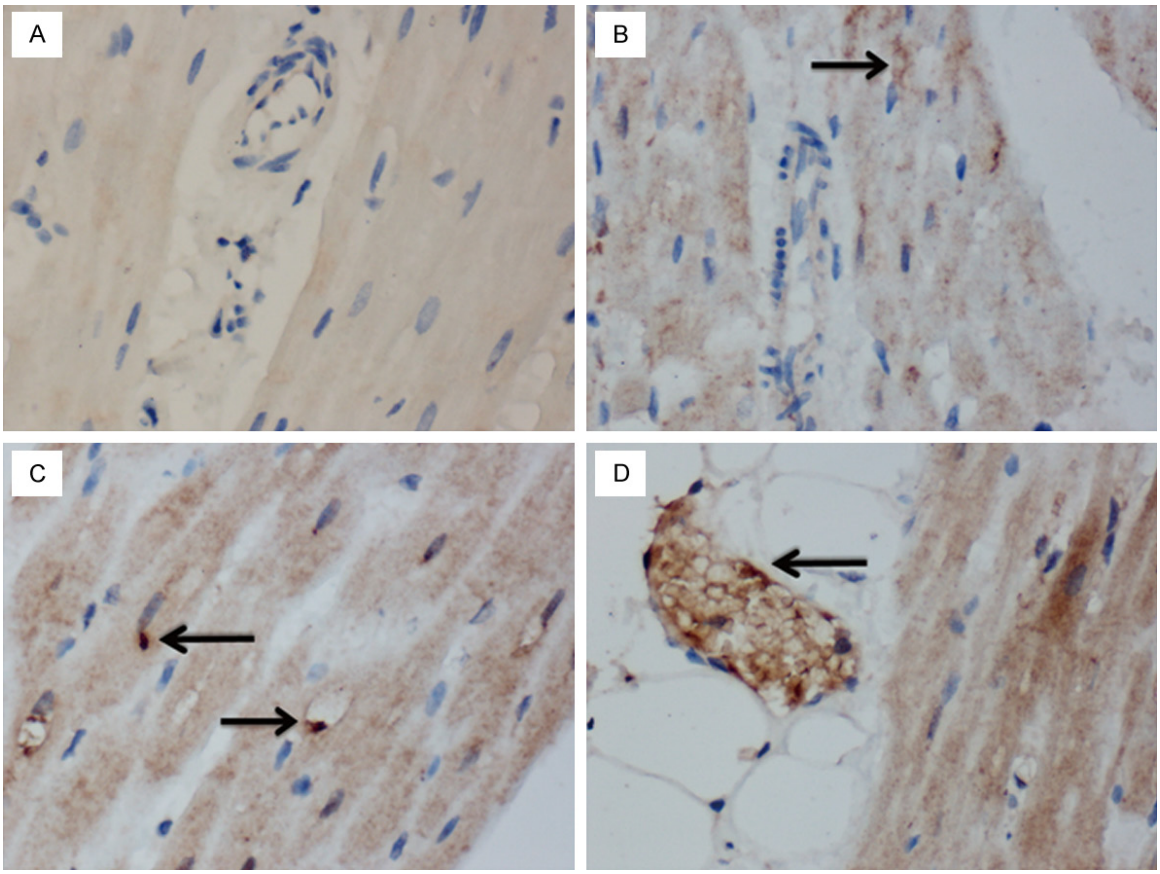

Figure 4. Immunohistochemical analysis of ICAM-1 expression in cardiac tissue. A. Normal cardiac tissue from the sham-operated group (×400). B-D. Cardiac tissue after 4 h of IAH at 15 (Bx400), 20 (Cx400) or 30 (Dx400) mmHg IAP.

tration of TNOS from the 15 mmHg IAH subgroup to the 30 mmHg IAH subgroup were 1.769±0.208 U/mg, 1.475±0.111 U/mg, 0.930±0.153 U/mg and 0.788±0.101 U/mg, respectively), which were significantly lower than the values of the sham-operated group (2.578±0.520 U/mg). The concentration of TNOS at 2 hrs after decompression in each IAH decompression subgroup increased (to 2.478±0.100 U/mg, 2.118±0.149 U/mg, 1.761±0.091 U/mg, and 1.566±0.092 U/mg, respectively), which was significantly different compared with the concentration of TNOS in each IAH subgroup. In contrast, a comparison with the sham-operated group showed that the concentration of TNOS 2 hrs after decompression in each IAH decompression subgroup appeared significantly lower, except that from the 15 mmHg IAH decompression subgroup (**Figure 5B**).

The levels of induced nitric oxide synthase (iNOS) in each graded IAH subgroup increased gradually and were 0.475±0.025 U/mg, 0.482±0.053 U/mg, 0.501±0.022 U/mg and 0.517±0.088 U/mg, respectively, but they were not significantly different than the value of iNOS in the sham- operated group (0.468±0.130 U/mg). The levels of iNOS 2 hrs after decompression continued to increase and were significantly higher than those of both the sham-operated group and the graded IAH subgroups (with exception of the 15 mmHg subgroup) (**Figure 5C**).

Discussion

Elevated IAP directly or indirectly causes the dysfunction of whole-body organ systems [7, 8]. Emerson et al. found that the elevated IAP caused animal death because of cardiovascular events rather than because of respiratory failure. Mahjoub et al. [9] found that the mean arterial pressure did not significantly change after IAP at 20 mmHg that was maintained for 1 h, but the left ventricular diastolic function

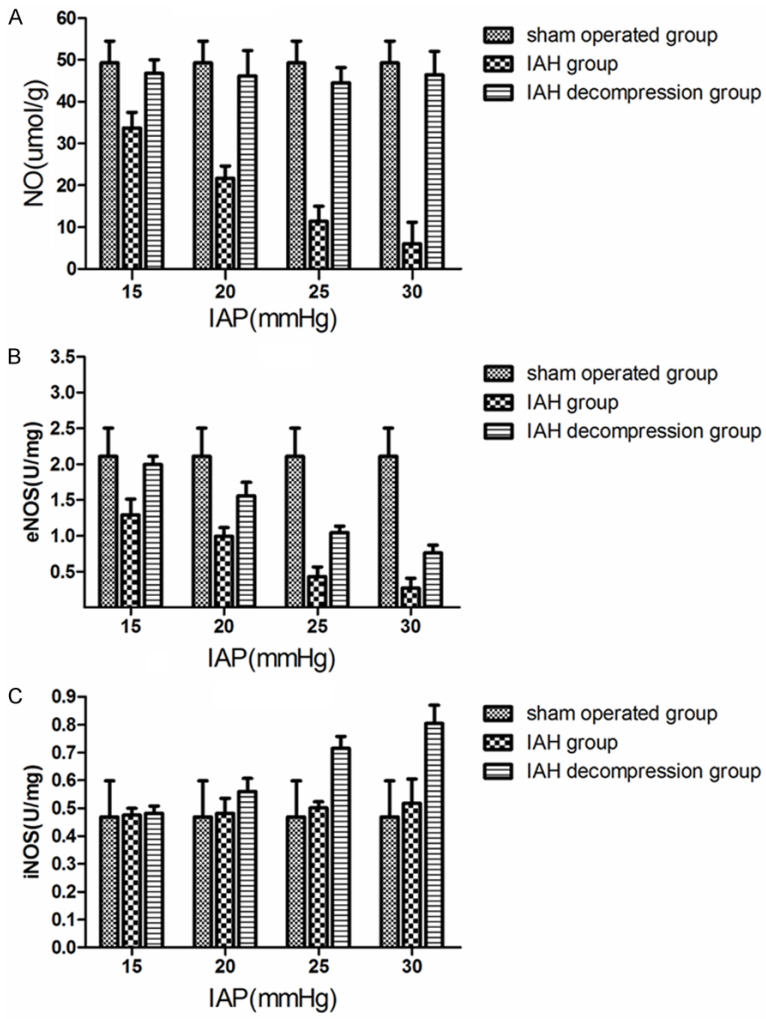

Figure 5. A. Comparison of the NO levels in canine myocardial tissue; B. Comparison of the eNOS levels in canine myocardial tissue; C. Comparison of the iNOS levels in canine myocardial tissue.

was impaired. Vivier et al. [10] observed that when the intra-abdominal pressure gradually increased to 30 mmHg, even under normal conditions, the blood volume of the left ventricular end-diastolic area (LVEDA) had decreased by 78%, which severely affected its function. IAH can cause cardiac dysfunction, but there have been no reports about whether it can cause myocardial tissue damage and related pathologies.

In this study, the pathological findings demonstrated no obvious ischemia or necrosis in the myocardial tissue after IAH. The serum markers of myocardial injury were negative after the IAH was maintained at levels 1-3 for 4 hours, whereas the troponin T levels (cTnT) were positive after the IAH was maintained at 30 mmHg for 4 hours; this value remained positive even at 2 hours after decompression, which suggests that severe IAH can cause myocardial tissue injury due to the long maintenance period of IAH at a high level. The microstructure and ultrastructure of myocardial tissue indicated that intra-abdominal pressure can cause pathological changes in myocardial cells to varying degrees. The H&E staining and light microscopic analysis of the canine myocardial tissue found that the maintenance of IAP at 15 mmHg for 4 h can cause myocardial microvascular congestion and leakage of a small amount of red blood cells, and can even cause "Rouleaux-like changes" of myocardial microvascular erythrocytes when IAP is maintained at 20 mmHg for 4 h. This implies the induction of precursor signs of myocardial microcirculation thrombosis. With the gradual increase of IAP, the degree of damage to the myocardium gradually worsened. When the IAP was increased to 30 mmHg and maintained for 4 h, the canine cardiac tissue indicated the adherence of erythrocytes and neutrophils as well as exudation. These parameters worsened, and even myocardial microvascular endothelial discontinuity was observed. The microstructure of the myocardial tissue by light microscopy showed obvious myocardial injuries that were induced by IAH and which were most likely due to the damaged microvascular endothelial cells.

Through further observation of the ultrastructure of the myocardial tissue, we found evidence of myocardial microvascular congestion, swelling of myocardial cell membranes and mitochondria, and even dissolved mitochondria after IAP was maintained at 15 mmHg for 4 hours. As the extent of IAP increased, we observed myocardial interstitial inflammatory leakage, dissolved filaments, Z line breakage,

and even cardiac microvascular endothelial cell edema after IAP was maintained at 30 mmHg for 4 hours.

Considering the above morphological observations of myocardial tissue, we found that the occurrence of IAH and the high level of IAP (especially that greater than 20 mmHg) can cause myocardial injury, which was associated with the extent and duration of IAP. The main cause of myocardial injury in this case was most likely due to damage of the cardiac microvascular endothelial cells, which resulted from the increased IAP. The impaired cardiac microvascular endothelial cells will also damage the tight junctions between the cells and further lead to a series of pathological changes within the endothelial cells; this may result in the dysfunction and even the failure of the cardiovascular system. Our experiments showed that abdominal decompression at an early stage (2 hours in this experiment) may aggravate myocardial injuries due to ischemia-reperfusion injury, but this does not prevent the favorable recovery of heart function.

In our experiment, the IAH induced pathological changes such as "Rouleaux-like changes" of myocardial microvascular erythrocytes, leukocyte adherence and neutrophil exudation, which indicates that the increased IAP induced inflammatory changes in the myocardial tissue.

In the present study, immunohistochemistry was used to detect the expression of ICAM-1 in canine cardiac tissues. We found that the ICAM-1 was expressed in myocardial cells, especially on the endothelial cell surface after the occurrence of IAH. Furthermore, the more severe the abdominal hypertension, the more significant the ICAM-1 expression; this indicates that IAH leads to the damage of myocardial cells, especially to the damage of endothelial cells. This result in an inflammatory response, which is similar to that seen in the microstructure observed under a light microscope. In this experiment, the morphological observation showed no significant pathological changes of the myocardial tissue as a result of IAH, which suggests that maintenance of IAH for a short period of time is not sufficient to cause myocardial histopathological changes such as ischemia and infarction, even with the 30 mmHg level of IAH. The microscopic pathology observed by light microscopy showed that IAH can cause congestion of myocardial tissue, inflammatory exudation, vascular endothelial discontinuity and other pathological changes. Further, the observations by electron microscopy revealed cardiac microvascular endothelial edema. Hence, we believe that damage to cardiac microvascular endothelial cells may be a key point of IAH-induced myocardial injury.

Numerous studies have confirmed that nitric oxide (NO) is a type of highly active fat-soluble free radical that has a strong resistance to the cell membrane and a strong vasodilatory function [11, 12]. Currently, nitric oxide synthase is divided into three types: neuronal nitric oxide synthase (nNOS), which is mainly distributed in the brain, cerebellum and spinal cord; endothelial nitric oxide synthase (eNOS), which is mainly found in vascular endothelial cells, endocardial cells, myocardial cells, and special conduction tissues (atrioventricular node); and inducible nitric oxide synthase (iNOS), which is mainly found in immunocompetent cells such as monocytes, mast cells and neutrophils [13, 14].

In this experiment, we found that the levels of NO and total nitric oxide synthase (TNOS) in cardiac tissues were decreased after the occurrence of IAH and that this decrease was more significant with a higher level of IAH. Therefore, we believe that the concentrations of NO and eNOS in the myocardial tissue were decreased after the occurrence of IAH; however, as eNOS is primarily found in the vascular endothelial cells, endocardial cells, and myocardial cells, the endothelial cells and cardiac cells that generated the eNOS would be undoubtedly damaged.

Accordingly, through a combination of the change in troponin cTnT and observations through cardiac tissue microscopy and ultrastructural analysis, we confirmed that IAH can cause substantial damage to cardiac cells, especially to the vascular endothelial cells. Through the determination of the levels of NO, NOS in myocardial tissue and the expression of ICAM-1 in a large number of myocardial vascular endothelial cells, we have demonstrated that cardiac microvascular endothelial injury triggers changes in heart function, myocardial damage and inflammation at the molecular level due to IAH. The abdominal pressure inter-

vention can significantly improve the indicators, which suggests that decompression remains an effective means for the treatment of IAH/ACS.

However, this study also has some deficiencies, (e.g., the maintenance period of the IAH was short, and the IAH animal model is a simple model, which does not involve even basic lesions). In a future study, we will focus on the injuries to the cardiac vascular endothelial cells to clarify the specific signaling pathways that are involved. We also plan to reverse the IAH-induced heart dysfunction at an early stage by an intervention in the microvascular endothelial injury pathway, thereby providing new ideas for clinical treatment strategies.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Lianyang Zhang, Trauma Center, State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing 400042, China. E-mail: dpzhangly@163.com

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