Developing a new experimental model of abdominal compartment syndrome

Desenvolvimento de um novo modelo experimental de síndrome do compartimento abdominal

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A B S T R A C T

Objective: To describe an experimental, unprecedented model that mimics the abdominal compartment syndrome (ACS). **Methods**: twenty rats were randomly divided into four groups. To simulate ACS intra-abdominal hypertension (IAH) was induced by inserting cotton surgical dressing (Zobec®), 15x15cm (intra-abdominal pressure constant and equal to 12mmHg) associated with hypovolemia induced by withdrawing blood, keeping mean arterial pressure (MAP) around 60mmHg (HYPO). To dissociate the effects of those IAH-induced hypovolemia per se, two other groups were analyzed: one with only with IAH and another with only hypovolemia. The simulation group (sham) underwent the same surgical procedure performed earlier, however, the levels of intra-abdominal pressure and MAP were kept in 3mmHg and 90mmHg, respectively. **Results**: By analyzing the impact of IAH on the small intestine, we observed necrosis of the villi, congestion, and neutrophilic infiltration. Hypovolemia induced only inflammation and edema of the villi. However, the association of IAH and HYPO led to hemorrhagic infarction, besides worsening of the aforementioned parameters. **Conclusion**: This model was effective in inducing ACS expressed by the effects found in the small intestine.

Key words: Abdominal cavity. Hypovolemia. Surgical procedures, operative. Epidemiology, experimental. Rats.

INTRODUCTION

The escalation of conflicts in the twenty-first century, along with the significant increase in violence, caused an increase in severity of trauma and resulted in a growing number of surgical interventions in patients with hemodynamic instability, among which the damage control operations. This procedures consists in performing life-saving maneuvers in critical conditions and is designed to shorten surgical time so that the risk of death does not exceed the benefit of the procedure, by controlling the bleeding, preventing the exit of the intestinal contents, postponing visceral resections, sutures and reconstructions[1].

With the advent of this surgical procedure there was an increased incidence of abdominal compartment syndrome and, consequently, the need for monitoring the intra-abdominal pressure. This allowed, during the last decade, the signs and symptoms of intra-abdominal hypertension and abdominal compartment syndrome to be reported after different surgical procedures and also to be recognized as prevalent among non-surgical patients in intensive care units[2,3] .

Since it is not possible to study the abdominal compartment syndrome by double-blind controlled prospective studies with patients, it is necessary to develop experimental models that mimic this syndrome, i.e., where there is a possible association of etiologic agents, such of intra-abdominal hypertension (IAH) associated with hypovolemia.

For the development of models of IAH usually air or liquid is insufflated in the abdomen. However, the intra-abdominal pressure tends to reduce over time, minimizing the deleterious effects on the abdominal organs. Therefore, in order to simulate human IAH it is essential to develop a model where the levels of intra-abdominal pressure remain constant. Moreover, there is need to

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develop a model that reproduces more faithfully the morphological and pathophysiological alterations of the abdominal compartment syndrome.

Thus, this study aimed to develop an experimental model of intra-abdominal hypertension associated with hypovolemia in rats, simulating the abdominal compartment syndrome.

METHODS

Was used 20 Wistar rats (*Rattus norvegicus albinus*) of both sexes, weighing 300-350g, from the Animal Laboratory of the Pulmonary Research of Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro – UFRJ. The study was approved by the Ethics Committee for Animal Use of the Center for Health Sciences, UFRJ (CEUA-019).

The animals were sedated (diazepam 5mg diluted in 5ml saline and 1ml injected intraperitoneally) and anesthetized (sodium thiopental 20mg/kg intraperitoneal) dose sufficient to maintain the anesthesia (suppression of corneal-palpebral reflex). Every hour a third of the initial dose was applied, up to a total of three hours.

The animals were immobilized in supine position with the forearm in abduction at 90 degrees to the body, and later extended. We performed a tracheotomy with the introduction of a polyethylene tube (PE240, Clay-Adams Inc., New York, USA) of 1.5mm internal diameter and 7.5cm long, attached to the trachea with 3-0 cotton thread.

A PE10 polyethylene catheter was then introduced in the carotid artery to induce hypovolemia and continuous measurement of mean arterial pressure (MAP SCIREQ, Montreal, Canada).

This was followed by the induction of neuromuscular paralysis with pancuronium bromide (2mg/kg) intravenously in the tail vein in sufficient quantity to keep it for an hour, with replication of half of the initial dose, whenever necessary.

The animals were then connected to a Servo® ventilator and kept on mechanical ventilation for three hours, with the following parameters: tidal volume = 8ml/kg, PEEP = 5cm H_2O, respiratory rate = 80rpm; FiO_2 = 0.4.

Next, we performed an abdominal puncture in the left flank of the animals, by Seldinger method, with a deep vein catheter (Cook Inc.) 20cm in length and 7.5 French double lumen, attached to the skin with nylon 4-0 suture. This catheter was connected to a pressure transducer (SCIREQ, Montreal, Canada) for continuous intra-abdominal pressure measurement.

The rats were then randomly distributed into four groups consisting of five animals each. In the IAH group, intra-abdominal hypertension was induced by midline laparotomy, followed by insertion of a cotton surgical dressing (Zobec®) 15x15cm cut in order to cover 3/4 of the abdominal cavity. After checking the position of the intra-abdominal catheter we measured the intra-abdominal pressure, which should remain around 12mmHg throughout the experiment, i.e., three hours after the synthesis of the abdominal wall by planes (Figure 1). In the HYPO group there was initially laparotomy and manipulation of the cavity without introducing a surgical dressing. Then he withdrew 1ml of blood through the catheter located in the carotid artery in two steps (0.5ml + 0.5ml) with an interval of 10 minutes, in order to reduce the MAP values to 60mmHg, in contrast to previous groups, in which MAP was maintained at 90mmHg. If MAP was increased after the closure of the cavity, we withdrew 0.5ml more of blood, with the same technique described above. When the pressure decreased to values lower than 60 mmHg, the animal received heparinized blood stored in the mean arterial pressure system tube, and then, if necessary, we administered 0.5ml of Ringer lactate. The intra-abdominal pressure was maintained at about 3mmHg. In the IAH HYPO group we combined the procedures of the groups mentioned above. Hence, in this case, the IAP and MAP remained constant, with values of 12mmHg and 60mmHg, respectively. In the simulation (sham) group a midline laparotomy was performed and the abdominal cavity manipulated, simulating an inventory, the position of the catheter being verified and the intra-abdominal pressure measured (close to 3mmHg), followed by abdominal wall synthesis in plans and carotid puncture only for monitoring of MAP, without drawing blood.

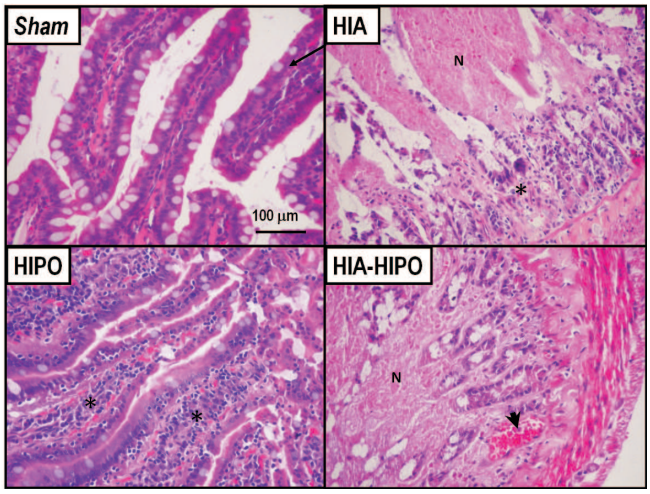

Figure 1 - *Photomicrograph of the small intestine stained with HE - 200X increase. The simulation/sham group exemplifies the normal architecture, contrasting with the IAH group that showed intense congestion and inflammation of the intestinal mucosa. The HYPO group revealed congestion (*). In the IAH-HYPO group there was a total disruption of villous architecture, with marked necrosis and hemorrhagic infiltration.*

After three hours of mechanical ventilation, all animals were heparinized (0.1ml) and ten minutes later, subjected to death without pain induced by section of the abdominal aorta and vena cava. Samples of the small intestine were removed, fixed in buffered 4% formaldehyde for 48 hours. Then the material was gradually dehydrated by immersion in solutions with increasing concentrations of ethanol, reaching 100%. After dehydration, the material was immersed in paraffin, sectioned in sections of 5mm thickness and stained with hematoxylin and eosin (HE).

Histological analysis of the small intestine was performed using an optical microscope (BX51, Olympus Latin America Inc., Miami, Florida, USA) according to their qualitative and semiquantitative features. For descriptive analysis, we observed the whole surface of the slide, including all structures of the intestine, in 100X and 400X augmentations. For each field, the following parameters were analyzed: necrosis of the villi, hemorrhagic infarction, congestion and the presence of inflammatory cells. We used a semi-quantitative scoring of five points, based on the severity and graded as negative = 0 (normal intestine); 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%, 15 non-coincident microscopic fields being quantified for each animal, a total of five animals per group (400X increase).

The histological parameters were compared by analysis of variance on ranks followed by Dunn's test. In all tests, the significance level was 5% ($p < 0.05$ and CI = 95%).

RESULTS

IAH induced necrosis of the villi, congestion, and neutrophilic infiltration, and the intensity of these lesions were larger than the simulation/sham group ($p < 0.05$). Hypovolemia caused only inflammation and swelling of villi. However, in the IAH and HYPO group there were more severe histological changes for all parameters studied when compared with the findings of the simulation group / sham (Figure 1 and Table 1). In addition, there was a greater degree of hemorrhagic infarction in relation to IAH group, and necrosis of villi, hemorrhagic infarction, and congestion when compared to the HYPO group ($p < 0.05$).

DISCUSSION

This study presents a new model of abdominal compartment syndrome, characterized by intra-abdominal hypertension and hypovolemia, that caused histological changes in the small intestine, characterized by necrosis of the villi, hemorrhagic infarction, congestion, and neutrophilic infiltration.

In most experimental studies, intra-abdominal hypertension is induced by insufflation of air (or CO_2), or instillation of fluid[4,6]. However, both techniques require constant administration of the substrate to maintain the desired levels of intra-abdominal pressure (IAP), since peritoneal absorption progressively dilutes their baric effects. New materials have been proposed, like a synthetic gelatin (polisuccinate), that although costly, seem to be able to maintain levels of IAP under more controlled conditions[7].

Schachtrupp *et al*. published a review on different experimental models of abdominal compartment syndrome in 2007 and concluded that, to obtain reliable scientific evidence, it would be necessary to create a model of this disease that associated hemorrhage or inflammation with a component of resuscitation volume related to edema of the bowel[8]. Thus, the experimental model used was designed aiming to: 1) Simulate the abdominal compartment syndrome by inserting a solid material that caused IAH, as well as keep constant pressure levels, without the variations reported, when liquid or gas was used associated with hypovolemia; 2) Clarify the pathophysiological role played by hypovolemia alone or combined with IAH; and 3) Demonstrate the precocity of the injuries caused by the model of the small intestine.

In histological analysis, it is clear that the presence of a small degree of congestion and inflammation in the small intestine of the Simulation/sham group was probably due to the laparotomy trauma itself, followed by manipulation of the intestinal contents. Hypovolemia caused, by itself, congestion and neutrophilic infiltration and that may be related to the reduction of intestinal perfusion.

In the IAH group we found necrosis of the villi, congestion and inflammation. In this context, Toens *et al.*[4] also found serious damage to the intestinal mucosa in a

Table 1 - Semiquantitative analysis of histological changes of the small intestine.

Groups	Necrosis Of Villi	Hemorrhagic Infarction	Congestion	Inflammatory Cells
SIMULATION (*Sham)*	0 (0 - 0)	0 (0 - 0)	1 (0 - 1)	1 (1 - 1)
IAH	2 (2 - 2,5)*	0 (0 - 0)	3 (2,75 - 3)*	3 (2,75 - 3,25)*
HIPO	0 (0 - 0)**	0 (0 - 0)	2 (1,75 - 2)*.**	2 (1,75 - 2)*.**
IAH-HIPO	4 (3 - 4)*#†	2 (1,75- 3)*#†	4 (3,75 - 4)*†	4 (3 - 4)*†

*The values represent the median of five animals (25% -75% percentiles). * Significantly different from the Simulation/sham ($p < 0.05$); # HIA-HYPO versus HIA ($P < 0.05$); † HIA-HYPO vs. HYPO ($p < 0.05$), ** HIA versus HYPO.*

pig model, although they used an intra-abdominal pressure of 30mmHg for a period of 24 hours, maintained with the use of CO_2 insufflation. Certainly, the consequences of the levels of IAP on the intestine are variable, depending on the species, since the abdominal wall compliance is variable, i.e., much larger in rats than in pigs, suggesting that the consequences of 12mmHg are much more deleterious in rats than in pigs. Samel *et al.*[10] used a fluorescent marker to quantify tissue perfusion in the gastrointestinal tract, and described decrease of functional capillary perfusion, the speed of red blood cells, in addition to decreasing the diameter of the capillaries and blood flow in rats with intra-abdominal hypertension (10 to 15mmHg insufflation with gas), suggesting a progressive dysfunction of the microcirculation of the mucosa. Similarly, Olofsson *et al.*, in 2009, observed that the reduction of microcirculation in the small intestine seromuscular layer is inversely proportional to the applied intra-abdominal pressure[6]. In his conclusion, the authors reported that even in the presence of intra-abdominal hypertension, blood flow in the mucosa of the small intestine, although small, seemed to be preserved when compared to other microvascular beds, probably due to rapid diffusion of the gas content. This confirms the need for experimental models with other causal agents besides the use of gas insufflation, capable of sustaining intra-abdominal hypertension.

Despite the importance of microcirculatory response to the release of humoral mediators in trauma, it is understood that the necrosis of villi, observed three hours after abdominal hypertension, is due also to to aggression by mechanical compression, as these viscera are in direct contact with the hypertension generator system.

Lou *et al*. evaluated the effects of intra-abdominal hypertension alone and associated with sepsis in the intestine of rats demonstrated by the injury to interstitial cells of Cajal and found disorders in the intestine peristaltic rhythmicity[10]. The combination of sepsis and IAH caused more intestinal damage compared with those arising from each trauma agent alone. Research on the effects of the IAH on the intestinal mucosa often associated other offending mechanism, such as sepsis. The same occurs with hypovolemia. There was no intention from our part to add this variable infectious, because its installation is a little later, fleeing the scope of an early assessment of the context of the Abdominal Compartment Syndrome. However, it is noteworthy that such etiologic associations usually result in a more severe bowel commitment.

In conclusion, this experimental model was able to produce clinical and surgical conditions for the study of Abdominal Compartment Syndrome in rats. In addition, histopathological changes were early observed on the small intestine.

RESUMO

Objetivo: *Este artigo objetiva descrever um modelo experimental, inédito, que mimetiza a síndrome do compartimento abdominal (SCA).* ***Métodos:*** *Foram utilizados 20 ratos distribuídos aleatoriamente em quatro grupos. Para simular a SCA foi induzida hipertensão intra-abdominal (HIA) através da inserção de curativo cirúrgico algodoado (Zobec®) de 15x15cm (pressão intra-abdominal constante e igual a 12mmHg) associada à hipovolemia induzida através da retirada de sangue, mantendo-se a pressão arterial média (PAM) em torno de 60mmHg (HIPO). Para dissociar os efeitos da HIA daqueles induzidos pela hipovolemia* per se*, dois outros grupos foram analisados: aquele com somente HIA e outro com hipovolemia. O grupo Simulação (*sham*) foi submetido ao mesmo procedimento cirúrgico anteriormente realizado; entretanto, os níveis de pressão intra-abdominal e PAM se mantiveram iguais a 3mmHg e 90mmHg, respectivamente.* ***Resultados:*** *Ao analisar o impacto da HIA sobre o intestino delgado, constataram-se necrose das vilosidades, congestão e infiltração neutrofílica. A hipovolemia induziu somente inflamação e edema do vilo. Entretanto, a associação de HIA e HIPO induziu, além de piora dos parâmetros supracitados, ao infarto hemorrágico.* ***Conclusão:*** *O presente modelo foi eficiente em induzir SCA expressa pelas repercussões encontradas no intestino delgado.*

Descritores: *Cavidade abdominal. Hipovolemia, Procedimentos cirúrgicos operatórios. Epidemiologia experimental. Ratos.*

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