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Early impact of abdominal compartment syndrome on liver, kidney and lung damage in a rodent model

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Abstract

Background: Abdominal compartment syndrome (ACS) sometimes occurs in critically ill patients following damage control surgery. The purpose of the present study was to develop a model of ACS and to evaluate its pathologic impact on liver, kidney, and lung morphology.

Methods: Twenty Wistar rats (mass 300–350 g) were randomly divided into four groups: 1) intra-abdominal hypertension (IAH): a laparotomy was performed and the abdomen packed with cotton until an intra-abdominal pressure (IAP) of 15 mm Hg was reached; 2) hypovolemia (HYPO): blood was withdrawn until a mean arterial pressure ~60 mm Hg was reached; 3) IAH + HYPO (to resemble clinical ACS); and 4) sham surgery. After 3 hours of protective mechanical ventilation, the animals were euthanized and the liver, kidney and lungs removed to examine the degree of tissue damage.

Results: IAH resulted in the following: oedema and neutrophil infiltration in the kidney; necrosis, congestion, and microsteatosis in the liver; and alveolar collapse, haemorrhage, interstitial oedema, and neutrophil infiltration in the lungs. Furthermore, IAH was associated with greater cell apoptosis in the kidney, liver and lungs compared to sham surgery. HYPO led to oedema and neutrophil infiltration in the kidney. The combination of IAH and HYPO resulted in all the aforementioned changes in lung, kidney and liver tissue, as well as exacerbation of the inflammatory process in the kidney and liver and kidney cell necrosis and apoptosis.

Conclusions: Intra-abdominal hypertension by itself is associated with kidney, liver and lung damage; when combined with hypovolemia, it leads to further impairment and organ damage.

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Key words: hypovolemia; intra-abdominal hypertension; apoptosis; necrosis; rodents, rats

In 2013, new consensus definitions for abdominal compartment syndrome (ACS) were published, defining it as a condition of sustained increase in intra-abdominal pressure (IAP) above 20 mm Hg with new-onset organ failure [1]. Increases in compartmental pressures and the resulting changes in interaction between different body compart-

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ments have a mechanical effect on vascular structures, preload, afterload and contractility of the heart, resulting in diminished venous return and poor organ perfusion [2]. Critically ill patients may experience intra-abdominal hypertension (IAH) and ACS (25–30% and 5% respectively), which, if left untreated, are associated with very high mortality [3]. When cardiac output (CO) drops in IAH, subsequent hypoperfusion results in sustained ischemia and the ischemia-reperfusion phenomenon, followed by alterations in the microbiome, intestinal epithelium and intestinal immune system, triggering the systemic inflammatory response and multiorgan dysfunction that appear in the final stages of ACS [4].

The management of catastrophic abdominal injuries has been described for many years [5]. In 1993, Rotondo *et al.* [6] introduced the term "damage control" to designate a staged repair procedure designed in an attempt to reduce the high mortality rates associated with this form of trauma. Although mortality decreased with this technique, damage control surgery induces IAH, which may progress to ACS and multiple organ failure [7]. On the other hand, in medical patients, the incidence of IAH and ACS has been declining since the introduction of the medical management algorithm [1].

Experimental research into IAH has flourished in recent years due to the advent of laparoscopic surgery, during which the effects of IAH may be observed. Although experimental models of IAH were developed using CO_2 insufflation, it is difficult to maintain high IAP levels for prolonged periods [8]. To address this issue, some authors have suggested the use of peritoneal saline infusion [9], rather than CO_2 insufflation to generate a lengthy IAH model [10]. Since the peritoneal membrane is an efficient system for drainage and absorption of liquids in the abdominal cavity, this system has also failed to maintain a stable IAP. Recently, another promising model has been introduced, the mechanical intestinal obstruction (MIO) model, which closely resembles the real situation of a patient with ileus and fluid-filled bowel dilation [7]. However, a more realistic ACS model cannot rely solely on increased IAP; it must also include hypovolemia. As the association of hypovolemia and IAH is frequent in various pathological conditions, including trauma, haemorrhagic shock and septic shock, a new ACS model seems warranted. This model should be able to maintain IAP values within an acceptable range during a prolonged interval, intending to maintain a level of IAH that, when combined with hypovolemia, would cause organ failure resembling human ACS [11–13]. We hypothesize that hypovolemia could compound the negative effects of IAH on the lungs and peripheral organs.

The aims of the present study were: 1) to develop a model of ACS in rats; 2) to analyse the morphological effects of IAH and/or hypovolemia on kidney, liver and lung tissue; and 3) to use apoptosis as an early marker of damage.

METHODS

This study was approved by the Ethics Committee of the Health Sciences Center, Federal University of Rio de Janeiro (CEUA-019). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences, USA.

ANIMAL PREPARATION AND EXPERIMENTAL PROTOCOL

Twenty Wistar rats (weight 300–350 g) were randomly assigned across groups (n = 5/group): 1) intra-abdominal hypertension (IAH), 2) hypovolemia (HYPO), 3) IAH plus hypovolemia (IAH+HYPO), and 4) sham surgery (Sham). The animals were sedated (diazepam 5 mg intraperitoneally), anaesthetised (thiopental sodium 20 mg kg^{-1} intraperitoneally), and tracheotomised. The depth of anaesthesia was similar in all animals. Mean arterial pressure (MAP) was continuously recorded (Networked Multi-Parameter Veterinary Monitor LifeWindow™ 6000V, Digicare Animal Health, Florida, USA). A polyethylene catheter (PE-50) was introduced into the carotid artery for blood sampling, monitoring of MAP, and administration of saline, if necessary, in order to maintain a MAP around 60 mm Hg in the HYPO group. We observed that, when we let MAP fall below this threshold in the IAH+HYPO group, the rats died before the 3-hour period defined for the experiment. Additionally, we observed in the group of rats used to construct the model that this level of MAP was optimal to induce changes in haemodynamic parameters (tachycardia) without the development of irreversible hypotension.

Animals were then paralyzed (vecuronium bromide 2 mg kg^{-1} intravenously) and mechanically ventilated (Servo-i, MAQUET, Sweden) in volume-controlled mode with the following settings: tidal volume (V_T) = 6 mL kg^{-1}, respiratory rate (RR) = 80 breaths per min, inspiratory-to-expiratory ratio = 1:2, fraction of inspired oxygen (FiO_2) = 0.4, and positive end-expiratory pressure (PEEP) = 5 cm H_2O. To induce IAH, a midline laparotomy (3 cm incision) was performed to expose the abdominal cavity, and four 15-cm cotton gauze packs (Cremer, Brazil) soaked in saline solution were placed in the four quadrants of the abdomen. A polyethylene catheter (BD™) was placed in the peritoneum for continuous measurement of IAP, as the stomach and bladder were compressed by the cotton packs and could not be used to measure IAP; a 2–0 silk suture was used to tie the catheter in place and prevent leaking. Both layers of the abdominal cavity were closed with 3–0 silk sutures until an IAP of 15 mm Hg was reached. IAP was maintained at this level throughout the experiment, considering we had obtained a large abdominal volume at this pressure consequent to the high abdominal compliance of the rat abdomen [14]. If necessary, more dressings were added or the abdominal suture was tied further to maintain IAP. Hypovolemia was induced by bloodletting in order to achieve a MAP around 60 mm Hg. In the other groups, MAP was maintained around 90 mm Hg. An MAP below this value in combination with IAH induced death within 3 hours. In the Sham group, rats underwent identical manipulation and instrumentation except for abdominal packing or blood drainage. After 3 hours of mechanical ventilation, the animals were euthanized and, the kidney, liver and lungs were extracted and prepared for histological examination and quantification of apoptosis.

HISTOLOGY

LIGHT MICROSCOPY

A laparotomy was performed at the end of the experiments. Heparin (1000 IU) was injected into the vena cava. The trachea was clamped at end-expiration (PEEP = 5 cm H_2O), and the abdominal aorta and vena cava were sectioned, yielding massive haemorrhage and rapid death. The kidneys, liver and lungs were removed, fixed in 3% buffered formaldehyde, embedded in paraffin, and stained with haematoxylin-eosin. Two investigators, unaware of the origin of the pathological material, examined the samples microscopically. The slides were coded and examined only at the end of all measurements.

APOPTOSIS ASSAYS

Apoptotic cells of kidney, liver, and lung were quantified using a terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) assay, performed in blinded fashion by two pathologists. Apoptotic cells were detected using the commercial In Situ Cell Death Detection Kit, Fluorescein (Boehringer Mannheim, Germany). Nuclei without DNA fragmentation stained blue as a result of counterstaining with haematoxylin. Ten fields per section were examined at a magnification of × 400. A five-point, semi-quantitative, severity-based scoring system was used to assess histological changes and apoptosis as follows: 0 = normal tissue throughout; 1 = changes in 1–25% of examined tissue; 2 = changes in 26–50% of examined tissue; 3 = changes in 51–75% of examined tissue; and 4 = changes in 76–100% of examined tissue.

STATISTICAL ANALYSIS

The normality of data was tested using the Kolmogorov-Smirnov test with Lilliefors' correction. The Levene median test was used to evaluate the homogeneity of variances. Comparisons among groups were performed using one-way analysis of variance (ANOVA) on ranks followed by Dunnett's post-hoc test. Data are expressed as median (interquartile range). All tests were performed using the SigmaStat 3.1 statistical software package (Jandel Corporation, San Rafael, USA). Significance was established at $P < 0.05$.

RESULTS

All animals with IAH, with or without hypovolemia, survived for the duration of the experiment (100% survival).

In the kidney, IAH led to oedema, inflammation, and apoptosis (Table 1, Fig. 1). Hypovolemia worsened all of these

Table 1. Semi-quantitative histopathological analysis of renal injury

Groups	Necrosis	Oedema	Inflammation	Apoptosis
Sham	0 (0-0)	0 (0–0)	0 (0–0)	1 (1–1)
IAH	0 (0–1)	3 (2.75–3)*	1 (1–2)*	3 (3–4)*
HYPO	3 (3–3)*	3 (2–3)*	2 (1.75–2)*	2 (1.75–2)*
IAH-HYPO	2 (1–2)*	3 (3–4)*	3 (3–4)*#†	3 (2.75–4)*†

*Significantly different from Sham group ($P < 0.05$). #Significantly different from IAH group ($P < 0.05$). †Significantly different from HYPO group ($P < 0.05$)

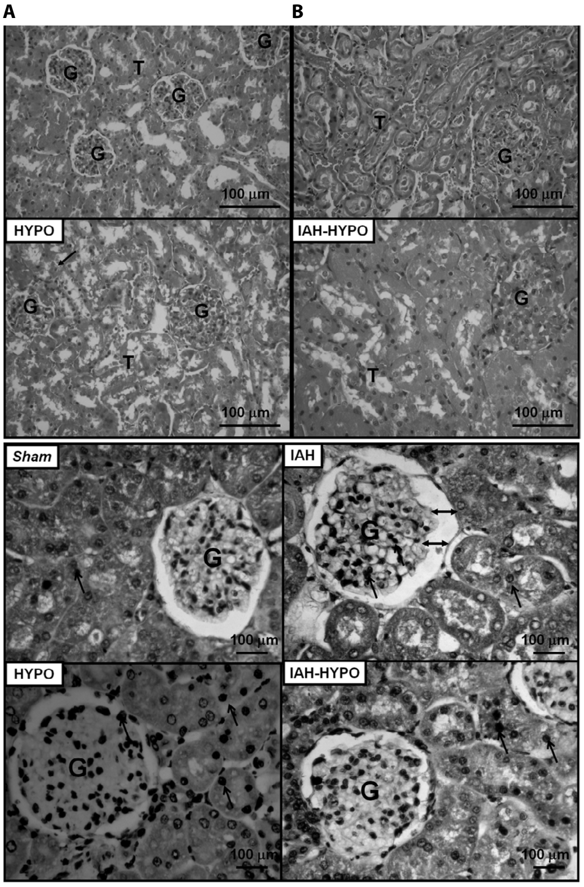

Figure 1. Panel A: Upper — representative photomicrographs of kidney tissue stained with haematoxylin-eosin (× 200). IAH — intra-abdominal hypertension; HYPO — hypovolemia. A sham-operated group was used as a control. In the Sham group, kidney histology shows glomeruli (G) and renal tubules (T) with preserved architecture. In the IAH group, there is visible disarrangement of renal tubules with degenerative cytoplasmic changes, oedema, and inflammation. In HYPO, necrosis is visible (arrow). In kidneys exposed to IAH-HYPO, inflammation and apoptosis were further increased. **Panel B:** Lower — representative photomicrographs of kidney tissue stained by TUNEL (× 400). In the Sham group, kidney histology shows glomeruli and brownish apoptotic renal cells. In the IAH group, kidney histology revealed numerous brownish apoptotic tubular cells. In kidneys exposed to IAH-HYPO, apoptosis was further increased.

Table 2. Semi-quantitative histopathological analysis of hepatic injury

Groups	Necrosis	Congestion	Microsteatosis	Apoptosis
Sham	0 (0–0)	1 (0–0)	0 (0–0)	1 (1–1)
IAH	2 (2–2)*	3 (2–3)*	3 (3–3)*	2 (2–3)*
HYPO	0 (0–0)	3 (2.75–3)*	2 (1.75–2)*	1 (1–1.25)
IAH-HYPO	2 (1.75–2.25)* †	4 (3.75–4)*	4 (3–4)*†	4 (4–4)*#†

*Significantly different from Sham group ($P < 0.05$). #Significantly different from IAH group ($P < 0.05$). †Significantly different from HYPO group ($P < 0.05$)

alterations, and was associated with the presence of areas of necrotic renal tissue. The combination of IAH and HYPO resulted in a further increase in inflammation-associated changes (Table 1).

Values are median (interquartile range) of 5 rats per group. A semi-quantitative severity-based scoring system was used: 0 = no visible injury (cell damage or apoptosis); 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% of examined tissue is injured or apoptotic. IAH — intra-abdominal hypertension; HYPO — hypovolemia; IAH-HYPO — combined intra-abdominal hypertension and hypovolemia in order to resemble abdominal compartment syndrome. A sham-operated group was used as a control.

In the liver, IAH led to necrosis, congestion, microsteatosis and increased apoptosis (Table 2, Fig. 2). HYPO alone resulted only in congestion and microsteatosis, but no necrosis. Animals in the IAH-HYPO group showed hepatocyte necrosis, congestion, microsteatosis, and a significant increase in hepatocyte apoptosis (Table 2, Fig. 2).

Values are median (interquartile range) of 5 rats per group. A semi-quantitative severity-based scoring system was used: 0 = no visible injury (cell damage or apoptosis); 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% of examined tissue is injured or apoptotic. IAH — intra-abdominal hypertension. HYPO — hypovolemia; IAH-HYPO — combined intra-abdominal hypertension and hypovolemia in order to resemble abdominal compartment syndrome. A sham-operated group was used as a control.

Changes in lung histology associated with IAH included atelectasis, interstitial oedema, thickened alveolar membranes, and increased cellularity, as well as increased apoptotic cell counts compared to the Sham group. HYPO alone resulted in increased alveolar collapse, interstitial oedema and neutrophil infiltration. IAH-HYPO led to a further increase in alveolar collapse, haemorrhage and apoptosis (Table 3, Fig. 3).

Values are median (interquartile range) of 5 rats per group. A semi-quantitative severity-based scoring system was used: 0 = no visible injury (cell damage or apoptosis); 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% of examined tissue is injured or apoptotic. IAH — intra-abdominal hypertension; HYPO — hypovolemia; IAH-HYPO — combined intra-abdominal hypertension and hypovolemia in order to resemble abdominal compartment syndrome. A sham-operated group was used as a control.

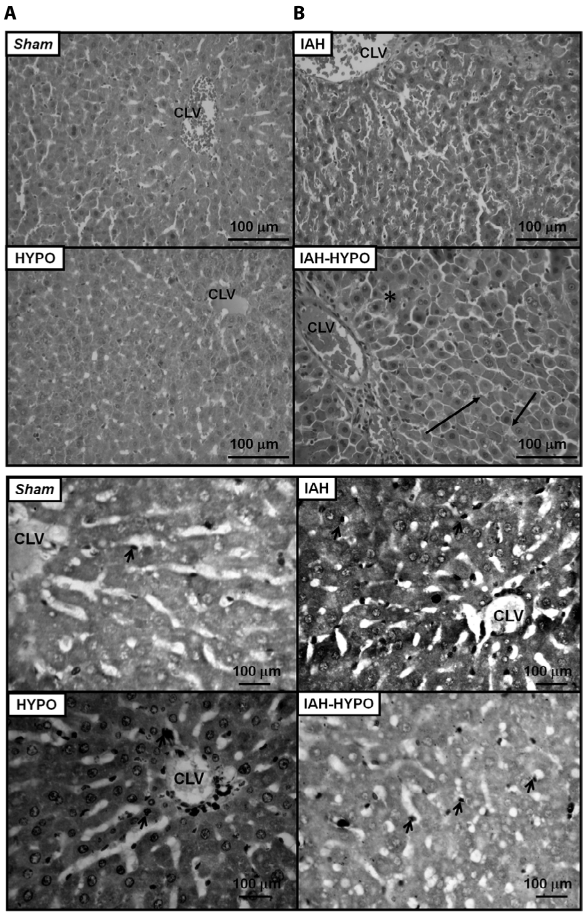

Figure 2. Panel A: Upper — representative photomicrographs of liver tissue stained with haematoxylin-eosin (× 200). IAH — intra-abdominal hypertension; HYPO — hypovolemia. A sham-operated group was used as a control. Note the normal arrangement of hepatocytes and centrilobular vein (CLV) in the Sham group. In IAH animals, liver histology revealed necrosis, congestion and microsteatosis (arrows). Hypovolemia led to distortion of the lobar architecture, with changes in trabecular spaces, congestions and microsteatosis. In IAH-HYPO, there is complete derangement of the parenchymal architecture, with a further increase in microsteatosis. **Panel B:** Lower — representative photomicrographs of liver tissue stained by TUNEL (× 400). Note the presence of apoptotic cells (arrows) resident on portal spaces in the Sham group. In IAH animals, liver histology revealed a great number of apoptotic hepatocytes (small arrows). In IAH-HYPO, there was no further increase in apoptosis

Table 3. Semi-quantitative histopathological analysis of lung injury

Groups	Alveolar collapse	Alveolar haemorrhage	Interstitial oedema	Inflammation	Apoptosis
Sham	0 (0–0)	0 (0–0)	0(0–1)	0 (0–0)	1 (1–2)
IAH	2 (2–2.25)*	2 (1.75–3) *	3 (2.75–3.25)*	2 (1.75–2.25)*	3 (3–3.25)*
HYPO	2 (1.75–2)*	0 (0–1)	3 (2.75–3.25)*	2 (1–2)*	2 (2–2)
IAH-HYPO	3 (2.75–3)*†	2 (1–2)*†	4 (3–4)*	2 (1.75–2)*	3 (3–4)*†

#Significantly different from IAH group ($P < 0.05$). †Significantly different from HYPO group ($P < 0.05$)

DISCUSSION

Although current treatment of ACS is based on consensus definitions and recommendations, several questions persist regarding fluid therapy, while the critical level of IAP that warrants intervention remains unknown [1]. In the vast majority of experimental studies, IAH has ranged from 20 to 50 mm Hg while the duration of IAH has ranged from 30 min to 24 h [7]. Organ dysfunction can develop as early as 15 min, while multiple organ failure may arise within 4 to 6 h. A few investigators have attempted to develop new experimental models that might mimic real-life conditions more closely [12, 15].

Our experimental rat model of ACS resembles the clinical picture of a human patient during the early post-operative course after damage control surgery [15]. This ACS model led to increased intrathoracic pressure with lung parenchyma impairment, resulting in interstitial oedema, alveolar collapse and haemorrhage, and neutrophil infiltration. Furthermore, there were histological signs of poor mesenteric perfusion, which may be associated with direct pressure on the surgical wound interfering with the local blood supply, and may have induced renal and hepatic oedema, inflammation, and necrosis.

In some previous studies, ACS was simulated by associating an experimental model of IAH with haemorrhage [1, 16]. In the present study, IAH was induced by packing the four quadrants of the peritoneal cavity with saline-soaked gauze dressings and closing both layers of the abdominal cavity until an IAP of 15 mm Hg was achieved [17]. This technique has some advantages over existing models. Intraperitoneal fluid infusion is likely to be absorbed and therefore may interfere with the pathophysiological response to IAH [10], while the use of CO_2 insufflation may represent an additional physiologic variable [8]. Furthermore, CO_2 may be absorbed and the gas volume can be compressed, whereas saline and cotton dressings cannot.

In contrast to other clinical studies that considered ACS levels at an IAP threshold higher than 20 mm Hg, in the present study, the threshold was set at 15 mm Hg, for two main reasons: 1) as the abdominal compliance of rats is high, a 15 mm Hg IAP cannot be compared in absolute values to humans [18]; and 2) pilot studies showed that all

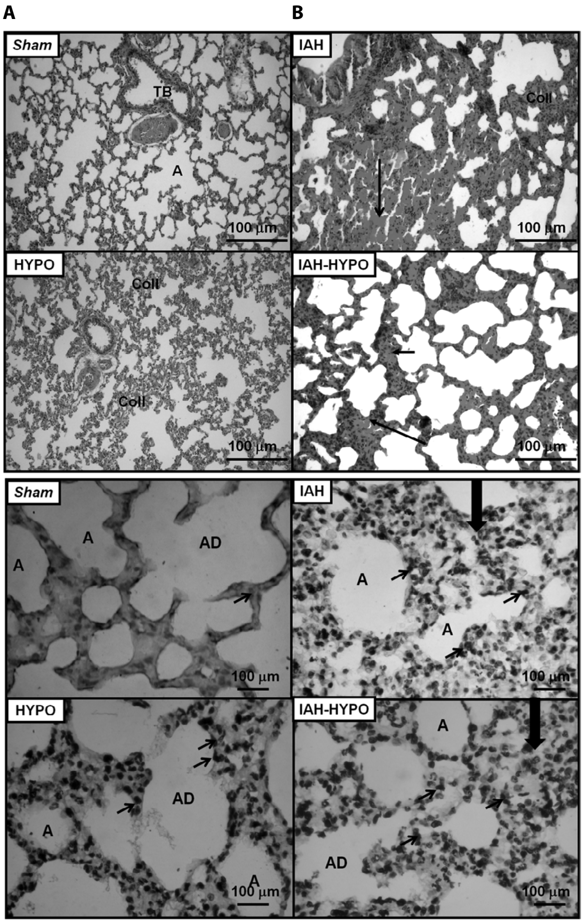

Figure 3. Panel A: Upper — representative photomicrographs of lung tissue stained with haematoxylin-eosin (× 200). IAH: intra-abdominal hypertension. HYPO — hypovolemia. A sham-operated group was used as a control. Note preserved cellular architecture with normal alveoli (A) and ducts (AD) in the Sham group. Conversely, in the IAH group, lungs presented alveolar collapse (coll), interstitial alveolar oedema, alveolar haemorrhage and neutrophil infiltration. Panel B: Lower — representative photomicrographs of lung tissue stained by TUNEL (× 400). Note sparse brownish apoptotic lung cells (arrows) in the Sham group. In contrast, in the IAH group, lungs exhibited numerous brownish apoptotic pneumocytes (arrows). In IAH-HYPO, the number of apoptotic epithelial cells was increased further.

animals died at 3 hours with an IAP higher than 15 mm Hg, while keeping them alive during this period required high doses of fluids and norepinephrine. Since these therapies may interfere with histopathological findings and thus limit understanding of the consequences of ACS *per se* in the various affected organs, we chose to keep IAP at 15 mm Hg, a level that was survivable without interventions that may affect subsequent histological analysis. Early changes in liver, kidney and lung tissue were evaluated using a semi-quantitative analysis of histological damage and apoptosis. Furthermore, to minimise the impact of mechanical ventilation on the lungs and peripheral organs, the animals were ventilated for 3 hours with a protective strategy (V_T = 6 mL kg^{-1} and PEEP = 5 cm H_2O).

IMPACT OF INTRA-ABDOMINAL HYPERTENSION ON THE LIVER, KIDNEYS, AND LUNGS

IAH led to kidney and liver damage with oedema, inflammation, and necrosis. Our results are in line with the previous literature reporting renal [19-22] and hepatic [21] dysfunction (Tables 1, 2, Figs 1, 2). Toens *et al.* [23] used a porcine model with IAP ~30 mm Hg for 24 h, induced by CO_2 insufflation, and observed necrosis in the central vein of the liver, as well as tubular and glomerular necrosis. Studies with lower levels of IAP (10 mm Hg) have also reported cellular dysfunction [24]. These morphological changes in the liver and kidneys may be associated with increases in IAP and pleural pressure leading to a decrease in venous return, direct compression of the heart, and increased afterload (especially in the right ventricle) [25]. The decrease in cardiac output and increase in interstitial pressure and outflow pressure may have reduced perfusion of the intra-abdominal organs, resulting in splanchnic ischemia.

In a murine model of IAH, Gong *et al.* observed that an IAP of 20 mm Hg maintained for 4 hours could lead to a condition comparable with ACS in humans. Additionally, they reported persistent respiratory acidosis even after decompression, reductions in renal blood flow and urine output and lung damage [26]. Meier *et al.* observed that a similar IAP level led to haemodynamic deterioration and organ dysfunction, with parenchymal injury in the liver, lung, bowel and myocardium [27].

The aggressive damage observed in the lungs, liver and kidneys in this experiment showed that the effects of IAH may extend beyond the peritoneal cavity.

IMPACT OF HYPOVOLEMIA ON THE LIVER, KIDNEYS, AND LUNGS

Hypovolemia can lead to severe impairment in liver, kidney and lung function [28–34]. Li *et al.* observed that haemorrhagic shock releases mediators resulting in lung inflammation [28]. Experimental models have also demonstrated that haemorrhage impairs endothelial cell function [29] and depresses intrinsic myocardial contractility [30], suggesting reduced perfusion. Histological alterations in the liver after autologous whole blood transfusion were attributed to hypoxic hepatocellular damage associated with the severity of the shock model rather than to the administration of fluid itself [31, 32]. Mayeur *et al.* demonstrated tissue effects in the renal outer medulla 2 days after a haemorrhagic insult [34]. Similarly, in our study, hypovolemia resulted in kidney, liver and lung impairment with congestion, necrosis and interstitial oedema (Tables 1–3 and Figs 1–3).

IMPACT OF COMBINED INTRA-ABDOMINAL HYPERTENSION AND HYPOVOLEMIA ON THE LIVER, KIDNEYS AND LUNGS

Combined IAH with hypovolemia led to interstitial oedema and alveolar collapse (Table 3). Similarly, Oda *et al.* observed that the combination of haemorrhagic shock and ACS resulted in greater cytokine activation and neutrophil-mediated lung injury than no combination at all [35]. Moreover, in a small-animal model of ACS (haemorrhagic shock and IAH induced by intraperitoneal air injection), Rezende-Neto *et al.* also observed lung damage [19] (Table 3, Fig. 3).

The association of IAH and hypovolemia led to liver congestion, cellular necrosis, microsteatosis and apoptosis (Table 2). Conversely, Mogilner *et al.* [36] found only mild liver histological alterations with an IAP of 60 mm Hg for 2 hours. These differences may be attributed to the timing of analysis (2 vs. 3 hours), as well as the method used to induce IAH (CO_2 insufflation *vs.* abdominal packing). The combination of IAH and hypovolemia also led to a reduction in liver mitochondrial function, leading to liver damage [24, 37], as well as lung parenchymal impairment. These changes may be attributed to an increase in intrathoracic pressure, followed by a decrease in venous return and ventricular compliance. Consequently, cardiac output reduces, decreasing perfusion of the lungs, kidney and other retroperitoneal and intraperitoneal organs [38, 39].

In the kidneys, IAH and hypovolemia led to apoptosis, tubular necrosis, oedema and inflammation. These changes may also be caused by reduced renal perfusion and increased renal vascular resistance, resulting in kidney damage [9, 40, 41].

The degree of apoptosis is associated with cellular stress and organ failure [24, 37]. In line with this, according to some authors [36], IAH leads to visceral apoptosis. In combination with hypovolemia, however, it increased lung and liver apoptosis. Apoptosis is one of the major pathways that lead to cell death. One may draw a parallel between IAH leading to low abdominal perfusion and subarachnoid haemorrhage (SAH) — which involves a rapid rise of intracranial pressure and a reduction in cerebral perfusion pressure – leading to brain oedema, oxidative and nitrosative stress, as well as neural apoptosis [38]. It has been shown that treatment with melatonin after experimental SAH induced by endovascular perforation ameliorated brain oedema, decreased mortality and improved neurological outcome. This finding also supports the hypothesis that melatonin acts as an anti-apoptotic agent that downregulates caspase-3 expression and reduces cell apoptosis to improve neurological outcome in rats subjected to SAH [42]. It is an interesting hypothesis that the use of apoptosis as a marker of precocity and grade of inflammatory response may also uncover possible treatments to modulate inflammatory response and enable an earlier diagnosis, even in primary or secondary ACS, beyond other clinical approaches [43].

LIMITATIONS

The principal limitation of this model is the compliance of the rat abdomen. For this reason, we validated the desired level of IAH via the onset of ventilator alterations in the plateau pressure curve, considering this to be in line with development of organ failure. Although the sample size also appears to be quite small, it was calculated on the basis of the experience of our laboratory in previous studies of IAH. The combined IAH and hypovolemia model induces a highly unstable condition that usually requires volume resuscitation. The idea behind the creation of this group (IAH-HYPO) was to replicate the damage control situation seen in an ICU environment and discover how soon the deleterious effects of this combination occur. As one of the aims of our experiment was the evaluation of the precocity of morphologic changes, we could not evaluate other outcomes, such as the severity of inflammation or the degree of immunosuppression. In our opinion, the major endpoints of this study – validation of a new model, demonstration of early onset of the effects of IAH, and the exponential deleterious effect of hypovolemia in combination with IAH — have all been well documented. We understand that other experimental models have been described in the literature; however, as these are all very unstable, we did not include any other model for comparison in this experiment. As our IAH/ACS model without hypovolemia was quite stable, it can be used for future research, including in combination with other procedures, such as blood sampling to assess inflammatory response, calculation of organ function scores (e.g., SOFA), or monitoring of extravascular lung water (in a larger animal model). Unlike in previous IAH models, an IAP of 15 mm Hg rather than 20 mm Hg was used, considering the compliance of the rat abdominal wall and the large abdominal volume obtained. As the gauze packing model causes stomach and bladder compression, traditional methods for IAP monitoring (such as gastric or bladder pressure measurement) cannot be used; therefore, we performed continuous direct intraperitoneal pressure measurement instead. Although MAP values in the normal arterial pressure group (~90 mm Hg) and in the HYPO group (~60 mm Hg) were very high compared to those obtained in established IAH models, this comparatively mild hypotension was necessary to keep the animals alive for 3 hours without interventions that might have interfered with histopathological findings.

CONCLUSIONS

We have described a novel experimental model for the induction of ACS in the rat, based on packing of the four abdominal quadrants with saline-soaked gauze, associated with hypovolemia in order to simulate damage control surgery in human abdominal trauma. We also found that IAH induced more deleterious effects than hypovolemia alone with regard to cellular damage; these effects were more significant in extraperitoneal organs, particularly the kidneys. On the basis of our findings, we also conclude that apoptosis is an interesting biomarker for early identification of pathological processes. Its incorporation into clinical use may allow development of future interventions for the restoration of normal cellular kinetics after organ damage, perhaps even reducing morbidity and mortality.

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