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Intestinal Ischemia, Bacterial Translocation, and Oxygen Free-Radical Production in Abdominal Compartment Syndrome

Niedokrwienie jelit, translokacja bakteryjna i wydzielanie wolnych rodników tlenowych w zespole ciasnoty wewnątrzbrzuszej

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Abstract

Objectives. The aim of this experimental study was to evaluate the consequences of increased intra-abdominal pressure on the small bowel and whether this pressure creates intestinal ischemia leading to oxygen free-radical production and bacterial translocation.

Material and Methods. Twenty Sprague-Dawley rats weighing 275–300 g were used. Group 1 rats (n = 10) were subjected to 20-mm Hg pneumoperitoneum pressure for 60 minutes. In group 2 rats (n = 10, controls) the intra-abdominal pressure was not increased. In all rats the following parameters were investigated: mean arterial pressure after carotid catheterization, histopathological examination of the intestinal mucosa evaluated with a scoring system, malondialdehyde production in the liver and small bowel, and bacterial translocation towards the mesenteric lymph nodes, liver, and spleen 24 hours after pneumoperitoneum deflation.

Results. The mean arterial pressure exhibited no alterations. Histological analysis mainly showed extensive epithelial separations from the lamina propria down the sides of the villi and ulceration at the villus tips in the rats with increased intra-abdominal pressure. Bacterial translocation occurred to the mesenteric lymph nodes, spleen, and liver after 60 minutes of increased intra-abdominal pressure of 20 mm Hg ($p < 0.05$). Malondialdehyde increased in the liver and small bowel mucosa ($p < 0.05$ for both).

Conclusions. Increased intra-abdominal pressure in rats leads to intestinal ischemia and mild histological changes in the small bowel and to oxygen free-radical production and bacterial translocation (*Adv Clin Exp Med* 2009, 18, 2, 115–120).

Key words: abdominal compartment syndrome, intestinal ischemia, bacterial translocation, rat.

Streszczenie

Cel pracy. Ocena wpływu zwiększonego ciśnienia wewnątrz jamy brzusznej na jelito cienkie. Zbadano ponadto, czy zwiększone ciśnienie wewnątrz jamy brzusznej wywołuje niedokrwienie jelit prowadzące do wydzielania wolnych rodników tlenowych i translokacji bakteryjnej.

Materiał i metody. Badanie doświadczalne przeprowadzono w grupie 20 szczurów Sprague-Dawley o masie 275–300 g. U szczurów w grupie 1 (n = 10) wytworzono na 60 minut odcięcie otrzewnową (20 mm Hg) i badano wpływ zwiększonego ciśnienia wewnątrz jamy brzusznej na błonę śluzową jelita cienkiego, wytwarzanie wolnych rodników tlenowych i translokację bakteryjną. W grupie 2 (n = 10, grupa kontrolna) ciśnienie wewnątrz jamy brzusznej nie było zwiększone. U wszystkich szczurów oceniano średnie ciśnienie tętnicze; wykonano badanie histopatologiczne błony śluzowej jelita cienkiego z zastosowaniem systemu punktowego; badano wydzielanie aldehydu dimalonalowego w wątrobie i jelicie cienkim; translokację bakteryjną do krezkowych węzłów chłonnych, wątroby i śledziony 24 godziny po odcięciu otrzewnowej.

Wyniki. Nie wykazano różnic w średnim ciśnieniu tętniczym. Badanie histologiczne wykazywało przede wszystkim obfite oddzielanie komórek nabłonka od błazki właściwej, owrzodzenia na szczytach kosmków u szczurów poddawanych zwiększonemu ciśnieniu wewnątrz jamy brzusznej. Translokacja bakteryjna do krezkowych węzłów chłonnych, śledziony i wątroby pojawiała się po 60 minutach działania ciśnienia 20 mm Hg ($p < 0,05$). Wydzielanie aldehydu dimalonowego zwiększało się w wątrobie i błonie śluzowej jelita cienkiego ($p < 0,05$ dla obu).

Wnioski. Zwiększone ciśnienie wewnątrz jamy brzusznej u szczurów prowadzi do niedokrwienia jelit, łagodnych zmian histologicznych w jelicie cienkim, wydzielania wolnych rodników tlenowych oraz translokacji bakteryjnej (*Adv Clin Exp Med* 2009, 18, 2, 115–120).

Słowa kluczowe: zespół ciasnoty wewnątrzbzusznej, niedokrwienie jelit, translokacja bakteryjna, szczur.

Abdominal compartment syndrome (ACS) is a life-threatening clinical entity which can develop within the first 12 hours of intensive care unit admission in high-risk surgical patients [1]. Intra-abdominal pressure (IAP) may be increased by a variety of causes, including ascites and bowel distention owing to ileal or mechanical obstruction, abdominal trauma or surgery because of intraperitoneal or retroperitoneal bleeding, massive bowel distention from hemorrhagic shock/resuscitation, the placement of intraperitoneal packs to control residual bleeding, or laparoscopic surgery [2–7]. Abdominal compartment syndrome exists when IAP reaches a level at which derangement of normal physiological function ensues [4]. The adverse effects of increased IAP on cardiac, pulmonary, and renal functions have been described in both experimental and clinical settings [3, 4, 6]. Clinically, ACS consists of a need for increased ventilatory pressure and the presence of increased central venous pressure, decreased urinary output, and massive abdominal distention [4]. Cardiovascular, respiratory, and renal dysfunction become progressively difficult to manage unless the intra-abdominal pressure is reduced [3, 8, 9]. Abdominal decompression reverses all adverse effects of increased intra-abdominal pressure [10–12].

The adverse effects of raised IAP on intestinal mucosal blood flow have been described recently. Bacterial translocation (BT) is a well-described phenomenon after hemorrhagic shock, presumably because of the resultant intestinal ischemia [13–15]. The extent of damage done to intestine and liver attributable to free radicals produced by ischemia/reperfusion was indirectly assessed by measuring the level of malondialdehyde (MDA), an intermediate product of lipid peroxidation [5].

The purpose of this study was to investigate whether tension pneumoperitoneum leads to intestinal ischemia, the production of free radicals, and bacterial translocation in a rat model of the ACS.

Material and Methods

Twenty Sprague-Dawley rats weighing 275–300 g were used in this experiment. All the animals

were cared for in accordance with the institutional animal care and use committee guidelines. Food was removed 12 hours prior to the study, but all the animals were allowed free access to water. All the rats were anesthetized by intramuscular delivery of ketamine 50 mg/kg body-weight. The left carotid artery and jugular vein were dissected and cannulated by use of a 24-gauge heparinized catheter (Vasculon® 2, 24G/19 mm, I.V. Cannula, Helsingborg, Sweden) to monitor mean arterial pressure and to administer lactated Ringer's (LR) solution, respectively. After a 15-minute resting period, the baseline measurement was obtained and arterial pressure was monitored with a recorder (Pro-paq 106 EL, NELLCOR, USA) for the next 60 minutes. The rats were given a 1.5 ml/100 g bolus of LR solution and divided into two groups. In group 1 rats, pneumoperitoneum induction under sterile conditions was accomplished by peritoneal cavity puncture using an 18F Abocath connected to a mercury pressure gauge. The intra-abdominal pressure was elevated by insufflating CO₂ manually up to 20 mm Hg and maintained constant for 60 minutes. The rats in group 2 were similarly instrumented, but no pneumoperitoneum was created. Thus they served as controls. Baseline mean arterial pressure (MAP) was maintained by administration of additional LR solution. The rats were then allowed to recover from the anesthesia and allowed food and water.

Twenty-four hours later the animals were sacrificed. The mesenteric lymph node complex and a portion of the spleen and liver were obtained for culture. The extent of damage done to the intestine and liver attributable to free radicals that were produced by ischemia/reperfusion was assessed indirectly by measuring the malondialdehyde (MDA) level. A portion of the small intestine and liver were excised for measuring MDA.

MDA levels of the involved tissues were determined by the method of Ohkawa [16], then calculated as nmol/g tissue protein and compared with the same values of intact neighbor tissue as percentages. This method is based on measuring the concentration of the pink chromogen compound that forms when MDA couples to thiobarbi-

turic acid (TBA). Malondialdehyde was used as the standard and the estimation was performed using a standard curve obtained from the MDA-TBA reaction as described in the method [16]. The protein content of homogenates was determined according to the procedure of Lowry et al. [17] and the values were expressed as nanomoles of MDA per milligram of protein (nmol MDA/mg protein). All analyses were performed in duplicate.

A portion of the small bowel was removed for histopathological examinations. The tissue specimens were fixed in 10% formaldehyde, then dehydrated and embedded in paraffin wax. The samples were sectioned and stained with hematoxylin and eosin (H&E) and assessed in a blinded fashion by pathologists. Mucosal lesions were graded by a system described by Chiu et al. [18].

For the evaluation of bacterial translocation, the spleen and right hepatic lobe of all the rats were removed, followed by the mesenteric lymph node complex. The tissues were weighed and homogenized separately in sterile plastic bags (Stomacker, Lab-Blender 80) with Teflon-coated tissue-grinding rods in phosphate-buffered saline. Each homogenate was diluted 1:3, then plated on blood and MacConkey's agar culture plates. The plates were examined 24 and 48 hours after incubation at 37°C. The effect of increased IAP on bacterial translocation was studied in all rats. The bacterial count was calculated as the log of colony-forming units (CFUs) per gram of tissue.

Statistical Methods

Statistical analysis was performed using the SPSS 10.0 statistical software package. The paired Student's *t* test was used for the statistical evaluation of arterial pressure as well as MDA levels in liver and small bowel. Bacterial translocation from the gut lumen towards the lymph nodes, liver, and spleen was first assessed qualitatively by means of the chi-squared test and, after confirmation, one-way analysis of variance (ANOVA test).

Results

MAP was maintained at baseline levels when IAP was held at 20 mm Hg for 60 minutes (Fig. 1).

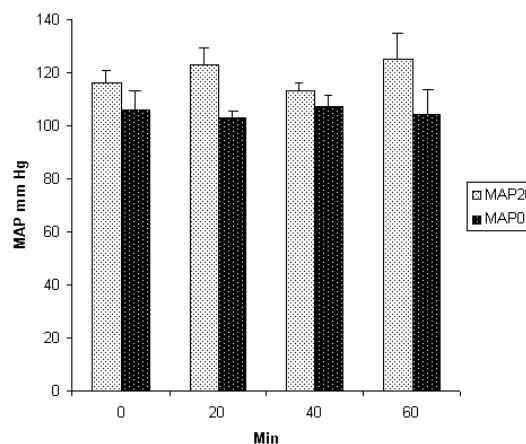


Fig. 1. The course of MAP in relation to IAP20. IAP: Intra-abdominal pressure, MAP: Mean arterial pressure, MAP0: MAP at baseline, MAP20: MAP with IAP 20 mm Hg

Ryc. 1. MAP w zależności od IAP20. IAP: ciśnienie wewnątrz jamy brzusznej; MAP: średnie ciśnienie tętnicze; MAP0: MAP początkowe; MAP20: MAP przy IAP 20 mm Hg

These animals received an average of 4 ml of LR solution per 100 g of body weight to maintain MAP during the study period.

Bacteria translocated primarily to the mesenteric lymph node in the rats with increased IAP (Tables 1, 2), whereas bacterial translocation did not occur in the control group. The most common bacteria were *Enterobacter*, *Enterococcus*, *Pseudomonas*, and *Staphylococcus*. Rats with increased IAP exhibited a significant amount of bacterial translocation towards the mesenteric lymph nodes, spleen, and liver compared with the controls ($p < 0.05$).

Malondialdehyde levels were measured in the small bowel and liver specimens of group 1 and group 2 rats. MDA levels, an indicator of free-radical production, were increased in the intestinal mucosa and liver of group 1 ($p < 0.05$). The mean malondialdehyde levels in the rats with increased IAP were 28.82 ± 1.12 and 37.84 ± 1.44 nmol/g of tissue in the liver and small bowel, respectively. The control group rats' mean malondialdehyde levels were 9.1 ± 2 and 13.2 ± 1.5 nmol/g of tissue in the liver and small bowel, respectively (Table 3). There was statistical significance when the two

Table 1. Incidence of bacterial translocation with increasing IAP ($p < 0.05$)

Tabela 1. Występowanie translokacji bakteryjnej wraz ze wzrostem IAP ($p < 0,05$)

Group (Grupa)	n	MLN	Spleen (Śledziona)	Liver (Wątroba)	Combined organs (Oba narządy)
1	10	4	1	1	6
2	10	0	0	0	0

Table 2. Number of viable bacteria with increasing IAP ($p < 0.05$). Results are expressed as mean CFU/g of tissue

Tabela 2. Liczba bakterii zwiększa się wraz ze wzrostem IAP ($p < 0,05$). Wyniki wyrażono w jednostkach tworzących kolonię na gram tkanki

Group (Grupa)	n	MLN	Spleen (Śledziona)	Liver (Wątroba)
1	10	30×10^3	19×10^3	33×10^2
2	10	0	0	0

Table 3. Malondialdehyde levels (mean $\pm$ SD) in the small bowel and liver of controls and rats with elevated IAP. Malondialdehyde level was measured as nmol/g of tissue, $*p < 0.05$

Tabela 3. Stężenie aldehydu dimalonowego (średnia $\pm$ SD) w jelicie cienkim i wątrobie w grupie kontrolnej i u szczurów ze zwiększonym IAP. Stężenie aldehydu dimalonowego wyrażono w nmol/g tkanki, $*p < 0,05$

Group (Grupa)	n	Small bowel (Jelito cienkie)	Liver (Wątroba)
1	10	$37.84 \pm 1.44^*$	$28.82 \pm 1.12^*$
2	10	13.2 ± 1.5	9.1 ± 2

groups' values were compared ($p < 0.05$ for both values).

Histological analysis of the rats with increased IAP mainly showed extensive epithelial separations from the lamina propria down the sides of the villi; ulceration at the villus tips in the rats with increased IAP and mucosal injuries were found to be of grades III and IV (Fig. 2A). The histopathology of the group 2 rats was normal and the mucosal injuries were mainly of grade I (Fig. 2B).

Discussion

There are many clinical situations, especially in managing patients with severe abdominal trauma, that can lead to increased intra-abdominal pressure. The physiological effects of IAP become problematic at > 20 to 25 mm Hg [19]. The cardiopulmonary effects of increased IAP are well described in animal models [20, 21] and were recently described in human studies of patients undergoing a laparoscopic procedure [22]. Increased intra-abdominal pressure decreases cardiac output; adverse effects are seen with intra-abdominal pressures as low as 10 to 15 mm Hg [1, 10, 11, 13]. Cardiac output (and stroke volume) is compromised through increased systemic vascular resistance, decreased venous return, and elevated intrathoracic pressure.

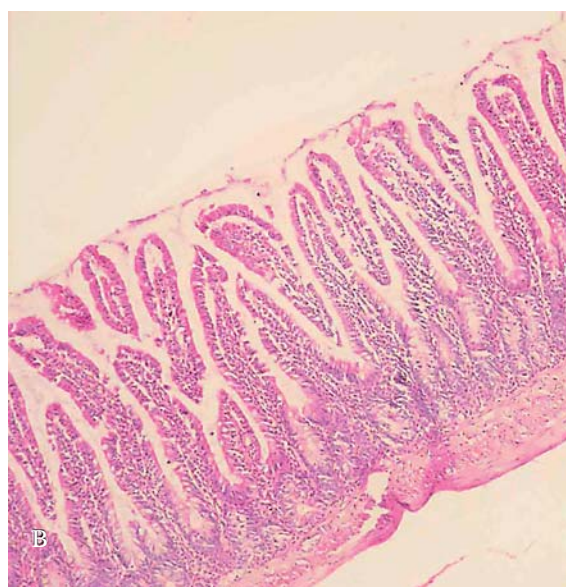
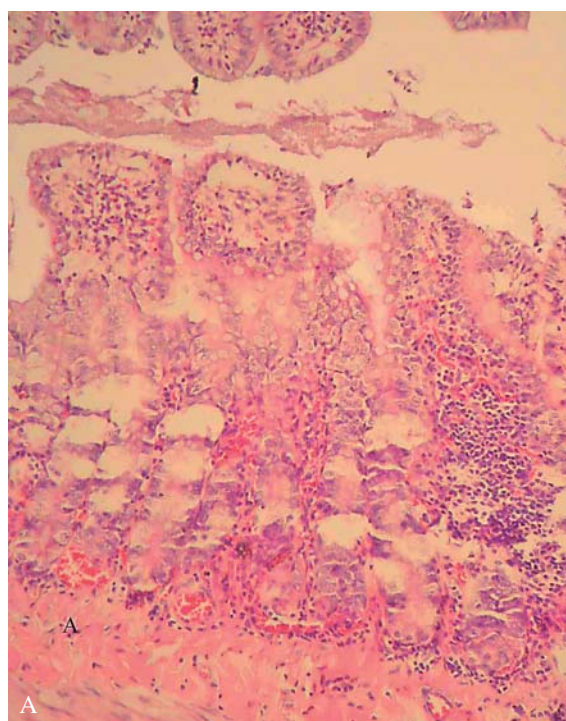


Fig. 2. (A) The group 1 rats, with increased intra-abdominal pressure, showed extensive epithelial separations from the lamina propria down the sides of the villi and ulceration at the villus tips (H&E, $\times 40$). (B) The histopathology of the group 2 rats was normal (H&E, $\times 40$)

Ryc. 2. (A) W grupie 1 u szczurów ze zwiększonym ciśnieniem w jamie brzusznej wykazano zwiększone oddzielanie komórek nabłonka od blaszki właściwej; owrzodzenie na szczytach kosmków (H&E, $40\times$). (B) Badanie histopatologiczne szczurów w grupie 2 nie wykazało nieprawidłowości (H&E, $40\times$)

An increase in IAP is associated with a reduction in visceral perfusion. Hepatic arterial, portal, and microvascular blood flow are affected [12, 14, 19]. The intestinal mucosa is highly sensitive to

various systemic and regional factors associated with ischemia, and the tips of the intestinal villi may be severely hypoxic even after short-duration microcirculatory disturbances [7, 23–25]. The results of this study in rats showed that 20-mm Hg intra-abdominal pressure for a 60-minute period leads to extensive epithelial separations from the lamina propria down the sides of the villi and ulceration at the villus tips in rats with increased IAP. These data suggest that during an increase in IAP, the splanchnic microcirculation is disturbed. Compartment syndrome is responsible for the ischemia of the visceral organs. Increased IAP reduces blood flow to all abdominal viscera except the adrenal glands [12, 26]. In animal experiments, when IAP is above 15 mm Hg, mesenteric arterial blood flow and intestinal mucosal flow decrease progressively despite preservation of a normal mean arterial pressure and cardiac output [14]. With increased IAP, oxygen delivery to visceral organs is compromised, causing intestinal ischemia, which may predispose to the production of oxygen free radicals and bacterial translocation [7, 16, 26, 27].

Oxygen-derived free radicals are known to be released during reperfusion after reversal of splanchnic ischemia and are important mediators of tissue injury [28–30]. Abdominal deflation at

the end of a procedure provides a model of reperfusion in previously ischemic organs. In this study, significant quantities of MDA were released from intestine and liver 24 hours after intra-abdominal pressure abolition. The quantity of reactive oxygen metabolites depends on the degree and duration of splanchnic ischemia. Biochemical and morphologic studies have established that the detrimental influence of oxygen free radicals occurs mainly during the period of reperfusion and reoxygenation, rather than during the ischemia [27, 31, 32].

Bacteria translocating from the gut after intestinal ischemia play a primary role in the development of septic complications [23–25]. Extensive studies in various experimental models have established that bacterial translocation takes place during burns, extended injuries, endotoxemia, hemorrhagic shock, and intestinal obstruction [33]. A significant factor facilitating translocation is disruption of the ecology of the indigenous gut flora resulting in intestinal bacterial overgrowth [34]. Independent of the mechanism of translocation, bacteria were found in the lymph nodes, liver, and spleen.

This experimental study has shown that increased IAP is responsible for gut ischemia, free-radical production, and bacterial translocation.

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