

Increased Intra-Abdominal Pressure Causes Bacterial Translocation in Rabbits

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Background: Abdominal compartment syndrome (ACS) is defined as intra-abdominal hypertension associated with organ dysfunction. Subsequently, increased intra-abdominal pressure (IAP) adversely affects the pulmonary, cardiovascular, renal, musculoskeletal/integumentary and central nervous systems. Bacterial translocation (BT), which is defined as the movement of viable enteric bacteria to the mesenteric lymph nodes, liver and spleen, occurs after various types of stress and results in splanchnic ischemia. In this experimental study, we aimed to investigate the effects of various levels of increased IAP on BT in rabbits; IAP was increased by the intra-abdominal balloon-insufflation method, thus simulating noncompliant abdominal-wall closure under tension.

Methods: Fifty rabbits were randomly assigned to 1 of 5 groups, with each group comprising 10 animals. In group I (control group), an intra-abdominal balloon was placed without inflation. In groups II, III, IV and V, IAPs of 10, 15, 20 and 25 mmHg, respectively, were established via inflation of the intra-abdominal balloon. All groups underwent laparotomy after 12 hours. Multiple biopsies were taken from ileocecal lymph nodes, the spleen and liver.

Results: BT was observed to some degree in all 4 experimental groups. A gradual increase in the phenomenon was noted as IAP increased from 10 to 15 mmHg; and BT was overt at 20 mmHg, and significant at 25 mmHg. *Klebsiella pneumoniae*, *Serratia marcescens*, and *Escherichia coli* were the predominant pathogens identified by culture.

Conclusion: We propose that an intravesical pressure (IVP) of 15 mmHg is the critical point for BT in patients with increased IAP. In this experimental study, BT occurred when IVP reached 20 mmHg. We suggest that IVP monitoring is desirable in the management of patients with ACS, and that decompressive laparotomy should be performed in patients with IVP > 20 mmHg. [*J Chin Med Assoc* 2005;68(4):172–177]

Key Words: bacterial translocation, compartment syndromes, intra-abdominal pressure

Introduction

Abdominal compartment syndrome (ACS) has been broadly defined as organ dysfunction attributed to increased intra-abdominal pressure (IAP). The effects of ACS on abdominal contents and the respiratory system were of major interest to early investigators.^{1–3} In 1984, the first case series, in which IAP measurement was used as a criterion for abdominal decompression, was reported by Kron et al,⁴ who were the first to use the phrase “abdominal compartment syndrome”. A correlation between increased IAP and decreases in

cardiac output, total lung capacity, and functional residual capacity was well defined in animal models.^{5,6} Also, with advances in laparoscopic procedures, many investigators attempted to demonstrate altered hemodynamics associated with increased IAP in humans.^{7,8}

Markedly increased IAP occurs widely after extensive abdominal trauma. Many factors contribute to this phenomenon, such as accumulation of blood and clot, bowel edema or congestion resulting from injury to mesenteric vessels, and perihepatic or retroperitoneal packing after damage-control laparot-

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omy. In these cases, closure of a noncompliant abdominal wall under tension may further lead to ACS.⁹ Using radioactive microspheres, Caldwell and Ricotta¹⁰ determined that elevated IAP led to reduced organ blood flow (OBF) to all intra-abdominal organs, except the adrenal glands, where OBF was increased.

Diebel et al^{11,12} showed that increased IAP could cause severe intestinal ischemia, and significant decreases in mesenteric artery blood flow, and hepatic artery and portal venous blood flow, although cardiac output and mean arterial blood pressure remained normal. The investigators also showed that a continuous IAP of 20–25 mmHg for 60 minutes caused deterioration of intestinal-barrier function, and bacterial translocation (BT) occurred from intestinal sites. In sepsis, intestinal bacteria and endotoxins resulting from BT arise and result in multiple organ failure. Thus, the early detection of increased IAP and its management before it reaches a critical level at which ACS occurs are particularly important.¹³

IAP can be measured by various methods. However, direct measurement using an intra-peritoneal catheter, as done during laparoscopy, and measurement of urinary bladder and/or gastric pressures, are the most common clinical applications. The estimation of IAP by bladder-pressure measurement is a noninvasive, easy, and highly reproducible method; the bladder volume that best approximates IAP has been confirmed as 50 mL.^{14,15}

In this experimental study, we tried to establish a model, using intra-abdominal balloon-insufflation, that simulated noncompliant abdominal-wall closure under tension after damage-control laparotomy, a procedure for temporary stabilization of critically injured patients. Our aim was to define the critical IAP level at which BT led to ACS, and to determine, by intravesical (IVP) pressure monitoring, the critical pressure level for decision-making about abdominal decompression.

Methods

Study animals

Fifty male, New Zealand rabbits weighing approximately 2500–3000 g were used in the study. The animals were randomly assigned to 1 of 5 groups (10 rabbits in each group). All animals were anesthetized by intramuscular injection of xylazine 10 mg/kg and ketamine hydrochloride (Ketalar®; Parke-Davis, Morris Plains, NJ, USA) 100 mg/kg. The incision side of the abdomen was shaved just before operation, cleaned with 10% povidone-iodine,

and covered with a sterile drape. The bladder was emptied via an 8-fr Nelaton urinary catheter attached to a pressure-measuring manometer, and the top of the pubic symphysis was used as the zero point. An infusion line was placed in the right external jugular vein. Electrocardiogram and noninvasive blood pressure monitors were used to monitor animals during surgery, and baseline mean arterial pressure was obtained by instilling crystalloid solution as necessary. Group I animals (control group) underwent laparotomy, without any IAP change, whereas IAPs of 10, 15, 20 and 25 mmHg were applied to animals in groups II, III, IV and V, respectively.

The study protocol was approved by the appropriate institutional committee, and adhered to good-practice guidelines for the use of experimental animals.

ACS model

ACS was established in a manner similar to that in the model of Engum et al,¹⁶ but with some modifications. Laparotomy was performed via a midline incision under sterile conditions. A 30-cm Penrose drain (Rüsch Inc, Duluth, GA, USA), with its lower tip tied, was placed in the abdominal cavity through the incision. The open tip of the drain was attached to a pressure manometer, and the abdominal cavity was inflated until the target pressure level was attained. Thereafter, the open tip was tied, and the inflated drain was left in the abdominal cavity by suturing the incision. IAP was monitored by direct measurement of bladder pressure, and measurements were repeated every 4 hours.

All rabbits were reanimated, fed with standard rabbit nourishment, and allowed access to tap water *ad libitum*. Twelve hours later, laparotomy was performed under anesthesia, using sterile instruments, and samples of the peritoneal cavity were obtained for aerobic and anaerobic culture to detect any accidental bacterial contamination. Spleen and liver biopsies were performed, and several mesenteric lymph nodes were harvested for quantitative culture. Biopsy materials were separately wrapped in aluminum foil, under sterile conditions, and transferred to our laboratory. The samples were weighed (0.5–1.0 g) and homogenized in sterile trypticase soy broth for 15 minutes at room temperature. After homogenization, 1:10 serial dilutions were obtained, and 0.1-mL portions of each homogenate were plated on 5% defibrinated sheep blood and eosin methylene-blue agar culture plates. All cultures were incubated at 37°C under aerobic conditions and examined for bacterial growth after 24–72 hours. Colonies of 30–300 were counted, and results were multiplied by dilution ratio and divided by tissue weight. The results were recorded

as colony-forming units per gram of tissue (CFUs/g). Conventional microbiologic methods were used for bacterial identification, and, if needed, API® strips (bioMérieux sa, Marcy l'Etoile, France) were used for confirmation.

Statistical analyses

All data were expressed as mean ± standard deviation. Mean values for CFUs and number of bacterial growths for each group were compared using the Kruskal-Wallis test, and subgroups were compared using the Mann-Whitney U-test, while *p* values were subjected to Bonferoni correction, and *p* values less than 0.05 were considered significant. The compatibility of groups to a normal distribution was evaluated with a 1-sided Kolmogorov-Smirnow test, and appropriate non-parametric tests were selected.

Results

Bacterial cultures

All animals that died during the experiments were replaced, and procedures were reapplied to new animals so that the groups were complete. Bacterial growth was not observed in cultures from rabbits in group I (control group). However, the evaluation of BT in groups subjected to various levels of IAP (groups II–V) showed a linear correlation between BT and IAP. We observed bacterial growth in cultures from 2 organ samples in group II, 4 in group III, 22 in group IV and 24 in group V, thus giving a total of 52 cultures from 150 samples.

Organ involvement

Figure 1 depicts the organs affected by bacterial infection (CFUs per gram of tissue) after BT. Clearly, BT started in animals subjected to IAP levels of 10 and 15 mmHg, but differences were not statistically significant relative to controls ($p > 0.05$). Group IV animals (IAP 20 mmHg) exhibited a significant amount of BT towards the mesenteric lymph nodes, spleen and liver relative to controls ($p = 0.02$), but this difference was more prominent in group V animals (IAP 25 mmHg) relative to controls ($p = 0.001$).

Principal pathogens

Klebsiella pneumoniae, *Serratia marcescens*, and *Escherichia coli* were the principal pathogens identified by culture. Positive cultures with Gram-negative bacteria were more frequently observed than those with Gram-positive bacteria, and this difference was more prominent when IAP increased to 25 mmHg (Figure 2).

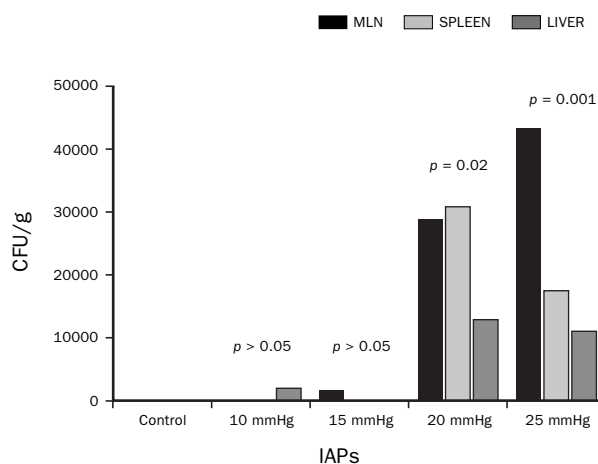


Figure 1. Number of bacterial colony-forming units (CFUs) per gram of tissue in the mesenteric lymph nodes (MLN), spleen, and liver of rabbits subjected to various levels of intra-abdominal pressure (IAP). Bacterial translocation was statistically evident at IAP values of 20 and 25 mmHg ($p = 0.014$ for group IV vs controls; $p = 0.006$ for group V vs controls).

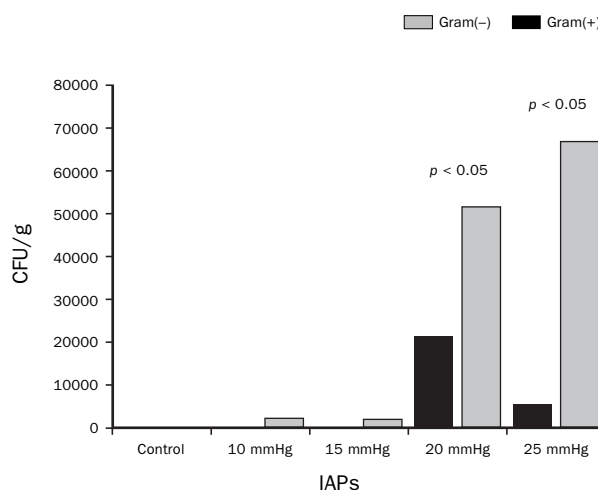


Figure 2. Culture results for Gram-positive and Gram-negative bacteria after bacterial translocation at various levels of intra-abdominal pressure (IAP). The total number of Gram-negative bacteria was significantly ($p < 0.05$) greater in groups IV (IAP 20 mmHg) and V (IAP 25 mmHg) than in the control group.

Discussion

ACS was recognized well over a century ago, yet the pathophysiologic implications of IAP were only discovered in the last 20 years. Initially, ACS was considered to manifest only in trauma patients, but we now recognize that it may have significant reverberations throughout the surgical specialties, and that it may also occur in medical patients.

Three types of ACS have been defined. Primary ACS is essentially associated with organ dysfunction and elevated IAP in the presence of direct injury to the abdominal contents. It is a common complication of damage-control laparotomy, and may also occur in patients for whom non-operative management of abdominal-organ injuries fails because of ongoing bleeding. Secondary ACS typically occurs in patients with severe shock requiring massive resuscitation (e.g. burns patients). Recurrent ACS, which has a very high mortality rate, may occur in patients who have recovered from either primary or secondary ACS.^{2,17}

Patients with massive abdominal injuries managed by damage-control surgery (DCS) are at high risk of developing intra-abdominal hypertension (IAH) because of resuscitation with massive crystalloids, blood products, and intra-abdominal packing. Various studies have shown that ACS occurs in high proportions of such patients (6–36%). This has led surgeons to adopt a policy of urgent re-exploration of the abdomen to avoid adverse effects of IAH, such as diminished hepatic and gut perfusion, increased acidosis, and coagulopathy.^{9,18–20}

BT can be defined as the passage of viable indigenous bacteria and their endotoxins through the intestinal epithelium and lamina propria to sterile body sites, such as mesenteric lymph nodes (MLNs), the spleen, liver, and bloodstream, during damage of the intestinal mucosal barrier. Impaired intestinal perfusion at the mucosal and submucosal levels causes reductions in tissue oxygen tension, anaerobic cell metabolism, acidosis, and free-radical generation. The potential mechanisms involved in splanchnic hypoperfusion, and the deterioration of gut mucosal stability, include inadequate tissue and cellular oxygen delivery, the presence of oxygen-derived, toxic free radicals generated by activation of the xanthine-oxidase system after reoxygenation, and the production of inflammatory mediators. The abovementioned factors that cause enteric mucosal damage play the principal pathogenetic role in BT.^{21,22}

Diebel et al¹³ demonstrated that a continuous IAP of 20–25 mmHg for 60 minutes led to deterioration in intestinal-barrier function and BT from the intestinal site. Further, Ivatury et al^{9,23} suggested early decompressive laparotomy at the critical levels of IVP < 20 mmHg and intramucosal pH < 7.3. However, this proposal has not been widely accepted, although it has led to investigation of the pathophysiologic changes occurring at an IAP of less than 20 mmHg.

Recent animal studies evaluating the systemic inflammatory response and remote organ dysfunction in laboratory models of hemorrhagic shock and ACS

suggest that IAH can provoke the systemic release of pro-inflammatory cytokines, which may serve as a second insult for the induction of post-injury multiple organ failure (MOF). Indeed, results showed that an IAP of 20 mmHg for 90 minutes induced a significant, systemic increase in plasma levels of interleukin (IL)-1 and IL-6, and reperfusion after ACS promoted the secondary release of tumor necrosis factor- α . Results from myeloperoxidase assay and histopathologic analysis also demonstrated that ACS with decompression induced a greater degree of lung polymorphonuclear neutrophil (PMN) accumulation; these findings corroborated the potential for ACS to promote remote organ damage.^{24,25}

The effect of various IAP values on BT in a carbon-dioxide pneumoperitoneum model was investigated by Polat et al,²⁶ who suggested that BT started when IAP reached 14 mmHg, but became more prominent when IAP reached 20–25 mmHg. Although our experimental model was different, our results corroborated this starting IAP level for BT.

More recently, a clinically relevant, small-animal model, with laparotomy/hemorrhagic shock as the initial insult, and ACS as a timed secondary insult, was used to search for evidence of remote organ dysfunction.²⁷ The introduction of ACS 8 hours after shock, which was suggested as a maximal priming period for circulating PMNs, provoked acute-lung and liver injury, and resulted in 33% mortality; PMNs appeared to be a central effector associated with ACS-provoked MOF.²⁷

In our experimental study, we tried to simulate noncompliant abdominal-wall closure under tension, a potentially life-saving procedure in severely injured patients, after damage-control surgery. The model was designed to try to simulate laparotomy, followed by handling of the intestinal segments and establishment of a constant IAP, by leaving an inflated balloon (with different pressures) in position before laparotomy closure. By monitoring IVP in the animals, we tried to determine the following: the critical levels of IAP that lead to BT in cases of ACS; and the decision-making point at which decompressive laparotomy should be performed. Models using hypertonic saline or carbon-dioxide pneumoperitoneum to establish increased IAP were not preferred in our study because we assumed that peritoneal absorption might result in unsteady IAP levels over the lengthy period of 12 hours.

Analyzing the results, there was bacterial growth only in some liver-biopsy cultures at 10 mmHg IAP, and in some MLN-biopsy cultures at 15 mmHg IAP; however, these results were not statistically

significant. At an IAP level of 20 mmHg, significant bacterial growth was noted in cultures from the MLNs ($p = 0.034$ vs controls), spleen ($p = 0.014$) and liver ($p = 0.014$); this was also true at an IAP level of 25 mmHg (respective p values were again 0.034, 0.014 and 0.014). Thus, we can postulate that BT starts at an IAP level of 10 mmHg, and increases at 15 mmHg; BT reaches statistical significance at an IAP level of 20 mmHg, and attains its maximum value at 25 mmHg. These results might suggest that, even when clinical deterioration is not obvious, decompressive laparotomy should be applied in patients with IVP of 20 mmHg, since the damaging effects of ACS are already likely to have occurred.

In our study, bacterial growth was evident in 52 cultures from a total of 150 organ specimens. For each animal, various bacteria were grown in the different biopsy materials. *K. pneumoniae*, *S. marcescens* and *E. coli* were the principal pathogens identified, although other pathogens included *Klebsiella rhinoscleromatis*, *Streptococcus viridans*, Group D non-hemolytic streptococci (non-enterococcal), *Klebsiella oxytoca*, *Klebsiella ozanae*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*.

In this experimental study, we tried to simulate a low-tension laparotomy closure, with massive intestinal edema, or a salvaged abdomen by the damage-control approach, in rabbits. We also tried to address the critical levels of IAP for the timely recognition of ACS and for the prompt initiation of abdominal decompression. The study findings reveal that BT started as IAP increased from 10 to 15 mmHg; BT became significantly evident at an IAP level of 20–25 mmHg. When bladder pressure exceeds 20 mmHg, decompressive surgery should be considered imperative.

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