PAPER

Hemorrhage Exacerbates Bacterial Translocation at Low Levels of Intra-abdominal Pressure

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Background: It has been shown previously that the adverse cardiopulmonary sequelae of increased intra-abdominal pressure (IAP) are worsened by hemorrhage and resuscitation. Bacterial translocation (BT) to the mesenteric lymph nodes (MLNs), liver, and spleen has also been shown to occur with increased IAP.

Objective: To investigate the hypothesis that BT associated with elevated IAP would be significantly increased after hemorrhage and resuscitation.

Materials and Methods: Anesthetized adult male rats had femoral artery and vein catheters placed, and an intra-abdominal catheter placed to measure IAP. Group 1 underwent surgery only and served as controls. Group 2 had IAP raised to 10 mm Hg by infused lactated Ringer's solution for 40 minutes. Group 3 had a 25% hemorrhage, followed by resuscitation by infused lactated Ringer's solution and shed blood. Group 4 first had a 25% hemorrhage, resuscitated using infused lactated Ringer's solution and shed blood, and then had IAP raised to 10 mm Hg by infused lactated Ringer's solution for 40 minutes. All groups were killed after 2 hours, and had MLNs, liver, and spleen harvested for quantitative cultures.

Results: Hemorrhage and resuscitation alone did not increase BT to the MLNs, liver, or spleen. An increase in IAP to 10 mm Hg resulted in a significant level of BT to the MLNs and liver on MacConkey II agar ($P<.05$), and a significant increase in the level of BT only to the liver on trypticase soy agar with 5% sheep's blood ($P<.05$). Hemorrhage and resuscitation did increase the level of BT to the liver and spleen when IAP was increased to 10 mm Hg ($P<.05$).

Conclusions: In this model, hemorrhage and resuscitation alone did not increase BT to the MLNs, liver, or spleen. However, hemorrhage and resuscitation increased BT to the liver and spleen when IAP was increased to 10 mm Hg. This supports the concept that prior hemorrhage and resuscitation exacerbates the effects of increased IAP.

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Sepsis is the most common cause of death 72 hours after trauma.[1] It has been reported that 30% of patients with sepsis and multiple organ system failure have no septic focus identified.[2] Bacteria normally found within the gastrointestinal tract, however, are the most common organisms infecting these patients.[3]

Ravin and Fine[4] implicated the gastrointestinal tract as the source for bacteria and/or endotoxins that might contribute to systemic infection, and this marked the advent of the concept of bacterial translocation (BT). They demonstrated that radioactively labeled *Escherichia coli* fed to hemorrhaged rabbits were found in considerable abundance in the liver and spleen when compared with unshocked rabbits.[5] Twenty-five years later, Baker et al[6] used a rodent model to demonstrate BT to the mesenteric lymph nodes (MLNs), liver, and spleen following hemorrhage. In addition, histological preparations of ileum and cecum were made to demonstrate bacteria penetrating the damaged intestinal mucosa following hemorrhage.

In 1911, Emerson[7] was one of the first investigators in the United States to conduct experiments observing the respiratory variations of intra-abdominal pressure (IAP). Bradley and Bradley[8] studied the renal effects of increased IAP on human volunteers, and reported the

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MATERIALS AND METHODS

SURGICAL PROCEDURES

The experimental protocol was approved by the Animal Research Committee of the Albert Einstein College of Medicine, Bayside, NY. All animals were maintained in accord with the recommendations of the National Institutes of Health guidelines for the care and use of laboratory animals. Twenty-seven male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) (approximately 375-450 g) were fed ad libitum prior to surgery and were anesthetized with intraperitoneal injection of combined ketamine hydrochloride (40 mg/kg) and pentobarbital sodium (20 mg/kg). Surgical anesthesia was confirmed by continuous monitoring of heart rate, mean arterial pressure, and corneal and toe-pinch reflexes. All animals were given supplemental oxygen (3 L by nasal cannula) throughout the experiment.

The hind limbs and abdomen were shaved and scrubbed for sterile preparation. A right femoral artery cannula was placed for continuous monitoring of the heart rate and mean arterial pressure. A right femoral vein cannula was placed for hemorrhage and resuscitation. An intra-abdominal catheter was placed via a minilapartomy and was closed with a purse-string suture ensuring a watertight seal for the instillation of lactated Ringer's solution.

Following the administration of heparin sodium (2000 U/kg), all animals were given a 20-minute recovery period under anesthesia. An animal warmer and warmed intravenous fluids were employed to maintain body temperature between 35°C and 37.5°C that was measured with a rectal thermometer.

The animals were then divided into 4 groups: group 1 (n=6), controls; group 2 (n=7), IAP increase of 10 mm Hg; group 3 (n=7), 25% hemorrhage and resuscitation; and group 4, 25% hemorrhage and resuscitation followed by an IAP increase of 10 mm Hg. Group 1 animals were killed 2 hours after undergoing the aforementioned surgical procedure and served as controls. Group 2 animals had IAP increased to 10 mm Hg with the instillation of warmed lactated Ringer's solution for 40 minutes. Group 3 animals were subjected to a moderate hemorrhage. From the femoral vein cannula, 25% of the estimated blood volume was removed during a 30-minute period. The blood was heparinized and saved for reinfusion. After the hemorrhage period, the animals were given a 30-minute equilibration period prior to resuscitation. Twice the volume of blood removed was given as lactated Ringer's solution during 15 minutes followed by the shed blood during another 15 minutes. Group 4 animals were subjected to the same hemorrhage protocol as group 3 animals. Following the hemorrhage and resuscitation period, the animals were given a 30-minute equilibration period. They were then subjected to the same IAP protocol as group 2. The animals were killed 2 hours after the completion of each protocol.

MICROBIOLOGICAL ANALYSIS

Samples of MLNs, liver, and spleen, weighing 0.25 to 0.5 g were harvested and homogenized in sterile trypticase soy broth for 10 minutes at room temperature. Serial dilutions were then plated onto MacConkey II agar (Difco Laboratories, Detroit, Mich) and trypticase soy agar with 5% sheep's blood (TSA II, BBL Microbiology Systems, Cockeysville, Md). The plates were examined and the colonies counted 24 and 48 hours after incubation at 37°C.

STATISTICAL ANALYSIS

Statistical calculations were made using a commercially available computer software (Statview 4, Abacus Concepts Inc, Berkeley, Calif) and a personal computer (Performa 6116CD model, MacIntosh, Apple Computers, Cupertino, Calif). All data were recorded as colony-forming units per gram $\times 10^3$, and reported as mean ± SEM. Differences in the means of the 4 groups were determined using 1-way analysis of variance (ANOVA) and the Fisher protected least significant difference test. Statistical significance was defined as $P<.05$.

decline in renal plasma flow and glomerular filtration rate that occurs with an IAP of 20 mm Hg. Richardson and Trinkle[9] showed the adverse cardiopulmonary effects of increased IAP in dogs.

The relationship between increased IAP and visceral blood flow was initially studied by Caldwell and Ricotta.[10] In their experiments, radioactive microspheres were injected into the left atrium of dogs after raising IAP to 20 and 40 mm Hg. A reduction in microsphere uptake in almost all intra-abdominal organs, except for the adrenal gland, occurred which correlated with a 25% and 50% reduction in visceral blood flow at an IAP of 20 and 40 mm Hg, respectively. Diebel et al[11] used a laser probe to report a 40% reduction in intestinal mucosal blood flow (MBF) at an IAP of 20 mm Hg. Eleftheriadis et al[12] and Diebel et al[13] also demonstrated a significant reduction in gastrointestinal MBF with increased IAP that correlated with a significant increase in the level of BT to the MLNs, liver, and spleen.

Several investigators have shown the adverse effects of hemorrhage on increased IAP.[14-16] Simon et al[17] were the first to investigate how hemorrhage and resuscitation affected the sequelae of elevated IAP. It was shown that the adverse cardiopulmonary sequelae of increased IAP were worsened by prior hemorrhage and resuscitation. In addition, Friedlander et al[18] observed that the reduction in intestinal MBF associated with elevated IAP was also worsened by prior hemorrhage and resuscitation. These experimental conditions more closely mimicked the acute trauma scenario, and supported the prompt and aggressive management of those trauma patients with increased IAP.

The association of BT and trauma continues to be controversial.[19-22] Clinical data on MLN positivity in trauma patients are inconsistent with that obtained from trauma scenarios created in the laboratory. To our knowledge, no clinical study to date, however, has measured BT in trauma patients developing increased IAP after hemorrhage and resuscitation. In addition, no-

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Agar Colony Counts*

Group	MLN	Liver	Spleen
		MacConkey II Agar	
1	0	0.9 ± 0.3	4.3 ± 3.4
2	0.8 ± 0.3	3.8 ± 0.7	14.0 ± 1.8
3	0	3.0 ± 1.7	5.5 ± 3.3
4	0.8 ± 0.3	10.6 ± 2.2	28.0 ± 2.1
		Trypticase Soy Agar With 5% Sheep's Blood	
1	0	0.8 ± 0.3	4.4 ± 3.4
2	0.7 ± 0.4	7.4 ± 2.2	19.3 ± 3.8
3	0.2 ± 0.1	5.9 ± 3.1	9.9 ± 3.6
4	0.5 ± 0.3	15.9 ± 3.1	32.7 ± 3.6

*Values expressed as mean ± SEM (colony-forming units per gram × 10^3). Braces indicate statistically significant intergroup differences at P<.05, 1-way analysis of variance and Fisher protected least significant difference test. Rodent groups were categorized as follows: group 1 (n = 6), controls; group 2 (n = 7), intra-abdominal pressure increase of 10 mm Hg; group 3 (n = 7), 25% hemorrhage and resuscitation; and group 4 (n = 7), 25% hemorrhage and resuscitation followed by an intra-abdominal pressure increase of 10 mm Hg. For more details see "Surgical Procedures" subsection of "Materials and Methods" section. MLN indicates mesenteric lymph nodes.

laboratory investigation has measured the effects of hemorrhage and resuscitation on the degree of BT associated with increased IAP. We therefore used a rodent model to test the hypothesis that the level of BT associated with elevated IAP would be significantly increased after hemorrhage and resuscitation.

RESULTS

GROUP 2: IAP OF 10 mm Hg

When the IAP was raised to 10 mm Hg, a significant level of BT to the MLNs and liver was observed in group 2 animals when compared with controls on MacConkey II agar ($P<.05$; **Table**). The Table compares the levels of BT in the same groups of animals on trypticase soy agar with 5% sheep's blood ($P<.05$).

GROUP 3: 25% HEMORRHAGE

The Table gives the levels of BT to the MLNs, liver, and spleen in group 3 animals on MacConkey II agar and trypticase soy agar with 5% sheep's blood, respectively. The level of BT after a 25% hemorrhage and resuscitation, although increased, was not significant when compared with controls ($P>.05$).

GROUP 4: 25% HEMORRHAGE 25% AND IAP OF 10 mm Hg

Group 4 animals had an approximately 3-fold and 2-fold increase in the level of BT to the liver and spleen when compared with group 2 animals, respectively ($P<.05$; Table and **Figure 1**). On trypticase soy agar with 5% sheep's blood, there was an approximately 2-fold increase in the levels of BT to the liver and spleen in the same groups of animals ($P<.05$; Table and **Figure 2**).

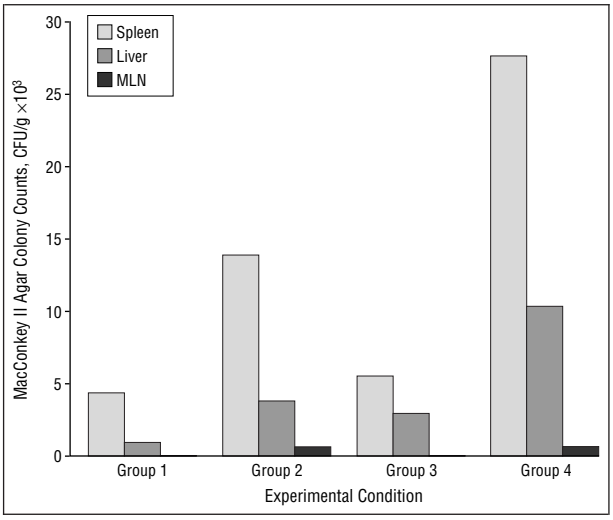

Figure 1. *MacConkey II agar colony counts. MLN indicates mesenteric lymph nodes. Rodent groups were categorized as follows: group 1 (n=6), controls; group 2 (n=7), intra-abdominal pressure increase of 10 mm Hg; group 3 (n=7), 25% hemorrhage and resuscitation; and group 4 (n=7), 25% hemorrhage and resuscitation followed by an intra-abdominal increase of 10 mm Hg. For further detailed description of the groups see "Surgical Procedures" subsection of "Materials and Methods" section.*

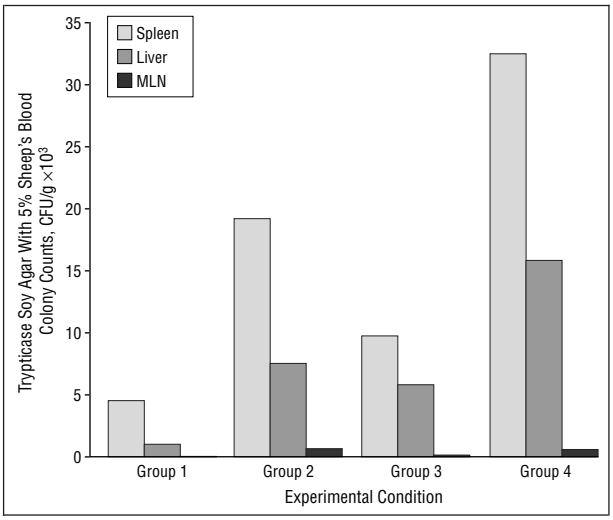

Figure 2. *Trypticase soy agar with 5% sheep's blood (TSA II, BBL Microbiology Systems, Cockeysville, Md) and colony counts. MLN indicates mesenteric lymph nodes. Rodent groups were categorized as follows: group 1 (n=6), controls; group 2 (n=7), intra-abdominal pressure increase of 10 mm Hg; group 3 (n=7), 25% hemorrhage and resuscitation; and group 4 (n=7), 25% hemorrhage and resuscitation followed by an intra-abdominal increase of 10 mm Hg. For further detailed description of the groups see "Surgical Procedures" subsection of "Materials and Methods" section.*

COMMENT

Data reported herein again confirm our hypothesis that prior hemorrhage and resuscitation exacerbates the effects of increased IAP. Simon et al[17] evaluated the effects of a severe hemorrhage model on increased IAP for cardiopulmonary sequelae. It was found that bleeding these animals to 40% of their blood volume, and resuscitating them with lactated Ringer's solution and their own shed blood, worsened the effects of increased IAP on pulmonary function. At an IAP of 20 mm Hg prior hemorrhage significantly increased the $PaCO_2$,

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Clinical Relevance Statement

Sepsis and multiple organ system failure are the leading causes of death in trauma patients after hemorrhagic shock. In addition, bowel edema and intra-abdominal ascites from hemorrhage and resuscitation contribute to increased IAP that may progress to the abdominal compartment syndrome. It has been observed clinically that prior hemorrhage and resuscitation worsens the sequelae of increased IAP that includes the development of the acute respiratory distress syndrome, acute renal failure, sepsis, and multiple organ system failure. The proposed mechanism is BT from the gastrointestinal tract to lymphatics and/or major reticuloendothelial organs, such as the spleen or liver, and the blood. These data presented herein show that prior hemorrhage and resuscitation worsens BT associated with increased IAP. The exacerbation of BT results from the loss of gastrointestinal mucosal integrity secondary to a decrease in MBF and ischemia/reperfusion injury. Perhaps pharmacological agents that specifically vasodilate the gastrointestinal microvasculature and reduce ischemia/reperfusion injury may supplement the aggressive resuscitation of trauma patients who develop increased IAP after hemorrhage and resuscitation, and thereby reduce the incidence of sepsis and multiple organ system failure.

decreased the tidal volume, and decreased the partial pressure of arterial oxygen–fraction of inspired oxygen ratio. Furthermore, an approximately 60% and 75% reduction in intestinal MBF was found in those animals hemorrhaged and resuscitated prior to increasing IAP to 30 and 40 mm Hg, respectively.[18]

Two previous studies report that increased IAP results in BT to the MLNs, liver, and spleen. Diebel et al[13] subjected rats to an IAP between 20 and 25 mm Hg for 60 minutes, and also measured ileal MBF while maintaining mean arterial pressure.[13] They found a significant increase in the level of BT to the MLNs, liver, and spleen in those rats subjected to the increased IAP which correlated with a significant reduction in MBF. Eleftheriadis et al[12] also found a significant level of BT in those rats subjected to an IAP of 15 mm Hg for 60 minutes, and this also correlated with a significant decline in jejunal microcirculation.

In this model, hemorrhage and resuscitation alone did not increase BT. This finding was inconsistent from the hemorrhage model of Baker et al[6] that found an increase in the level of BT. In this study, rats were hemorrhaged to a mean arterial pressure of 30 mm Hg for 30, 60, and 90 minutes, and were resuscitated with their own shed blood. A significant level of BT to the MLNs occurred in all the rats when compared with controls. Those rats subjected to 90 minutes of hemorrhagic shock also had significant levels of BT to the liver and spleen.

The most plausible explanation for the difference in our results resides in the degree and technique of hemorrhage. Our hemorrhage protocol fixed the volume of hemorrhaged blood to 25% and did not subject the rats to a prescribed period of hypotension. Furthermore, our model included a more traditional resuscitation with crystalloid and shed blood rather than shed blood alone.

An IAP of 10 mm Hg for 40 minutes increased BT to the MLNs and liver on MacConkey II agar, but the liver only on trypticase soy agar with 5% sheep's blood. An explanation for this disparity is that greater numbers of gram-negative bacteria are found within the gastrointestinal tract, and therefore have a greater chance of translocation. In this experiment, MacConkey II agar reflects translocation of many gram-negative bacteria such as *E coli*, *Klebsiella* species, and *Enterobacter* species. As a result, MacConkey II agar probably recovered more of the translocating bacteria to the MLNs than did trypticase soy agar with 5% sheep's blood.

Hemorrhage and resuscitation significantly augmented the level of BT to the liver and spleen when the IAP was raised to 10 mm Hg. The magnitude of bacteria translocating to the liver and spleen on trypticase soy agar with 5% sheep's blood was approximately 1.5 times that on MacConkey II agar. An explanation for this disparity resides in previous experiments done by Wells et al.[23] Their findings support a hypothesis that organisms residing within macrophages, such as *Salmonella* species and *Listeria monocytogenes* translocate more easily. In addition, aerobic and facultative bacteria translocate more easily when compared with anaerobic bacteria. In this experiment, trypticase soy agar with 5% sheep's blood reflects translocation of both gram-positive and *E coli*, one of the most abundant, aerobic facultative organisms in the gastrointestinal tract. As a result, this might explain why the magnitude of BT on trypticase soy agar with 5% sheep's blood was greater than on MacConkey II agar.

Hemorrhage and resuscitation did not significantly increase the level of BT to the MLNs when the IAP was raised to 10 mm Hg. This finding was consistent on both MacConkey II agar and trypticase soy agar with 5% sheep's blood. It might be postulated that when killed, bacteria already translocated to other systemic organs, such as the liver and spleen from the MLNs.

The clinical literature supports laboratory data regarding the principle of BT in those patients with intrinsic gastrointestinal pathologic conditions.[22] Evidence for BT associated with trauma in the clinical literature, however, argues against that found in the laboratory and continues to be an area of debate.[18-21] Several investigators[20-22] have found no association between trauma patients and MLN culture positivity. In one study done by Moore et al,[20] only 2% of 412 portal vein blood cultures obtained from 20 severely injured patients were positive for organisms. In another study by Pietzman et al,[22] all MLN cultures obtained from 15 trauma patients undergoing reexploration 2 to 5 days after admission to the hospital were negative, whereas all the MLN cultures obtained from 3 patients with intrinsic gastrointestinal pathologic conditions were positive (ie, bleeding, ulcerative colitis, and sigmoid volvulus). Only one of the trauma patients became bacteremic with *Enterobacter cloacae* without a source being identified.

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Other clinical investigators have employed more sophisticated techniques to measure BT, but still have been unsuccessful in corroborating the laboratory data. Brathwaite et al[21] used immunofluorescence techniques for mouse monoclonal antibody to *E coli* β-galactosidase, and then goat antimouse IgG to detect BT in 18 blunt trauma patients. They found only 3 portal venous blood cultures positive for organisms and 1 positive MLN. *Escherichia coli* β-galactosidase, however, was found within the cytoplasm of macrophages in all MLNs. Only one patient died of multiple organ system failure.

Splanchnic hypoperfusion has been implicated as a mediator for BT.[6,11] The purpose of our study, however, was not necessarily to determine a mechanism for the significant levels of BT occurring with increased IAP after hemorrhage and resuscitation since previous work done by Friedlander et al[18] measured intestinal MBF in pigs subjected to a similar protocol. As previously mentioned, the splanchnic hypoperfusion associated with increased IAP was worsened by hemorrhage and resuscitation at an IAP of 30 and 40 mm Hg. This may be a plausible explanation for the increase in BT occurring in our model.

Whether BT contributes to posttraumatic septic complications and multiple organ system failure will continue to be controversial. It is evident from this study, however, that prior hemorrhage and resuscitation increases the degree of BT associated with increased IAP.

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