Free Radical Research, May 2009; 43(5): 470–477

informa
healthcare

The role of oxygen-free radical in the apoptosis of enterocytes and bacterial translocation in abdominal compartment syndrome

GUANWEN GONG, PENGFEI WANG, WEIWEI DING, YUNZHAO ZHAO, & JIESHOU LI

Department of Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu Province, PR China

(Received 30 December 2008; revised 4 February 2009)

Abstract

Background: The purpose of this study was to study the impact of intra-abdominal hypertension (IAH) on the intestine.

Materials and methods: One hundred and twenty Sprague-Daley rats were divided into four groups. In the ACS group, the intra-abdominal pressure (IAP) was increased to 20 mmHg. In the ACS/DE group, increased IAP was followed by decompression. In the control1 and control2 groups, the IAP remained unchanged. Malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH) and glutathione peroxidase (GSH-Px) enzymes of the intestine were measured. Additionally, ileal tissues were obtained for histopathological examinations and apoptosis detection. Liver, spleen and mesenteric lymph nodes were obtained for microbiological analysis.

Results: In the presence of IAH, MDA and MPO were increased, while GSH and GSH-Px were decreased. Microbiological analysis suggested bacterial translocation across the gut. Morphological examinations indicated that the Chiu's score and apoptotic index in the ACS/DE group were the highest in the four groups.

Conclusions: Oxidative stress plays an important role in the intestinal damage and bacterial translocation in abdominal compartment syndrome. Additionally, the influence of oxygen free radicals occurs mainly during the period of reperfusion rather than during the IAH period.

Keywords: *Abdominal compartment syndrome, intra-abdominal hypertension, intra-abdominal pressure*

Introduction

The detrimental effect of intra-abdominal hypertension (IAH) was first reported about the association between intra-abdominal hypertension and oliguria in the late 19th century. However, it was not until the 1980s that clinicians woke up to the significance of intra-abdominal hypertension. Intra-abdominal hypertension is a potentially lethal syndrome that is seen most commonly in surgical patients who have suffered some form of abdominal catastrophe such as necrotizing pancreatitis, intra-peritoneal or retro-peritoneal haemorrhage, grossly oedematous bowel secondary to massive fluid resuscitation, ileus and intra-abdominal sepsis [1–4]. It occurs in up to 41% of surgical patients [5]. Without intervention, it can develop to abdominal compartment syndrome (ACS), a life-threatening condition that leads to renal, pulmonary, and circulatory failure [6–8]. The term 'abdominal compartment syndrome' was first used by Kron et al. [9] to describe the deleterious effects of intra-abdominal hypertension after abdominal aortic aneurysm surgery in 1984. In 2004, the consensus conference of the World Society on Abdominal Compartment Syndrome (WSACS) defined intra-abdominal hypertension as an intra-abdominal pressure (IAP) $\geq$ 12 mmHg and abdominal compartment syndrome as a condition with an IAP $\geq$ 20 mmHg in combination with previously not present organ system failure [10].

Since increased intra-abdominal pressure reduces blood flow to intra-abdominal organs and the decompression results in instantaneous restitution of the feeding blood supply, prolonged IAH followed by decompression has to be considered as a classical

Correspondence: Jieshou Li, MD, Research Institute of General Surgery, Jinling Hospital, No. 305 East Zhongshan Road, Nanjing, Jiangsu Province 210002, PR China. Tel: +86-25-80860137. Fax: +86-25-480396. Email: lijieshou@yahoo.cn

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760902846157

ischemia-reperfusion (I/R) insult. Kacmaz et al. [11] studied IAH for 1 h followed by decompression for another 1 h. However, the vast majority of the animal experiments concentrated only on the increased abdominal pressure without the succedent reperfusion injury by abdominal decompression.

It is widely accepted that reperfusion of the ischemic tissue may promote the generation of various reactive oxygen metabolites, which are known to have deleterious effects on various cellular functions [12]. Lipid peroxidation mediated by oxygen-free radicals (OFR) is believed to be an important cause of destruction and damage to cell membranes. Besides their direct damaging effects on tissues, free radicals can also trigger the accumulation of leukocytes and thus cause tissue injury indirectly. However, what role OFR plays in the bowel injury and bacterial translocation in ACS is still not known.

ACS has a high mortality, most deaths resulting from sepsis and multi-organ failure. There is still no human data existing to suggest that elevated intra-abdominal pressure leads to the translocation of bacteria across the gut mucosa. However, data from some animal work suggests that this is a possibility [13,14] while others cannot [15].

The purpose of the present study is to investigate the role of oxidative stress in ACS-induced intestinal damage and examine whether ACS will lead to bacterial translocation across the intestinal mucosal barrier.

Materials and methods

Animals

This research complied with regulations regarding animal care as published by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. One hundred and twenty male Sprague-Daley rats weighing 250 ± 25g were used in our study. Rats were kept on a 12-h light/dark cycle with free access to standard laboratory chow and water. Humidity was maintained at 50% and the temperature at 25°C. Animals were not fasted before experiments.

Abdominal compartment syndrome model

The rats were weighed and anaesthetized with 50 mg/ kg ketamine and 0.5 mg/kg xylazine, which were administered intraperitoneally. Additional doses were administered intravenously as needed (12.5 mg/ kg ketamine and 0.5 mg/kg xylazine). The trachea was exposed and cannulated with a 14-gauge catheter. The animals were then mechanically ventilated on a rodent ventilator at a rate of 60 breaths per minute and a tidal volume of 1.5 ml/100 g.

The right carotid artery was cannulated with heparinized polyethylene-50 tubing connected to pressure transducers for continuous monitoring of the mean arterial pressure (MAP). The right jugular vein was cannulated to administer 0.9% saline. Rectal temperature was monitored and maintained between 36–38°C throughout the experiment, using a heating plate.

The abdomen was shaved and a 14-gauge plastic catheter with small side holes was inserted into the abdominal cavity through a lower-abdominal midline puncture for nitrogen gas insufflation. The punctured abdominal wall was tightly closed with a purse-string suture around the catheter to ensure an airtight seal. The catheter was connected to a nitrogen gas bag and to a pressure gauge to monitor IAP. IAH was induced by insufflating of nitrogen gas intraperitoneally through the catheter.

Experimental procedures

Animals were randomly divided into four groups. All animals were allowed to stabilize on 100% oxygen for 30 min after completion of the preparation. Once a steady-state was reached, continuous basal fluid substitution with 0.9% saline was given (1 mL/100g bw*h) through the jugular vein. In the whole experiment, the fraction of inspired oxygen was 1.0 to provide optimal oxygenation. In group I (ACS group, $n = 30$), nitrogen gas was slowly insufflated through the plastic catheter until IAP reached 20 mm Hg. IAP was initially increased to 10 mmHg for abdominal wall adaptation. After 5 min, it was further elevated to 20 mmHg. During the next time, gas was either added or removed, to keep the IAP at 20 ± 1 mmHg for 4 h. In group II (ACS/De group, $n = 30$), elevated IAP applied for 4 h was decompressed by pumping out the nitrogen gas from the abdomen slowly and a 4-h reperfusion period was allowed. In group III (control1 group, $n = 30$), animals which underwent an identical 4-h procedure (anaesthesia, intubation, instrumentation, laparotomy, intravenous fluid substitution, etc.), however, without induction of IAH, served as sham-operated controls. In group IV (control2 group, $n = 30$), animals underwent an identical 8-h procedure without IAH, and also served as sham controls. At the end of the experiments, a midline laparotomy was performed and mesenteric lymph nodes (MLN), spleen, and liver specimens were obtained under sterile conditions. Then, samples of ileum were harvested.

A MAP of 100 mmHg was determined as fluid resuscitation end point. Whenever the MAP decreased beyond a threshold of 100 mmHg, the continuous intravenous fluid substitution was increased to 2 mL 0.9% saline/100 g bw*h. With persisting hypotension, the fluid substitution was increased to a maximum of 4 mL/100 g bw*h.

Tissue preparation for biochemical analysis

All tissues were washed twice with cold saline solution and homogenized using a glass Teflon homogenizer (B. Brawn, Germany) in buffer at a ratio of 1/10 (50 mM potassium phosphate buffer pH 7.8, containing 0.5 mmol/L PMSF, 10 μg/mL aprotinin) after cutting the tissue into small pieces with scissors and centrifuged at 2500 g. Malondialdehyde (MDA) analyses were measured at this homogenate stage. The homogenate was then centrifuged at 45 000 g for 30 min. The supernatant was used for colourimetric determination of glutathione peroxidase (GSH-Px) enzyme activities. For glutathione (GSH) and myeloperoxidase (MPO) assay, tissue preparation details were mentioned in the analysis section. All preparation procedures were performed at $+4^{\circ}$C. All homogenates were stored at -80°C prior to testing.

MDA assay

The MDA assay was based on the condensation of one molecule of malondialdehyde with two molecules of thiobarbituric acid (TBA) in the presence of reduced agents. The TBA + MDA complex was analysed by HPLC system as described by Tatum et al. [16]. Briefly, the HPLC system (Shimadzu VP Class, Shimadzu Corporation, Japan) consisted of a LC-10 ADVP pump system (Shimadzu VP) equipped with an automatic injector (SIL-10 ADVP), RF-10XL fluorescence detector and a personal computer using Class VP 6.1 Software. Aliquots of TBA + MDA samples were injected on a C18 column (Nucleosil 100-5, 150-4.6 mm; Macherey–Nagel Incorporation, Bethlehem, PA) maintained at 30°C, followed by fluorimetric detection at 550 nm after excitation at 340 nm. Serial concentrations (0.75–50 μM) of 1,1,3,3-tetraethoxypropane (TEP) were used as standard. Measurements were expressed in terms of MDA normalized to the tissue protein content.

Thiobarbituric acid (TBA), 1,1,3,3-Tetraethoxypropane (TEP), butylated hydroxytoluen (BHT), potassium monobasic phosphate (KH_2PO_4), potassium dibasic phosphate (K_2HPO_4), sodium hydroxide (NaOH), sodium dodecylsulphate (SDS), ethanol, pyridine, n-butanol, and HPLC grade methanol were obtained from Sigma Chemicals (Germany).

Determination of glutathione peroxidase activity

GSH-Px activity was measured by automated spectrophotometric method (Hitachi Modular Analytics, Roche Diagnostics Inc., Tokyo, Japan). The enzymatic reaction was initiated by the addition of cumene hydroperoxide (CuOOH) to the reaction mixture containing GSH, NADPH, EDTA, $NaNO_3$ and glutathione reductase. The change in the absorbance at 340 nm was monitored.

Determination of GSH concentration

Colourimetric assay for assessment of reduced glutathione concentration (Bioxytech, GSH-400, Oxis Research) was used. First, the tissue was homogenized in precipitation reagent (Bioxytech GSH-420, Oxis Research) and homogenate was centrifuged at 3000 g for 10 min at $+4^{\circ}$C and the upper aqueous layer was used for assay. Then, the level of reduced glutathione was measured at 412 nm by a spectrophotometer (Varian, Carry 50 UV-Visible, Australia). Results were expressed as μmol/mg protein.

Measurement of MPO levels

MPO activity was measured in tissues with a commercially available ELISA kit (Bioxytech MPO-EIA, Oxis Research, Portland, OR). Briefly, tissue samples were homogenized in 50 mM potassium phosphate buffer, pH 7.8, containing 0.5 mmol/L PMSF, 10 μg/mL aprotinin, 5% hexadecyltrimethylammonium bromide (HETAB) and centrifuged at 40 000 g for 15 min at $+4^{\circ}$C. Then, the supernatant was assayed according to the manufacturer's instructions. The absorbance was read at 405 nm using a Multi-Detection MicroPlate Reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT). Quantifications were achieved by the construction of a standard curve using known concentrations of MPO. Results were expressed as ng/mg protein.

Microbiological analysis

The blood samples of all animals were obtained through the vena cava, inoculated to blood culture medium including brain heart infusion (BHI) and incubated at 37°C for 7 days under aerobic conditions. The tissue specimens were extracted from liver, spleen and mesenteric lymph nodes (MLN), respectively, to indicate the bacterial translocation. Then they were weighed and placed into a glass homogenizer under sterile conditions. After the liver and spleen were homogenized into 2 mL BHI, 0.1 mL of these samples was inoculated with blood agar and McConkey agar. MLN was homogenized into 5 mL BHI and then its serial solutions were prepared from this homogenate. A 0.1 mL sample of each solution was inoculated with blood agar and McConkey agar. All cultures were incubated under aerobic conditions at 37°C and were examined at 24 h and 48 h for the presence of growth. The identification of bacterial species was performed by standard microbiological methods. Colonization was expressed as the number of colony-forming units per gram of tissue homogenate (CFU/g).

Histological examination

Ileal segments were excised and fixed immediately in 10% formalin, then embedded in paraffin wax, sectioned serially at 4 μm and stained with hematoxylin and eosin. Histological mucosal damage in each preparation was classified according to microscopic criteria set for the grading of intestinal tissue injury by Chiu et al. [17] as follows: grade 0, normal mucosa; grade 1, development of subepithelial spaces near the tips of the villi with capillary congestion; grade 2, extension of the subepithelial space with epithelial lifting from the lamina propria; grade 3, massive epithelial lifting down the sides of villi with few denuded villous tips; grade 4, denuded villi with exposed lamina propria and dilated capillaries; grade 5, digestion and disintegration of the lamina propria, haemorrhage and ulceration.

Situ detection of intestinal mucosal epithelial apoptosis

The ileal fragments were fixed in 4% formaldehyde polymerisatum and embedded in paraffin. The apoptosis of intestinal mucosal epithelial cell was detected by the terminal deoxynucleotidyl transferase (TdT)-mediated dUDP-biotin nick end labelling (TUNEL) method. Cell death was assessed using an assay kit from Roche Inc. (USA). Briefly, specimens were dewaxed and immersed in phosphate-buffered saline containing 3 g/L hydrogen peroxide for 10 min at room temperature and then incubated with 20 μg/ml proteinase K for 15 min at room temperature. Equilibration buffer (75 μl) was applied directly onto the specimens for 10 min at room temperature, followed by incubation with 55 μl of TdT enzyme at 37°C for 1 h. The reaction was terminated by transferring the slides to pre-warmed stop/wash buffer for 30 min at 37°C. The specimens were covered with a few drops of rabbit serum and incubated for 20 min at room temperature and then covered with 55 μl of anti-digoxigenin peroxidase and incubated for 30 min at room temperature. Specimens were then soaked in Tris buffer containing 0.2 g/L diaminobenzidine and 0.2 g/L hydrogen peroxide for 1 min for colour development. Finally, the specimens were counter-stained by immersion in hematoxylin. The cells with clear nuclear labelling were defined as TUNEL-positive cells. The rate of cell apoptosis (apoptotic index) was calculated as percentage of TUNEL-positive cells using the following formula: the number of TUNEL-positive cell nuclei/the number of total cell nuclei) × 100.

Statistical analysis

All data were expressed as the means ± SD. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The level of significance was set at $p < 0.05$. The statistical analyses were performed by SPSS, version 13.0 for Windows (SPSS Inc., Chicago, IL).

Results

Biochemical analysis of the intestine

Elevation of intra-abdominal pressure raised the MDA levels compared with the control1 group ($p < 0.05$). When decompression was applied for 4 h, the value of MDA was found to be substantially increased with respect to the control2 group ($p < 0.001$) and ACS group ($p < 0.01$) (Table I).

Elevated IAP in the ACS group led to an increase in MPO activity compared to the control1 group ($p < 0.05$). In the ACS/DE group, MPO activity was significantly higher than the control2 ($p < 0.001$) and ACS groups ($p < 0.001$) (Table I).

In the presence of IAH, GSH level in the intestine was decreased as compared to the control1 group ($p < 0.05$). This parameter was much lower after 4-h of decompression ($p < 0.001$ vs control2; $p < 0.05$ vs ACS group) (Table I).

The activity of GSH-Px in the intestine showed a tendency to decrease when IAP was elevated in the ACS group ($p > 0.05$). This reduction reached statistical significance following the decompression in the ACS/DE group ($p < 0.05$) (Table I).

The four parameters showed no statistical significance between the control1 and control2 groups ($p > 0.05$) (Table I).

Table I. Ileal tissue levels of malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH) and glutathione peroxidase (GSH-Px) in control1, control2, ACS and ACS/DE groups.

	MDA (mmol/mg/protein)	MPO (ng/mg/protein)	GSH (μmol/mg/protein)	GSH-Px (U/mg/protein)
Control1	0.415 ± 0.032	4.52 ± 0.61	0.381 ± 0.079	0.036 ± 0.007
Control2	0.487 ± 0.055	4.15 ± 0.72	0.422 ± 0.051	0.038 ± 0.007
ACS	0.512 ± 0.068⋆	6.77 ± 1.24⋆	0.302 ± 0.050⋆	0.034 ± 0.009
ACS/DE	0.727 ± 0.090###$$	10.28 ± 1.76###$$$	0.275 ± 0.025###$	0.027 ± 0.005#$

⋆$p < 0.05$, compared with the control1 group; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, compared with the control2 group; $$p < 0.05$, $$$p < 0.01$, $$$$p < 0.001$, compared with the ACS group.

The evaluation of bacterial translocation

Elevated IAP caused BT in the ACS group and the quantity of bacteria isolated from MLN and liver was significantly higher than that in the control1 group ($p < 0.05$). Also, IAH caused BT in the spleen, but this finding was not statistically significant compared to the control1 group ($p > 0.05$). The animals in the ACS/DE group exhibited a significant amount of bacteria translocated toward the MLN, spleen and liver compared to the control2 group and the ACS group. The predominating bacterium was Escherichia coli (Table II). There was no statistically significance between the control1 and control2 groups.

Histological changes in the intestinal tissue

Histological evaluation was performed according to the Chiu scoring method. Data related to scoring obtained by means of H&E staining as well as the microphotographs are shown in Figures 1 and 2. In the control group, the villus and glands were normal and no inflammatory cell infiltration was observed in mucosal epithelial layer (Figure 1A and C). In the ACS group, the surface epithelial cells lifted and sloughed off at the tip of the villus (Figure 1B). In the ACS/DE group, extensive denudation and collapse of the villus was observed (Figure 1D). According to the Chiu scoring system, the injury in the ACS group was found to have increased compared with the control group ($p < 0.01$). The Chiu's score in the ACS/DE group was the highest in the three groups (Figure 2).

Changes of intestinal mucosal epithelial apoptosis

TUNEL-positive epithelial cells at the villus surface were evidenced by dark brown nuclei. Microphotographs of apoptotic cells stained by the TUNEL method were shown in Figure 3. The apoptotic index of three groups was shown in Figure 4. As shown in Figure 3, in the control group, few TUNEL-positive cells were found (Figure 3A and C). Compared with the control, quite a few TUNEL-positive epithelial cells were found in the ACS group (Figure 3B) and the ACS/DE group (Figure 3D). The apoptotic index of the ACS was significantly higher than that in the control group and the index of the ACS/DE group was the highest in the three groups (Figure 4).

Discussion

IAH is a frequently encountered problem in critically ill patients and carries a high risk of morbidity and mortality [18]. There has been an exponential increase in investigations focused on increased IAP and subsequent adverse effects such as ACS [19]. Acute ACS is increasingly recognized as a cause for sepsis and multi-organ dysfunction.

When ACS develops, it is mandatory to decompress the abdomen surgically as soon as possible. However, this causes reperfusion of abdominal organs and also produces arterial hypotension. It is well known that reperfusion of the ischemic tissue may promote the generation of various reactive oxygen metabolites (ROM), which are known to have deleterious effects on various cellular functions [12]. In the intestinal tissue subjected to I/R, activated neutrophils induce tissue injury through the production and release of ROS and cytotoxic proteins (for example, proteases, MPO, lactoferrin) into the extracellular fluid, constituting the inflammatory cascades that trigger the radical-induced I/R injury [20–22]. In addition, several studies have demonstrated that ischemia/reperfusion in the viscera is associated with lipid peroxidation, which is an autocatalytic mechanism leading to oxidative destruction of cellular membranes, and their destruction can lead to the production of toxic, reactive metabolites and cell death [23,24]. In the present study, ACS followed by decompression caused an elevation in tissue MPO activity, indicating the presence of enhanced leukocyte recruitment in the inflamed tissue, while the increased intestinal MDA level, an indicator of lipid peroxidation, verified the oxidative damage in the intestinal tissue. Of interest, both parameters were shown to increase more during the decompression period as compared to the IAH period. These results suggested that oxidative damage, which involves the interaction of neutrophils and lipid peroxidation, plays an important role in the intestine injury in ACS.

In this study, we also observed a decrease in GSH and GSH-Px levels. These two parameters were

Table II. The quantitative results of bacterial translocation in tissue specimens.

Groups	MLN (CFU/g)	Liver (CFU/g)	Spleen (CFU/g)
Control1	185.58±105.22	35.24±23.16	50.20±40.33
Control2	124.76±86.35	23.36±15.43	41.78±32.27
ACS	566.37±237.46*	98.80±45.10*	65.32±44.15
ACS/DE	1210.83±952.52##$	280.79±131.04#$	209.75±107.56#$

*$p < 0.05$, compared with the control1 group; #$p < 0.05$, ##$p < 0.01$, compared with the control2 group; $$p < 0.05$, compared with the ACS group.

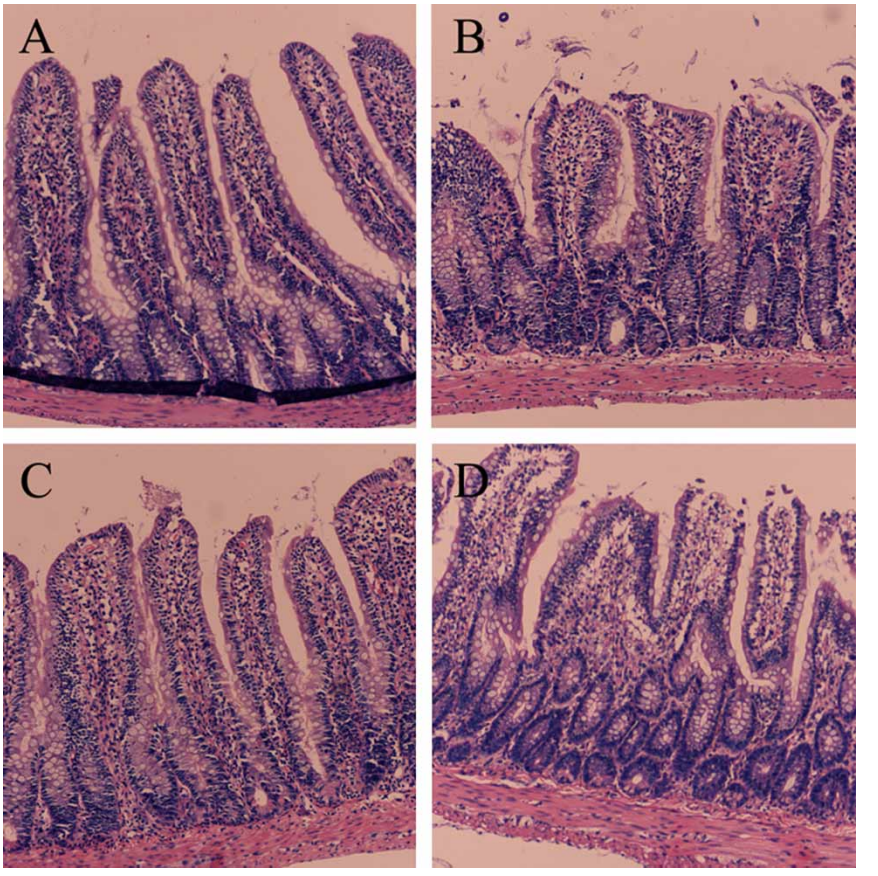

Figure 1. Photomicrographs of the ileal tissue stained by the hematoxylin and eosin in the control1 group, control2 group, ACS group and ACS/DE group.

increased after 4 h of intra-abdominal hypertension compared to the control values and they were increased more after 4 h of the reperfusion period. Glutathione provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage [25,26]. Several reports indicate that tissue injury induced by various stimuli are coupled with GSH depletion [27]. An earlier study proposed that tissue GSH is an indicator of post-ischemic tissue injury [27,28]. GSH scavenges O_2^- and protects protein thiol groups from oxidation. GSH also has a major role in restoring other free radical scavengers and antioxidants such as vitamin E and ascorbic acid to their reduced state [29]. It was reported that tissue GSH levels and the activity of GSH-Px, which are critical constituents of the GSH-redox cycle, were significantly reduced because of oxidative stress and the authors proposed that impairment of antioxidant defense mechanisms could permit enhanced free radical-induced tissue damage [28,30]. This indicated that the decrease of GSH and GSH-Px levels was related to the oxidative stress in ACS.

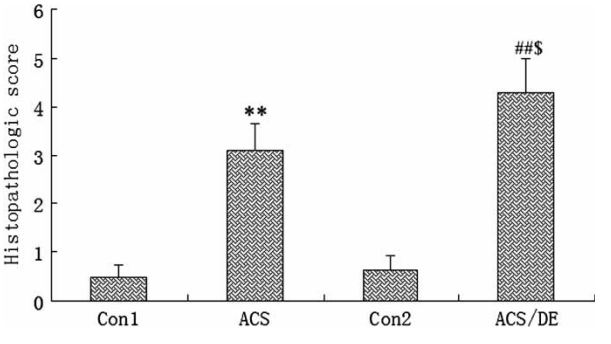

Figure 2. Intestinal mucosal injury evaluated by Chiu scoring system in control1 group, control2 group, ACS group and ACS/DE group. $^{**}p < 0.01$, compared with the control1 group; $\#\#p < 0.01$ compared with the control2 group; $\$p < 0.05$, compared with the ACS group.

Apoptosis, known as programmed cell death, is a form of cell death that serves to eliminate dying cells in proliferating or differentiating cell populations [23]. However, activation of apoptosis in pathologic states results in rapid and extensive cell death with consequent tissue dysfunction. Previous studies reported that apoptosis is a major mode of cell death in the intestinal damage induced by I/R [24,31]. It is well known that oxidative stress can induce apoptosis by damaging DNA, oxidizing membrane lipids and/or directly activating the expression of the genes/proteins responsible for

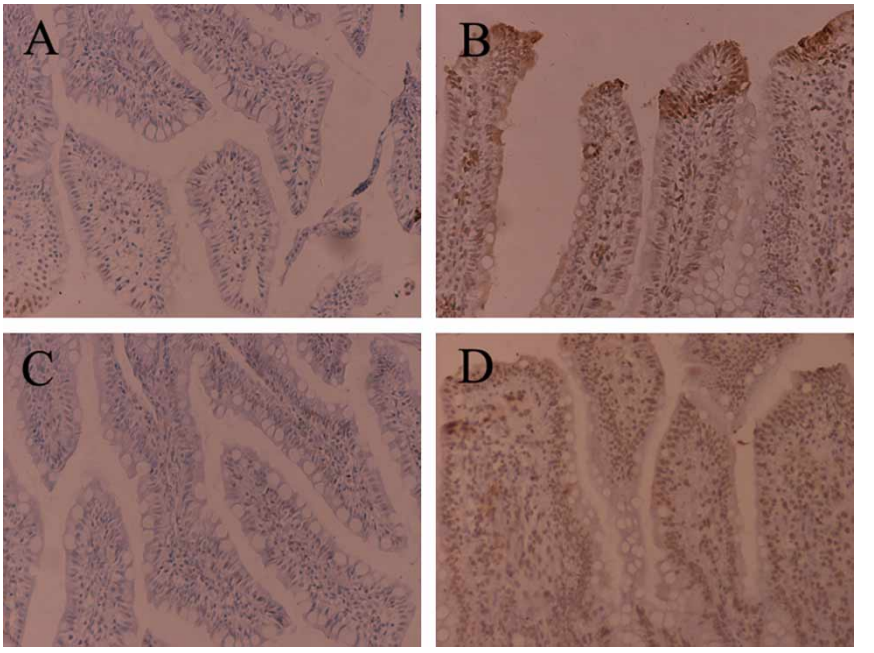

Figure 3. Light micrographs of the ileum stained using TUNEL method. Apoptotic nuclei are stained dark brown.

apoptosis [32–34]. Kojima et al. [35] reported that oxidative stress after I/R plays an important role in induction of apoptosis in the intestinal mucosa. In the present study, the apoptotic index as an indicator of apoptosis increased after IAH was induced and peaked after decompression, which was in accordance with the Chiu's scores.

In our study, we found a bacterial translocation toward the MLN, liver and spleen. In addition, the maximal incidence of BT occurred in the decompression period rather than the IAH period. Normally, in the gut, there is homeostasis between the intraluminal bacteria, their product and intestinal mucosal barrier. However, the intra-abdominal hypertension and the following decompression led an ischemia reperfusion injury to the intestine. The I/R injury to small intestine causes local production of the reactive oxygen species (ROS), such as hydroxyl radical, superoxide anion, hydrogen peroxide and reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite, which are thought to play a pivotal role in gut epithelial damage [36]. This in turn, creates a breakdown in the intestinal mucosal barrier and causes the distruption of mucosal integrity. This event may facilitate BT and release of endotoxins. This may explain why ACS is often complicated with sepsis and multiple organ failure.

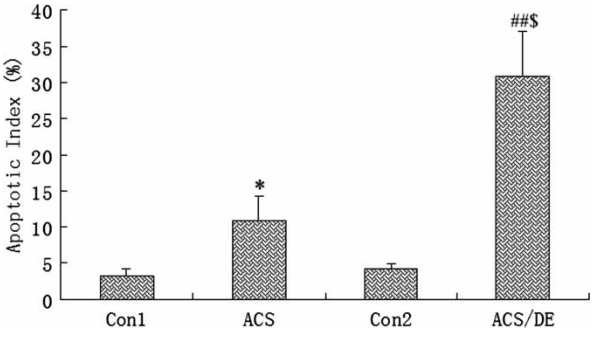

Figure 4. Apoptotic index in the ileal tissue determined by TUNEL staining in the control1 group, control2 group; ACS group and ACS/DE group. *$p < 0.05$, compared with the control1 group; ##$p < 0.01$, compared with the control2 group; \$$p < 0.05$, compared with the ACS group.

In conclusion, oxidative stress plays an important role in the apoptosis of enterocytes and the injury of the intestinal mucosa in ACS. ACS can also lead to bacterial translocation, which may be a contributory factor in the development of sepsis and multi-organ dysfunction syndrome in patients with intra-abdominal hypertension. Additionally, we have found that the detrimental influence of oxygen free radicals occurs mainly during the period of reperfusion and reoxygenation, rather than during the IAH period. Then, is it really mandatory to decompress the abdomen when ACS develops?

Acknowledgements

The study is supported by a grant from special project of Chinese Military Medicine Science and Technology Research '11.5' plan (No. 06Z017).

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Cullen DJ, Coyle JP, Teplick R, Long MC. Cardiovascular, pulmonary, and renal effects of massively increased intra-abdominal pressure in critically ill patients. Crit Care Med 1989;17:118–121.

[2] Fietsam R Jr, Villalba M, Glover JL, Clark K. Intra-abdominal compartment syndrome as a complication of ruptured abdominal aortic aneurysm repair. Am Surg 1989;55:396–402.

[3] Kashtan J, Green JF, Parsons EQ, Holcroft JW. Hemodynamic effects of increased abdominal pressure. J Surg Res 1981;30:249–255.

[4] Richardson JD, Trinkle JK. Hemodynamic and respiratory alterations with increased intra-abdominal pressure. J Surg Res 1976;20:401–404.

[5] Sugrue M, Jones F, Deane SA, Bishop G, Bauman A, Hillman K. Intra-abdominal hypertension is an independent cause of postoperative renal impairment. Arch Surg 1999;134(10):1082–5.

[6] Ivatury RR, Diebel L, Porter JM, Simon RJ. Intra-abdominal hypertension and the abdominal compartment syndrome. Surg Clin North Am 1997;77:783–800.

[7] Schein M, Wittmann DH, Aprahamian CC, Condon RE. The abdominal compartment syndrome: the physiological and clinical consequences of elevated intra-abdominal pressure. J Am Coll Surg 1995;180:745–753.

[8] Tons C, Schachtrupp A, Rau M, Mumme T, Schumpelick V. Abdominal compartment syndrome: avoidance and treatment [in German]. Chirurg 2000;71:918–926.

[9] Kron IL, Harman PK, Nolan SP. The measurement of intra-abdominal pressure as a criterion for abdominal re-exploration. Ann Surg 1984;199:28–30.

[10] Sugrue M. Abdominal compartment syndrome. Curr Opin Crit Care 2005;11:333.

[11] Kaçmaz A, Polat A, User Y, Tilki M, Ozkan S, Sener G. Octreotide improves reperfusion-induced oxidative injury in acute abdominal hypertension in rats. J Gastrointest Surg 2004;8:113–119.

[12] Werns SW, Lucchesi BR. Free radical and ischemic tissue injury. Trends Pharmacol Sci 1990;11:161–166.

[13] Diebel LN, Dulchavsky SA, Brown WJ. Splanchnic ischaemia and bacterial translocation in the abdominal compartment syndrome. J Trauma 1997;43:852–855.

[14] Eleftheriadis E, Kotzampassi K, Papanotas K, Heliadis N, Sarris K. Gut ischaemia, oxidative stress, and bacterial translocation in elevated abdominal pressure in rats. World J Surg 1996;20:11–16.

[15] Doty JM, Oda J, Ivatury RR, Blocher CR, Christie GE, Yelon JA, Sugerman HJ. The effects of hemodynamic shock and increased intra-abdominal pressure on bacterial translocation. J Trauma 2002;52:13–17.

[16] Tatum VL, Changchit C, Chow CK. Measurement of malonaldehyde by HPLC with fluorescence detection. Lipids 1990;25:226–229.

[17] Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states: a morphological, hemodynamic and metabolic reappraisal. Arch Surg 1970;101:478.

[18] Malbrain ML, Chiumello D, Pelosi P, Luciano G. Incidence and prognosis of intra-abdominal hypertension in a mixed population of critically ill patients: a multiple-center epidemiological study. Crit Care Med 2005;33:315–322.

[19] Rezende-Neto JB, Moore EE, Melo de Andrade MV, Teixeira MM, Lisboa FA, Arantes RM, de Souza DG, da Cunha-Melo JR. Systemic inflammatory response secondary to abdominal compartment syndrome; stage for muliple organ failure. J Trauma 2002;53:1121–1128.

[20] Mallick IH, Yang W, Winslet WC, Seifalian AM. Ischemia–reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci 2004;49:1359–1377.

[21] Zimmerman BJ, Granger DN. Mechanisms of reperfusion injury. Am J Med Sci 1994;307:284–292.

[22] Kettle AJ, Winterbourn CC. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep 1997;3:3–15.

[23] Sener G, Sehirli AO, Keyer-Uysal M, Arbak S, Ersoy Y, Yegen BC. The protective effect of melatonin on renal ischemia-reperfusion in rat. J Pineal Res 2002;32:120–126.

[24] Sener G, Paskalog˘lu K, Sehirli O, Ayanoglu-Dulger G, Alican I. The effects of melatonin on ischemia/reperfusion induced changes in rat corpus cavernosum. J Urol 2002;167:2624–2627.

[25] Mandel LJ, Schnellmann RG, William RJ. Intracellular glutathione in the protection from anoxic injury in renal proximal tubules. J Clin Invest 1989;85:316–324.

[26] Cuzzocrea S, Costantino G, Mazzon E, Micali A, de Sarro A, Caputi AP. Beneficial effects of melatonin in a rat model of splanchnic artery occlusion and reperfusion. J Pineal Res 2000;28:52–63.

[27] Paller MS, Patten M. Protective effects of glutathione, glycine, or alanine in an in vitro model of renal anoxia. J Am Soc Nephrol 1992;2:1338–1344.

[28] Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress: a review. J Biomed Sci 2000;7:444–458.

[29] Ross D. Glutathione, free radicals and chemotherapeutic agents. Pharmac Ther 1988;37:231–249.

[30] Reiter RJ, Tan DX, Acuna-Castroviejo D, Burkhardt S, Karbownik M. Melatonin: mechanisms and actions as an antioxidant. Curr Topics Biophys 2000;24:171–183.

[31] Omar R, Nomikos I, Piccorrelli G, Savino J, Agarwal N. Prevention of postischemic lipid peroxidation and liver cell injury by iron chelation. Gut 1989;30:510–514.

[32] Bai J, Cederbaum AI. Mitochondrial catalase and oxidative injury. Biol Signals Recept 2001;10:189–199.

[33] Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol Today 1994;15:7–10.

[34] Curtin JF, Donovan M, Cotter TG. Regulation and measurement of oxidative stress in apoptosis. J Immunol Methods 2002;265:49–72.

[35] Kojima M, Iwakiri R, Wu B, Fujise T, Watanabe K, Lin T, Amemori S, Sakata H, Shimoda R, Oguzu T, Ootani A, Tsunada S, Fujimoto K. Effects of antioxidative agents on apoptosis induced by ischaemiareperfusion in rat intestinal mucosa. Aliment Pharmacol Ther 2003;1:139–145.

[36] Willy C, Dahouk S, Starek C, Kaffenberger W, Gerngrob H, Plappert UG. DNA damage in humane leucocytes after ischemia/rperfusion injury. Free Radic Biol Med 2000;28:1–12.

This paper was first published online on iFirst on 31 March 2009.