Destructive Effects of Acidic Blood on the Intestines: Experimental Study

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ABSTRACT

Objective: This study aimed to investigate the destructive effects of acidic blood on the intestinal structures, which has been reported as the most hazardous biochemical result of vagosympathetic autonomic imbalances in intensive care unit patients with subarachnoid hemorrhage (SAH).

Materials and Methods: In total, 27 hybrid rabbits were used; 5 rabbits were used as a control group, 7 as the SHAM group into whom 1 cc saline was injected into the cisterna magna, and the remaining 15 were used as the study group. These animals received 1 cc of autologous arterial blood injection into the cisterna magna to create the SAH group. Blood pH values were recorded before, during, and after the experimental procedures. Computed tomography was performed to examine the intestinal morphology. Normal and degenerated epithelial cell densities of the intestine were estimated by stereological methods. The relationship between pH values and intestinal tissue changes was analyzed statistically using the Mann–Whitney U test.

Results: The mean blood pH values were 7.364±0.042 in the control group, 7.326±0.059 in the SHAM group, and 7.23±0.021 in the study group. Intestinal epithelial cell injury, desquamation of villus, and cell loss were observed. It is observed that the number of degenerated epithelial cells, fragmented villi numbers, and vacuoles significantly increased in the study group ($p<0.05$).

Conclusion: Acidotic intestinal injury secondary to blood pH changes following SAH may be considered as a generalized and dangerous complication with their multiorgan insuficiency effect.

Keywords: Subarachnoid hemorrhage, acidosis, intestines

Introduction

Acidosis is a life-threatening complication of autonomic network disruptions induced especially by subarachnoid hemorrhage (SAH) [1]. Carotid body and mesenteric paraganglia are the essential structures for pH regulation [2] and may prevent intestinal necrosis [3]. Disrupted and necrosed intestinal components [4] have been frequently observed in patients with untreatable acidosis [5]. Upper [6] and lower intestinal immune barriers may be destroyed by acidic blood [7], and bacterial translocation may occur [8]. Intestinal ischemia [9], endotoxemia [10], and mesenteric thrombosis may be caused by intestinal acidosis secondary to epithelial barrier disruption [11]. Karadeniz et al. [12] have shown that sacral parasympathetic insult causes mesenteric artery spasm and is a new cause of intestinal ischemic mucosal disruption following SAH. There is a linear relationship between sepsis-related complications and acidosis [13]. Children are more susceptible to acidosis than adults. This study aims to investigate the effect of intestinal acidotic disruption.

Materials and Methods

A total of 27 hybrid rabbits were used by the permission of the Ethical Committee on Animal Research of the university. Furthermore, 5 rabbits (n=5) were used as the control group, 7 rabbits (n=7) were used as the SHAM group in whom 1 cc of saline was injected into their cisterna magna, and 15 (n=15) rabbits used as the study group. The study animals received 1 cc autologous arterial blood injection into the cisterna magna to create the SAH group. Blood pH values of all the animals were measured with a pH meter (Mettler Toledo MP 220 pH Meter, Schwarzenbach, Switzerland). They were observed for 2 weeks and decapitated under

general anesthesia. Their intestinal tissues were extracted and fixed with 10% formalin solution. Tissue particles were located in paraffin blocks for consecutive 20 sections of 5 µm for stereological examinations. Specimens were stained with hematoxylin & eosin and aldehyde fuchsine immunostaining methods. The intestines were considered as cylindrical, and the intestinal villi were considered as stalactites. The numbers of normal and degenerated intestinal epithelial cells per cubic millimeter were estimated by stereological methods. Histopathologically, condensed cytoplasm, shrunken nucleus, angulated cells, and halo formation around the cytoplasmic mass were considered as degeneration criteria. Clinically, caseols resembling masses were observed by microscopic examination of the intestinal walls and considered as liquid cases that accumulate in the tissue site due to acidic plasma destruction. The numbers of vacuoles were counted by the stereological method as in epithelial cells.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 22.0 (IBM Corp.; Armonk, NY, USA), and Kruskal–Wallis test was used.

Results

Clinical Results

A total of 3 of 15 rabbits died because of cardiorespiratory irregularities. Meningeal irritation signs and forced/ischemic/autonomic discordance findings were detected in electrocardiography. The mean heart rate of the rabbits in the control group was 252±23/min, 224±14/min in the SHAM group; 182±15/min in the study group. In severe SAH, heart rhythm disorders were more apparent when the heart rate exceeded 142±12/min. Electrophysiological data of the heart and lungs showed that they had become acidotic and arrhythmic. Increase in acidosis causes increased acidic injury in the intestines. The mean blood pH values were 7.364±0.042 in the control group, 7.326±0.059 in the SHAM group, and 7.23±0.021 in the study group.

Histopathological Results

The intestines contained epithelial cells with large numbers of villi, and large capillaries were covered with cuboid epithelial cells having hairy extensions. The intestinal epithelial cells had spherical, cored nuclei in the rabbits of the control group. Cellular degeneration criteria, such as condensed cytoplasm, shrunken nucleus, angulated cells, and halo formation around the cytoplasmic mass, were detected in the study group. We suspected that carotid-body-like structures might be localized around the major intestinal arteries entering the intestinal wall. Our histopathological findings were as follows: Figure 1 shows the computed tomography and macroscopic view of the abdomen with dilated edematous intestinal wall and abdominoperitoneal fluid collections. Figure 2 shows the histological view of the normal carotid body and degenerated

Main Points

- There is no satisfactory information about how acidosis is dangerous.
- Blood acidosis induced organ deficiency-destruction has not been adequately defined.
- Acidotic blood induced intestinal injury is firstly described.

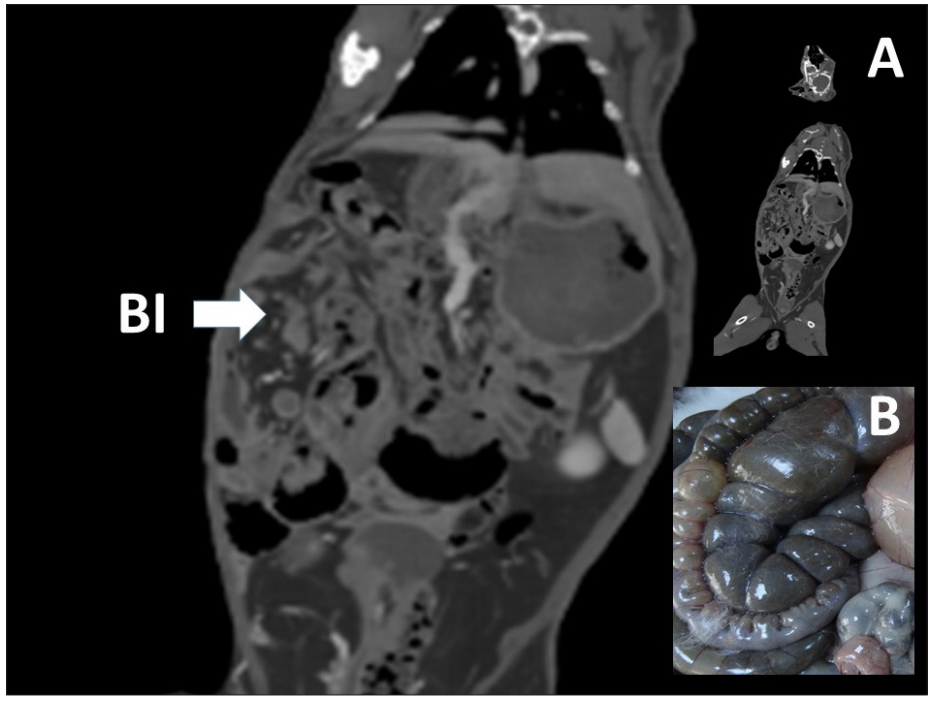

Figure 1. Computed tomography of the abdomen (A) showing dilated edematous intestinal wall and abdomino-peritoneal fluid collections (BI/Base)

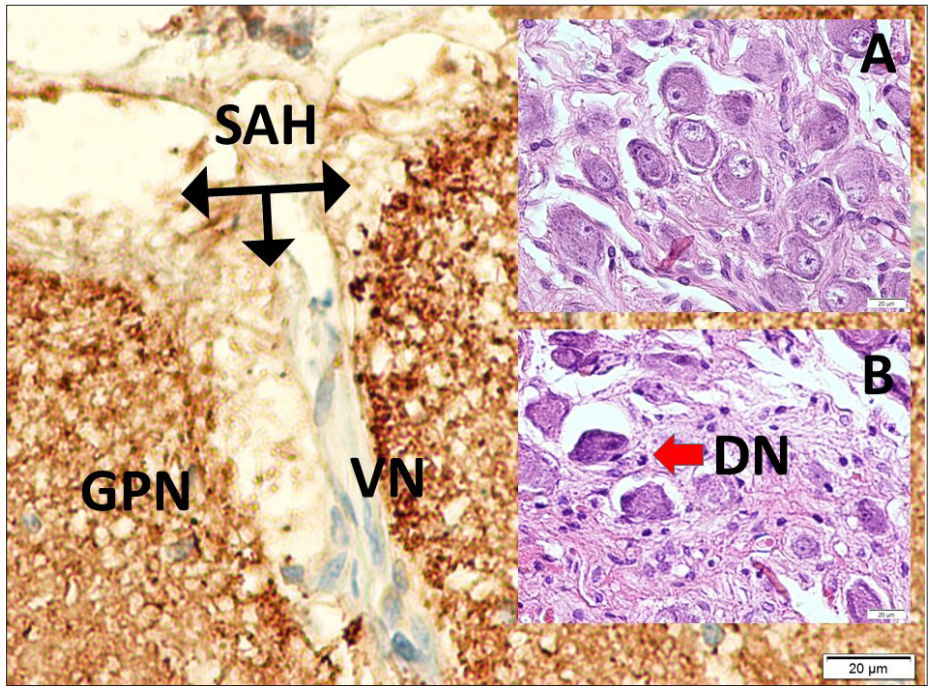

Figure 2. Histological view of the normal carotid body (LM, H&E, x40/A) and degenerated carotid body neurons (DN/LM, H&E, x40/B) and bloody and fibrotic bands developed subarachnoid space between glossopharyngeal and vagal nerve fibers entering the jugular foramen (LM, GFAP, x40/Base)

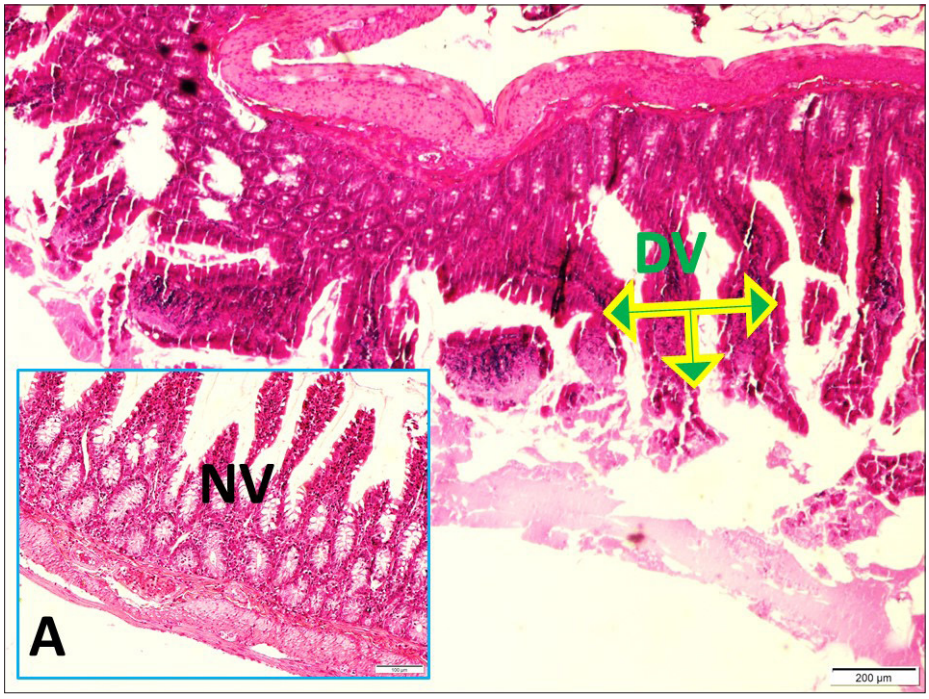

Figure 3. Histological view of the normal intestinal villi (NV) (LM, H&E, x10/A) and degenerated villi (Dv/LM, H&E, x4/B) of a SHAM animal

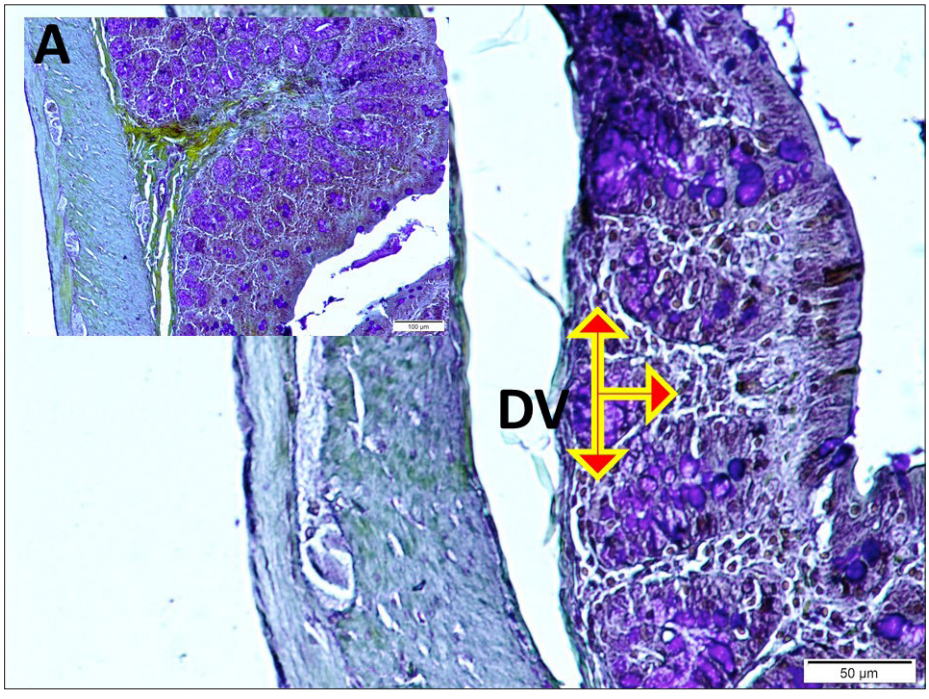

Figure 4. Histological view of the normal intestinal villi (NV) (LM, Aldehyde Fuchsin, x10/A) and degenerated epithelial cells and villi (Dv/LM, Aldehyde Fuchsin, x20/Base) of a study animal

carotid body neurons and bloody and fibrotic bands that developed a subarachnoid space between the glossopharyngeal and vagal nerve fibers that are just entering the jugular foramen. Figure 3 demonstrates the histological view of the normal intestinal villi and degenerated villi of a SHAM animal. Figure 4 shows the histological view of the normal intestinal villi and degenerated epithelial cells and villi of a study animal and histological view of the normal intestinal villi and degenerated villi of a SHAM animal. In the intestines considered to be cylinders, the number of cells per mm^2 was determined by dividing the inner wall surface areas by the cell surface area (Figure 5), histological view of the normal intestinal villi of a normal animal. To count the epithelial cells on the villi, the number of cells in a cubic millimeter was determined stereologically (Figure 6). Figure 7 shows the histological view of the fragmented intestinal villi and degenerated villi of a study animal. In the intestines considered to be cylinders, the number of cells per mm^2 was determined by dividing the inner wall surface areas by the cell surface area. Histological view of the dilated intestinal artery with degenerated endothelial and atrophic villi and paramount vacuoles of a study animal. In the intestines considered to be cylinders, the number of vacuoles per mm^3 was determined by dividing the wall surface volume by vacuole volume (Figure 8). We also suspected that carotid-body-like structures determined around the mesenteric arteries might be responsible for intestinal pH regulation, which has not been mentioned in the literature so far.

Numerical Results of Intestines

The mean villus number was 25±5 in the control group, 20±4 in the SHAM group, and 11±2 in the study group. The mean cell density of the intestines per mm^2 was estimated as 23.342±4.230 in the control group, 20.235±2.750 in the SHAM group, and 15.651±2.100 in the study group. The mean vacuole number was 12±3 in the control group, 27±6 in the SHAM group, and 65±12 in the study group (Table 1).

Discussion

Chemosensitive glomus cells of the carotid bodies [14] have the most sensitive structures for the blood pH changes [15, 16], and the most fatal complication of SAH is carotid body network insults induced acidosis [1]. Circulatory and respiratory autonomy is mainly regulated by the carotid bodies network [17]. We also detected carotid bodies like glomus corpuscles around the mesenteric circulation arteries described by Elliot GB et al. [18]. Acidosis destroys the immune barriers of intestines [7]. Intestinal ischemia may cause acidosis [9]. Endotoxemia may cause intestinal acidosis [10]. Our hypotheses were based on the mesenteric carotid body like structures-sacral parasympathetic circuitry degeneration induced acidosis related intestinal necrosis following SAH.

The gut is an important barrier for bacterial translocation during multiple organ dysfunction [6]. Intestinal alkaline secretion is decreased, which is an essential factor in the protection of the duodenum against luminal acidic fluid [19]. If severe acidosis occurs after the reservation of gut integrity has been disrupted, bacterial translocation will be developed inthe critically ill patients [8].

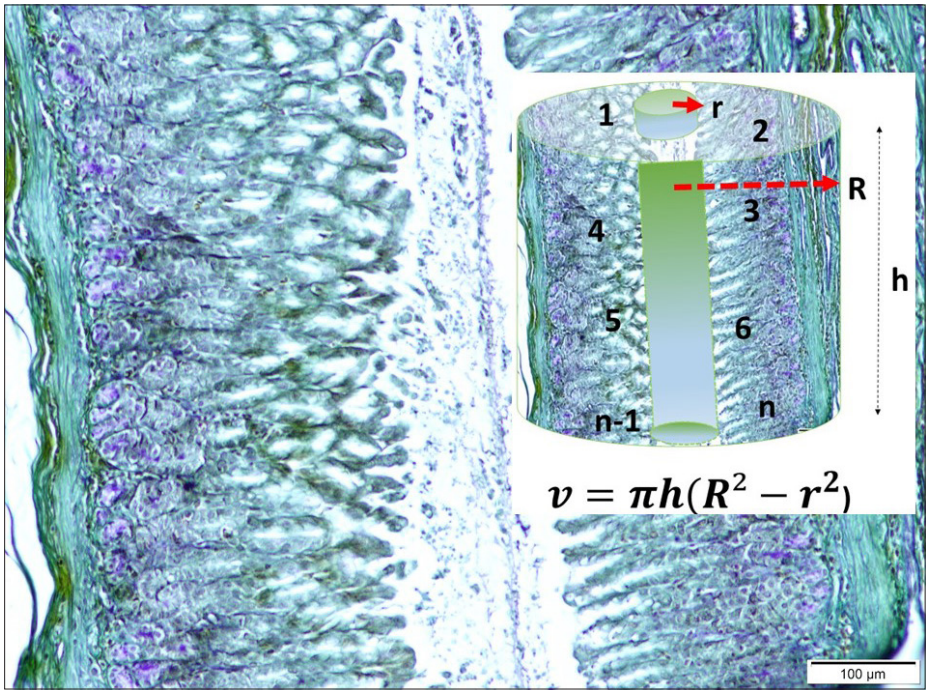

Figure 5. Histological view of the normal intestinal villi (NV) (LM, AF, x10/A) and degenerated villi (Dv/LM, H&E, x20/A) of a SHAM animal. In the intestines considered to be cylinders, the number of cells per mm2 is determined by dividing the inner wall surface area by the cell surface area

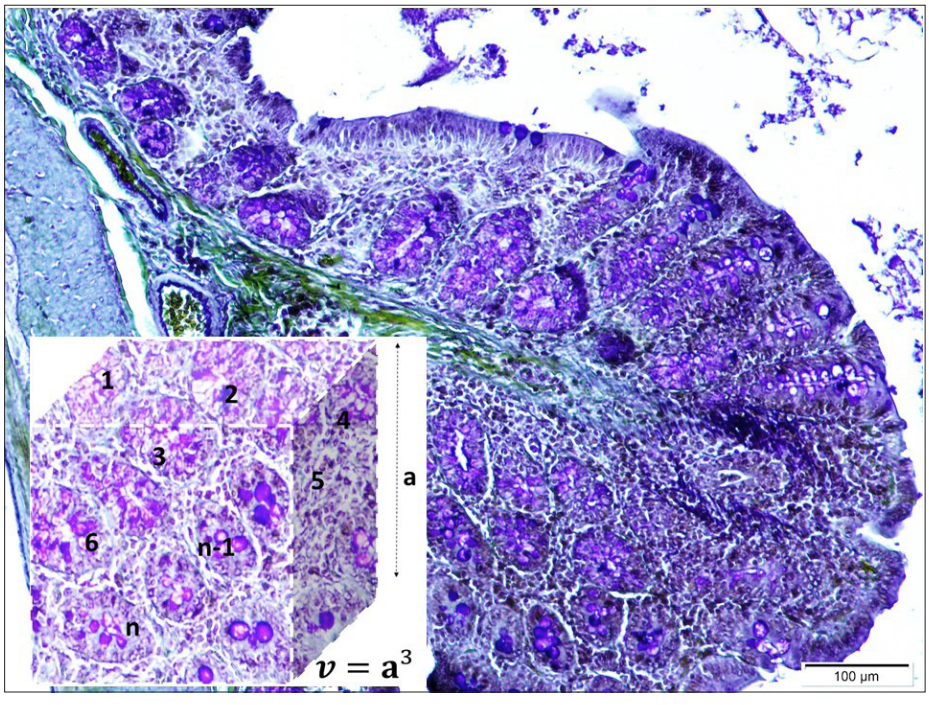

Figure 6. Histological view of the normal intestinal villi (NV) (LM, Aldehyde Fuchsin, x10/A), of a normal animal. To count the epithelial cells on the villi, the number of cells in a cubic millimeter is determined stereologically.

Stress ulcers and ischemic vagal network are the significant causes of stress ulcerations during SAH [20]. Interestingly, we showed that Onuf's nucleus cored network ischemia has an important role in the intestinal mucosal injury secondary to developing mesenteric artery spasm following SAH [12]. Acute superior mesenteric venous thrombosis may lead to intestinal epithelial barrier disruption [11]. Sepsis-related complications originally may result from acidosis [13]. Respiratory acidosis relies on intestinal acidosis [21]. Intestinal parasitic or bacterial infections trigger intestinal acidosis [22]. Water, electrolyte, and acid-base imbalances occur in rabbits during acidosis [5]. Interestingly, anosmia results in intestinal immunodeficiency-induced Peyer's patches insult [23]. Acidosis is not only a biochemical abnormality but also a dangerous histopathological problem for intestines, which has not been mentioned in the literature thus far.

Table 1. Numerical Results of Study

	Vi (n/mm^3)	EC (n/mm^3)	V (n/mm^3)
Normal	25	23.342	12
SHAM	20	20.235	27
Study	11	15.651	65

EC: Epithelial cells, Vi: Villus number, V: Vacuole numbers
Vi: p<0.0001 (Normal/Study)
p<0.0005 (SHAM/Study)
p<0.005 (Normal/Study)
EC: p<0.0001 (Normal/Study)
p<0.0005 (SHAM/Study)
p<0.005 (Normal/SHAM)
V: p<0.0001 (Normal/Study)
p<0.0005 (SHAM/Study)
p<0.005 (Normal/SHAM)

Although necrotizing enterocolitis accompanied by metabolic acidosis leading to multiple organ injury has been considered as a dangerous disorder for premature infants, our findings show that necrotizing enterocolitis should be accepted as a result of systemic acidosis following nervous system insult. Recent studies have explained that bacterial translocation could not be possible unless there is intestinal wall injury. Destroyed intestinal barrier with acidic blood pH is the most facilitating factor for bacterial translocation. Although pneumatosis intestinalis has been accepted as an essential and dangerous sign of necrotizing enterocolitis [24], histopathologically, we showed that the essential mechanism of pneumatosis intestinalis could be developing multiple intestinal vacuoles filled with intestinal air following acidotic injury. We explained that acidosis may be responsible for multiple organ failure syndromes in intensive care unit patients.

Kanat et al. [25] declared that acidosis is a dangerous complication of SAH, but the effect of acidosis on the intestine has not been adequately studied so far. In the light of that study, we reinvestigated the intestines and noticed that damaged carotid bodies induce acidosis and disrupt the autonomic network consisting of the vagal nerve, sacral parasympathetics, neuroenteric plexus, and detrimented target cells in the intestine, which could be named as intestinal acidotic disruption. The computed abdominal tomography shows dilated edematous intestinal wall and abdominoperitoneal fluid collections. Our limitations were decreasing number of biochemical parameters and lack of invasive intestinal pH determination.

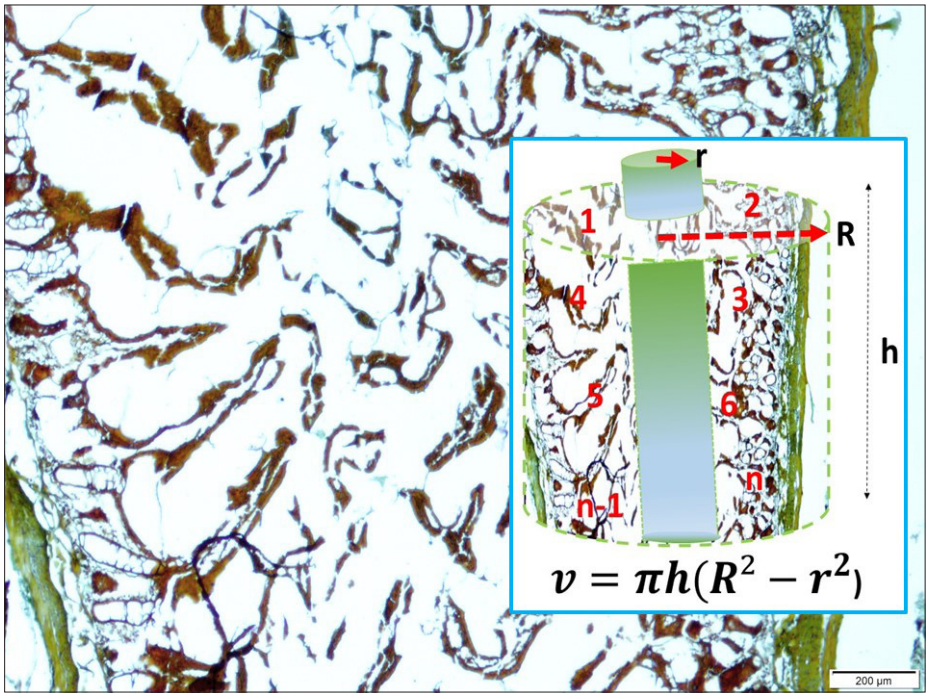

Figure 7. Histological view of the fragmented intestinal villi (LM, Aldehyde Fuchsin, x10/A) and degenerated villi (LM, Aldehyde Fuchsin, x4/Base) of a study animal. In the intestines considered to be cylinders, the number of cells per millimeter square was determined by dividing the inner wall surface area by the cell surface area

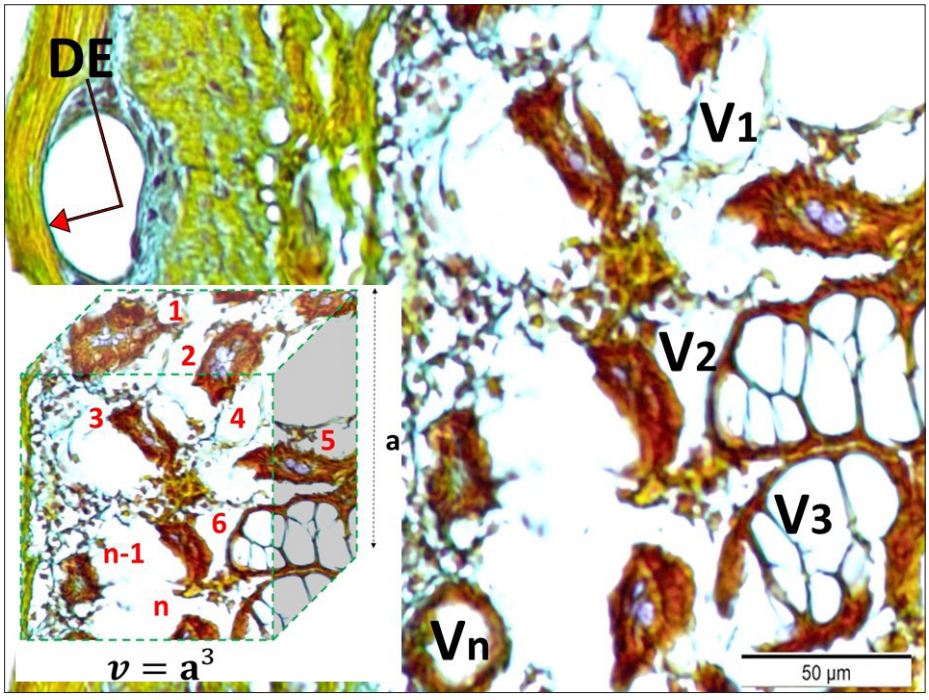

Figure 8. Histological view of the dilated intestinal artery with degenerated endothel (DE) and atrophic villi (LM, Aldehyde Fuchsin, x2/A), degenerated villi, and paramount vacuoles (V1-n) (LM, Aldehyde Fuchsin, x4/Base) of a study animal. In the intestines considered to be cylinders, the number of vacuoles per cubic millimeter is determined by dividing the wall surface volume by vacuole volume

Blood pH regulating paraganglia ischemia-induced acidosis is probably the most forgotten cause of intestinal ischemia and even necrosis.

Dilated intestinal arteries with degenerated endothelial atrophic villi and paramount vacuoles in intestinal walls destroyed by acidic blood were observed in the histopathological examinations of this study. Intestinal injuries have not been well investigated in ICU patients with SAH. The important role of intestinal injury has been underestimated. This experimental study implies that intestinal injury should be reevaluated with malnutrition, septicemia, perforation, absorption abnormalities, immune deficiencies, and necrosis. Intestinal injuries affect the liver, kidney, lungs, and nervous system. Many undocumented complications of SAH occur from intestinal insults, and further studies are required. Blood changes or paraganglia stimulation could be used for the treatment of intestinal acidosis in the future.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Ataturk University (19.09.2017/9-118).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - O.C., M.D.A., M.E.A.; Design - O.C., E.K.; Supervision - M.D.A., M.E.A.; Resources - O.C., M.D.A., E.K.; Materials - O.C., M.D.A., B.F., M.K.; Data Collection and/or Processing - M.D.A., O.C.; Analysis and/or Interpretation - M.D.A., M.K., B.F.; Literature Search - O.C., E.K., M.E.A.; Writing Manuscript - O.C., M.D.A.; Critical Review - M.D.A.

Conflict of Interest: The authors have no conflict of interest to declare.

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