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Intraluminal Tranexamic Acid Inhibits Intestinal Sheddases and Mitigates Gut and Lung Injury and Inflammation in a Rodent Model of Hemorrhagic Shock

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Abstract

Background—Intravenous tranexamic acid (TXA) is an effective adjunct after hemorrhagic shock (HS) due to its antifibrinolytic properties. TXA is also a serine protease inhibitor and recent laboratory data demonstrated that intraluminal TXA into the small bowel inhibited digestive proteases and protected the gut. ADAM-17 and TNF α are effective sheddases of intestinal syndecan-1 which when shed, exposes the underlying intestinal epithelium to digestive proteases and subsequent systemic insult. We therefore hypothesized that intraluminal TXA as a serine protease inhibitor would reduce intestinal sheddases and syndecan-1 shedding, mitigating gut and distant organ (lung) damage.

Methods—Mice underwent 90 minutes of hemorrhagic shock to a mean arterial pressure of 35 \pm 5 mm Hg following by the intraluminal administration of TXA or vehicle. After 3 hours, small intestine, lung, and blood were collected for analysis.

Results—Intraluminal TXA significantly reduced gut and lung histopathologic injury and inflammation compared to hemorrhagic shock alone. Gut, lung, and systemic ADAM-17 and TNF α were significantly increased by hemorrhagic shock but lessened by TXA. Additionally, gut and lung syndecan-1 immunostaining were preserved and systemic shedding lessened after TXA. TXA reduced ADAM-17 and TNF α , but not syndecan-1, in TXA-sham animals compared to sham vehicles.

Conclusions—Results of the present study demonstrate a beneficial effect of intraluminal TXA in the gut and lung after experimental hemorrhagic shock in part due to inhibition of the syndecan-1 shedding by ADAM-17 and TNF α . Further studies are needed to determine if orally

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administered TXA could provide similar intestinal protection and thus be of potential benefit to patients with survivable hemorrhage at risk for organ injury. This is particularly relevant in patients or soldiers who may not have access to timely medical care.

Level of evidence—NA

Keywords

hemorrhagic shock in mice; oral tranexamic acid; ADAM-17; TNF α ; syndecan-1

Introduction

Tranexamic acid (TXA) is a synthetic derivative of the amino acid lysine that blocks the lysine binding sites on plasminogen, thereby inhibiting fibrinolysis and decreasing bleeding (1). It demonstrates an extensive scope of utility as an antifibrinolytic agent in hemorrhagic conditions, including injured patients and soldiers after hemorrhagic shock (2,3). It is marketed in the United States as Cyklokapron in its intravenous form, but the oral formulation is also FDA approved for the management of acute abnormal uterine bleeding in nonpregnant reproductive-aged women (4). Oral TXA has significantly broadened bleeding indications in Europe and other countries where it has been shown to lessen postoperative blood loss and transfusion requirements and to reduce mortality rates in patients requiring urgent surgery (5). It has shown benefit after heart, orthopedic, and dental craniofacial surgery, as well in the treatment of menstrual bleeding, obstetrics, and hemophilia (6). Oral administration of TXA also takes advantage of its gut protease activity which has the potential to reduce gut barrier dysfunction and the subsequent development of multiple organ failure. One reason post-injury multiple organ failure remains an important cause of morbidity and mortality is the lack of early therapeutic interventions to prevent shock-induced gut dysfunction (7). In an elegant series of experiments in models of rodent peritonitis, endotoxemia, and hemorrhagic shock, DeLano et al. investigated whether enzymatic blockade of gut pancreatic enzymes by 6-amidino-2-naphthyl *p*-guanidinobenzoate dimethanesulfate, tranexamic acid, or aprotinin into the lumen of the small intestine could attenuate breakdown of the intestine and subsequent autodigestion with damage to the intestine, lung, and heart (8). The authors demonstrated a reduction in injury and mortality in animals receiving pancreatic enzyme blockade. Much of the pioneering work on pancreatic enzyme blockade has been done by Schmid-Schöbein et al. (9-13) They have demonstrated in a number of studies that digestive enzymes normally present in the gut, escape into the gut wall and then are released systemically with breakdown of the intestinal barrier as occurs in shock. We sought to extend these findings to investigate the mechanisms by which inhibition of intestinal proteases specifically by TXA after hemorrhagic shock would protect the gut and distant organ (lung) injury.

Syndecans are transmembrane glycoproteins that modulate binding and signaling of cytokines, chemokines, and adhesion molecules on the surface of both endothelial and epithelial cells. We and others have shown that syndecan-1 is shed after hemorrhagic shock from both the lung and gut (14,15). Shedding of the cell surface ectodomain of syndecan-1 is regulated by multiple intracellular signaling pathways converging on a diverse group of proteases, referred to as sheddases. The metalloproteinases of the ADAM Disintegrin And

Metalloproteinase (ADAM) family is the largest group of sheddases. ADAM17 is one member of the ADAM family that is co-expressed with syndecan-1 in the gut and mediates syndecan-1 shedding in the lung (16,17). Also known as TNF α -converting enzyme (TACE), ADAM17 cleaves pro-TNF α to release active TNF α . TNF α is a potent cytokine involved in systemic inflammation, is elevated in injured patients and in the laboratory in rodents after hemorrhagic shock, and is a known syndecan-1 shedding agonist (18,19). We hypothesized that intraluminal TXA, as a serine gut protease inhibitor, would reduce ADAM-17 and TNF α , and therefore attenuate intestinal syndecan-1 shedding, mitigating gut and distant organ (lung) injury and inflammation.

Materials and Methods

Mouse model of trauma/hemorrhagic shock (T/HS)

All procedures performed were protocols approved by the University of Texas Houston Medical School Animal Welfare Committee. The experiments were conducted in compliance with the National Institutes of Health (NIH) guidelines on the use of laboratory animals. All animals were housed at constant room temperature with a 12:12-h light-dark cycle with access to food and water ad libitum until the evening before surgery when food was withheld. Male C57BL/6J mice, 8-10 weeks of age and weighing approximately 22 grams, were used for all experiments. An established coagulopathic mouse model of trauma-hemorrhagic shock was utilized (14). Under isoflurane anesthesia, a midline laparotomy incision was made, the organs inspected, and then the incision closed. Bilateral femoral arteries were cannulated for continuous hemodynamic monitoring and blood withdrawal or resuscitation. After a 10-minute period of equilibration, mice were bled to a mean arterial pressure (MAP) of 35 \pm 5 mmHg and maintained for 90 minutes. Shams underwent anesthesia and placement of catheters but were not subjected to hemorrhagic shock. At the completion of the shock period, TXA was delivered intraluminally (25mg/ml, 0.7 ml/animal diluted in water) into the duodenum, jejunum, and ileum and compared to animals that underwent shock alone or shams that received TXA or vehicle. Injections were performed with a 25-gauge needle and were spaced in intervals of about 5 cm for a total of six injections, as previously described by Delano et al. (8). Vascular catheters were removed, incisions closed, and the animals were then awoken from anesthesia. After three hours, animals were sacrificed, the jejunum and lungs harvested, and blood obtained via cardiac puncture. This time point was chosen based on our previous investigation in rodent models of hemorrhagic shock showing near complete dissolution of the glycocalyx and enhanced systemic shedding of syndecan-1 (20).

Gut analyses

To verify protease inhibition by TXA, the small intestinal wall was analyzed for protease activity using the *Sigma-Aldrich Protease Fluorescent Detection Kit*.

Gut histopathology—Small bowel samples that had been fixed in formalin and embedded in paraffin. Tissue sections were deparaffinized and rehydrated, and then stained with hematoxylin and eosin. Five random fields with 100-250 villi/mouse were analyzed in a blinded fashion at 100X magnification to quantify morphologic damage to the villi.(21)

Damage was identified if any one of the following were present: a subepithelial space, moderate lifting of the epithelial layer from the lamina propria, massive epithelial lifting down the sides of the villi, denuded villi with lamina propria and dilated capillaries and/or digestion and disintegration of the lamina propria with hemorrhage, and ulceration. Results are expressed as the % of injured villi .

Gut inflammation—The *EnzCheck Myeloperoxidase Activity Assay Kit* (Enzo Life Sciences, ADI-907-029) was used to determine myeloperoxidase activity of gut lysates as an indicator of neutrophil infiltration. The fluorescence intensity of each sample was measured using excitation at 530 nm and emission at 590 nm. Samples were measured in duplicate with an average difference between measurements of 0.02 uU/mg.

Gut TNF α and A disintegrin and metalloproteinase (ADAM)-17—Levels of TNF α and ADAM-17, also known as TNF α converting enzyme (TACE), in small intestinal tissue were measured by mouse TNF α ELISA kit and mouse ADAM-17 ELISA kit (MyBioSource, San Diego, CA). The activity of ADAM-17 in the intestinal tissue was measured by SensoLyte 520 TACE (α -secretase) activity assay kit (AnaSpec, Fremont, CA) according to manufacturers' instructions.

Gut Syndecan-1—Small intestine was harvested and sections fixed with 10% buffered formaldehyde, then embedded in paraffin and sectioned. At least 3 sections from separate animals in each group were immunostained at the same setting with 1:100 rat anti-mouse syndecan-1 monoclonal antibody (BD Pharmingen, San Jose, CA), then incubated with 1:500 Alexa Fluor 488 goat anti-rat IgG (Invitrogen, Carlsbad, Calif). Images were obtained using an Olympus 1X71 microscope with SimplePCI6 software. The relative fluorescence intensity was quantified at 100x using Image J software (NIH) and reported as relative fluorescence units (RFU). Syndecan-1 protein was measured in the same samples using the *Mouse Syndecan-1 ELISA Kit* (Biobyte, San Francisco, CA).

Lung

Histopathology—Lungs were harvested and sections fixed with 10% buffered formaldehyde, then embedded in paraffin and sectioned. Sections were stained with hematoxylin and eosin, and then scored using a 3-point scale based on alveolar thickness, capillary congestion, and cellularity. The overall lung injury score was calculated by averaging these three indices of injury (22).

Inflammation—Infiltration of neutrophils was assessed by MPO immunofluorescence staining. Paraffin-embedded tissue was cut into 5- μ m-thick sections, then incubated with MPO primary antibody (1:100 MPO mouse monoclonal antibody; Abcam, Cambridge, Mass) followed by incubation with secondary antibody (goat anti-mouse; Alexa Fluor 568, Invitrogen, Carlsbad, Calif). Two random images were taken from each lung section with a fluorescent microscope (Nikon Eclipse Ti) at 200x and quantified using ImageJ software (National Institutes of Health). Results are reported as relative fluorescence units (RFU).

Syndecan-1, TNF α , and ADAM-17—To detect syndecan-1, lungs were sectioned and stained with rat anti-mouse syndecan-1 antibody (BD Biosciences, San Diego, Calif) and Alexa Fluor 488 goat anti-rat IgG (Invitrogen, Carlsbad, Calif) following deparaffinization and antigen retrieval with citrate buffer. Two random images were taken from each lung section with a fluorescent microscope at 100x and quantified using ImageJ software (National Institutes of Health)(14).

Pulmonary TNF α and ADAM-17 were measured by mouse TNF α ELISA kit and mouse ADAM-17 ELISA kit (MyBioSource, San Diego, CA). The activity of ADAM-17 in lung tissue was measured by SensoLyte 520 TACE (α -secretase) activity assay kit (AnaSpec, Fremont, CA) according to manufacturers' instructions. Lung MT-MMP-1 and MMP-9 were analyzed by Western blot.

Systemic Parameters—Blood was obtained at the time of sacrifice, and TNF α determined using the mouse TNF α ELISA kit (MyBioSource; San Diego, CA), ADAM17 using the Mouse A Disintegrin and Metalloproteinase 17 (ADAM17) ELISA Kit (MyBioSource, San Diego, CA) and syndecan-1 using the *Mouse Syndecan-1 ELISA Kit* (Biobyte, San Francisco, CA)

Statistical analysis—Data were analyzed by one-way ANOVA with Bonferroni correction. Data are expressed as mean \pm SEM with 8 mice/group.

RESULTS

Intraluminal TXA inhibits gut protease activity and mitigates gut and lung injury and inflammation

Intestinal protease activity was significantly reduced by TXA in sham treated animals (0.98 ± 0.13 ng trypsin/mg protein) compared to sham vehicle animals (1.68 ± 0.17 ng trypsin/mg protein). Hemorrhagic shock (3.60 ± 0.38 ng trypsin/mg protein) led to a marked increase in gut protease activity that was reduced to sham levels by intraluminal TXA (1.39 ± 0.30 ng trypsin/mg protein). Gut injury and inflammation (Figure 1) were markedly increased after hemorrhagic shock ($38.9 \pm 3.2\%$ injured villi and 1.37 ± 0.19 mU/mg MPO) compared to shams (injury: $1.7 \pm 0.6\%$ injured villi sham vehicle and $1.4 \pm 0.4\%$ injured villi sham TXA, $p > 0.05$ and inflammation: 0.25 ± 0.03 mU/mg MPO sham vehicle and 0.17 ± 0.04 mU/mg MPO TXA shams, $p > 0.05$) but was significantly reduced by intraluminal TXA ($11.8 \pm 2.0\%$ injured villi and 0.66 ± 0.09 mU/mg MPO).

In the lung (Figure 2), administration of intraluminal TXA was able to significantly lessen the lung histopathologic injury score (1.2 ± 0.2) compared to HS (1.8 ± 0.2) but remained increased compared to shams (0.17 ± 0.1 sham vehicle and 0.12 ± 0.1 shams TXA, $p > 0.05$). Hemorrhagic shock also led to a significant increase in lung inflammation ($816,040 \pm 61,658$ RFUs) compared to shams ($391,356 \pm 60,888$ RFUs sham vehicle and $3384,646 \pm 34,159$ RFUs sham TXA) but was significantly reduced by TXA ($530,917 \pm 71,632$ RFUs).

Intraluminal TXA inhibits intestinal ADAM-17 and TNF α , and restores intestinal syndecan-1—Intestinal ADAM-17 protein (Fig. 3A) was reduced by TXA in

sham treated animals (0.64 ± 0.14 ng/mg protein) compared to sham vehicles (1.14 ± 0.16 ng/mg protein). Hemorrhagic shock significantly increased ADAM-17 (2.24 ± 0.24 ng/mg protein) but protein levels were reduced by TXA (1.42 ± 0.12 ng/mg protein). To verify that protein levels reflected enzyme activity, ADAM-17 activity was also measured. As shown in Fig 3B, the trends were similar. ADAM-17 activity in sham animals treated with TXA was $49\pm 5\%$ vs sham vehicle at 100%. Hemorrhagic shock, however, resulted in an increase in activity to $446\pm 20\%$ while TXA administered after shock lessened activity to $237\pm 13\%$. Active TNF α was measured as an end product of ADAM-17 cleavage. As with ADAM-17, there was a significant increase in TNF α after hemorrhagic shock (34.13 ± 2.32 ng/mg protein) compared to shams (20.04 ± 0.92 ng/mg protein vehicle). TXA significantly reduced intestinal TNF α (23.98 ± 0.66 ng/mg protein) compared to HS animals. Similar to ADAM-17, TXA reduced TNF α in shams (20.04 ± 0.92 ng/mg protein vehicle and 14.49 ± 0.756 ng/mg protein sham TXA; $p<0.01$).

Lastly, we assessed the effect of TXA on intestinal syndecan-1 expression. As shown in Figure 4, syndecan-1 immunostaining was similar between shams ($100\pm 9\%$ sham vehicle vs $95\pm 8\%$ sham TXA, $p>0.05$), significantly reduced by HS ($39\pm 5\%$) but partially restored by TXA ($65\pm 8\%$). Protein levels were also measured and confirmed the results of immunostaining. Hemorrhagic shock reduced intestinal syndecan-1 (109 ± 10 pg/ml) compared to sham vehicle (240 ± 22 pg/ml) and sham TXA (230 ± 29 pg/ml) but was increased by TXA (179 ± 18 pg/ml). Importantly, unlike with ADAM-17 and TNF α , TXA did not alter syndecan-1 immunostaining or protein in shams.

Intraluminal TXA inhibits pulmonary ADAM-17 and TNF α and restores pulmonary syndecan-1—Similar to the intestine, pulmonary ADAM-17 protein was reduced by TXA in shams (1.04 ± 0.08 ng/mg protein sham-TXA vs 1.54 ± 0.06 ng/mg protein sham vehicle), significantly increased after hemorrhagic shock (2.41 ± 0.10 ng/mg protein) but reduced after TXA administration (1.79 ± 0.12 ng/mg protein)(Figure 5). Additionally, ADAM-17 activity (Figure 5) demonstrated comparable findings, with activity reduced by TXA in shams ($59\pm 4\%$) compared to sham vehicle (100%), elevated after hemorrhagic shock ($395\pm 19\%$), and lessened by TXA ($243\pm 13\%$). Although there was still a significant decrease by TXA, the reduction was not the same magnitude as in the gut.

For TNF α , there was a baseline amount in sham-vehicle animals (32.36 ± 4.03 ng/mg protein) that was reduced in the sham-TXA group (19.83 ± 1.66 ng/mg protein). The marked rise in TNF α after hemorrhagic shock (87.48 ± 4.80 ng/mg protein) was significantly reduced by TXA (47.76 ± 3.90 ng/mg protein).

As in the small bowel, pulmonary syndecan-1 immunostaining (Fig. 6) revealed that hemorrhagic shock ($484,698\pm 53,715$ RFU) resulted in a significant loss of cell-surface syndecan-1 compared to sham vehicle ($925,290\pm 47,593$ RFU) that was restored by TXA treatment ($857,961\pm 45,433$ RFU). Additionally, TXA had no effect on shams ($992,006\pm 62,749$ RFU) compared to sham vehicles ($p=0.41$).

Intraluminal TXA lessens systemic ADAM-17 and TNF α and reduces systemic syndecan-1 shedding—As expected, systemic levels of ADAM-17 and TNF α reflected

those of both the gut and lung (Table 1). Both ADAM-17 and TNF α were significantly increased after hemorrhagic shock compared to sham vehicle and sham TXA groups. Administration of TXA following shock resulted in a reduction of systemic levels of both. Similarly, protein levels of both were lessened by TXA in shams compared to vehicle treatment. Lastly, systemic syndecan-1 mirrored changes in the organs (Table 1). Higher systemic syndecan-1 was detected in plasma after hemorrhagic shock, reflecting lower levels in the lung and gut. Similarly, there was less shed syndecan-1 systemically after TXA treatment.

DISCUSSION

The important role of digestive enzymes in shock-induced gut dysfunction with subsequent systemic organ failure has been established by other investigators.(23-25). These studies led to concept of intestinal autodigestion from leakage of enzymes from the lumen into the wall of the intestine which then “autodigest” the intestinal wall (26). Using a model of pancreatic duct exteriorization, Fishman et al. recently determined that trauma/hemorrhagic shock alone is not sufficient to cause significant gut injury and dysfunction in the absence of intraluminal pancreatic proteases (27), highlighting the important role of these proteases in the systemic pro-inflammatory state following severe trauma.

In the current study we have shown that inhibition of intestinal proteases by the administration of intraluminal tranexamic acid following hemorrhagic shock reduces intestinal and distant organ (lung) histopathologic injury and inflammation, verifying and expanding on the work by DeLano et al.(8) Using an in vitro model of intestinal epithelial cells, Diebel et al recently demonstrated that TXA was protective to the mucus barrier function, suggesting an additional gut protective property of TXA.(28) We have additionally demonstrated for the first time that TXA’s protection is mediated in part by intestinal sheddase inhibition of ADAM-17 and by the intestinal shedding agonist, TNF α , both which preserved intestinal syndecan-1 and limited destruction of the intestine.

Ectodomain shedding of syndecan-1 is regulated by multiple signaling pathways converging on a diverse group of metalloproteinases, referred to as sheddases. The ADAM family comprises the largest group of these sheddases with metalloproteinases also playing a role (29). We sought to specifically investigate the role of intestinal sheddase inhibition as syndecan-1 has been showed to play a pathologic role after hemorrhagic shock and our recent work suggests that resuscitation strategies can differentially modulate syndecan-1 shedding (14,20,30,31). Whether intraluminal TXA represents an additional potential therapeutic to mitigate hemorrhagic shock –induced syndecan-1 shedding in patients is currently not known.

Although most studies on syndecan-1 refer to it as a marker of endothelial injury, it is actually found on both on endothelial and epithelial cells (32). ADAM17, also known as TNF α converting enzyme (TACE), as well as TNF α , are known modulators of syndecan-1 shedding. ADAM-17 is co-expressed with syndecan-1 on epithelial cells and TNF α is secreted by epithelial cells (11). TXA inhibited both ADAM-17 and TNF α , but not

syndecan-1, in sham treated animals suggesting that it acts to inhibit sheddase activity rather than at the level of syndecan-1.

The use of oral TXA as a gut protease inhibitor and an antifibrinolytic that lessens bleeding makes it an attractive agent to study for use in trauma. Studies to date have used intravenous TXA in patients in hemorrhagic shock.(33) However, the oral form can easily be translated to the battlefield as an early intervention for service members when timely evacuation is not possible or in civilian settings when transport times are prolonged in patients at risk for bleeding or in conjunction with the intravenous form in patients with active hemorrhage. Like intravenous TXA, oral TXA is also effective in controlling bleeding (34,35). Both the oral and IV forms are inexpensive, light weight, easy to administer, have minimal storage requirements and low volume requirements, and have good safety profiles. For oral therapy to be efficacious, adequate absorption from the gastrointestinal tract would need to occur and there is no data that the authors are aware of on the absorption of TXA in the setting of shock gut. However, we have shown in a model of severe gut ischemia/reperfusion that gut absorption can still occur, albeit at a slower rate (36). There are other limitations of the current model that could impair clinical translation. Studies to date have injected TXA directly into the small intestine and throughout its entire length and mixed in polyethylene glycol. (8,37) Fishman et al, when studying the effects of a mucus surrogate, injected polyethylene glycol into just the duodenum and demonstrated both gut (distal ileum) and lung protection (27), suggesting that direct contact of a protease inhibitor may not be required for protection. More proximal administration of TXA has not been tested and importantly, it is not known if oral (ie, gastric) administration would be effective in protecting the small intestine, particularly the ileum. Lastly the dose of enteral TXA used in the current study and the published literature (8) is higher than the FDA approved dose of oral TXA.

In summary, in a rodent model of hemorrhagic shock, intraluminal administration of TXA lessened gut and lung injury and inflammation. TXA inhibited gut activity of ADAM-17 and TNF α and lessened intestinal syndecan-1 shedding. Pulmonary and systemic inhibition followed the same pattern as the intestine. This study represents an early attempt to investigate the potential efficacy of oral TXA as a therapeutic adjunct to lessen gut and subsequent distant organ injury after hemorrhagic shock. Its use may be particularly relevant in patients or soldiers who may not have access to timely medical care.

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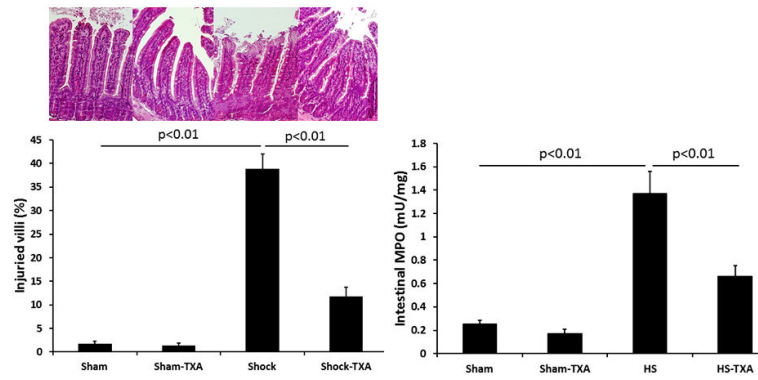


Figure 1. Intraluminal tranexamic acid reduces gut injury and inflammation following hemorrhagic shock

C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, small intestine was analyzed for histopathologic injury expressed as the % of injured villi with representative images shown. Inflammation was assessed by measuring myeloperoxidase activity. Results are expressed as mean \pm SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA= tranexamic acid

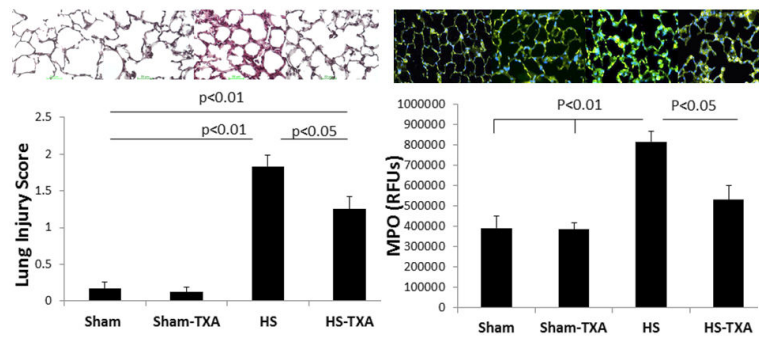


Figure 2. Intraluminal tranexamic acid reduces pulmonary injury and inflammation following hemorrhagic shock

C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, lungs were analyzed for histopathologic injury using a three point scoring system; representative photomicrographs are shown. Myeloperoxidase immunofluorescence staining was used to assess lung inflammation; representative images shown along with the corresponding images. Results are expressed as mean SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA- tranexamic acid; MPO=myeloperoxidase; RFU= relative fluorescent units

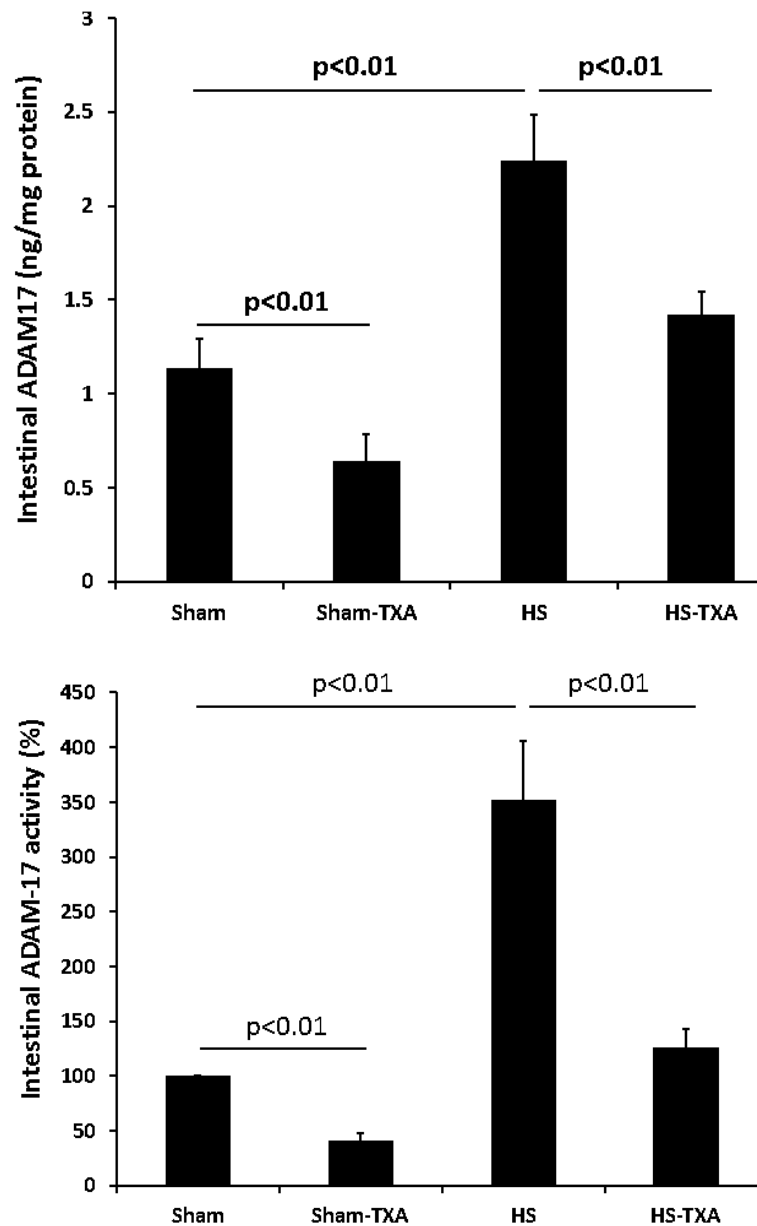


Figure 3. Intestinal ADAM-17 lessened by intraluminal tranexamic acid following hemorrhagic shock
 C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, small intestine was analyzed for A. Intestinal ADAM-17 protein and B. Intestinal ADAM-17 activity. Results are expressed as mean \pm SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA= tranexamic acid; ADAM-17= A disintegrin and metalloproteinase

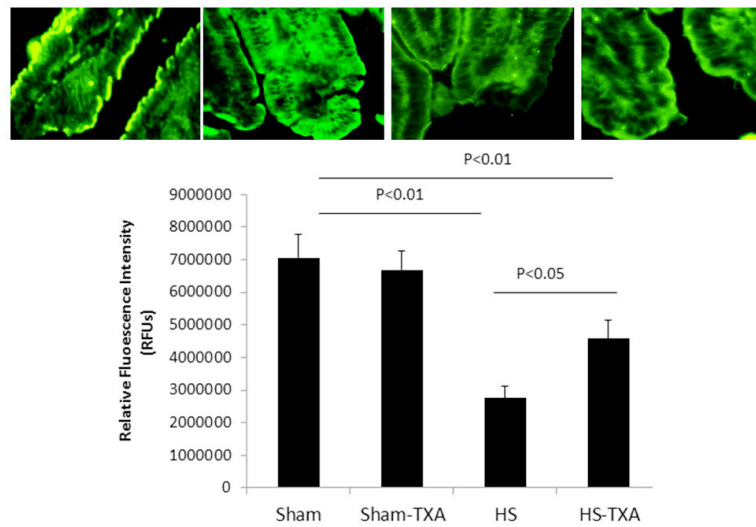


Figure 4. Intestinal syndecan-1 was preserved by intraluminal tranexamic acid following hemorrhagic shock

C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, small intestine was immunostained for intestinal syndecan-1. Shown are immunostained images (100x magnification) and the corresponding quantitation. Results are expressed as mean \pm SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA= tranexamic acid; RFU= relative fluorescent units

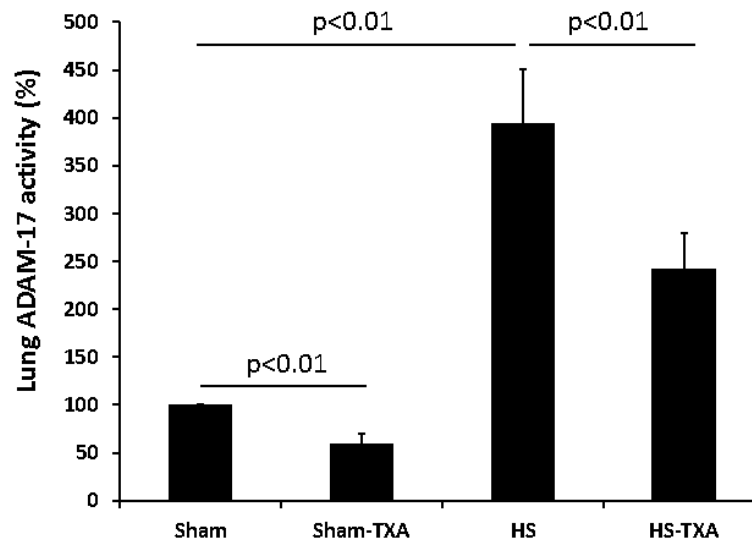


Figure 5. Pulmonary ADAM-17 is reduced by intraluminal tranexamic acid following hemorrhagic shock

C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, lungs were analyzed for A. Pulmonary ADAM-17 protein and B. Pulmonary ADAM-17 activity. Results are expressed as mean \pm SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA= tranexamic acid; ADAM-17= A disintegrin and metalloproteinase;

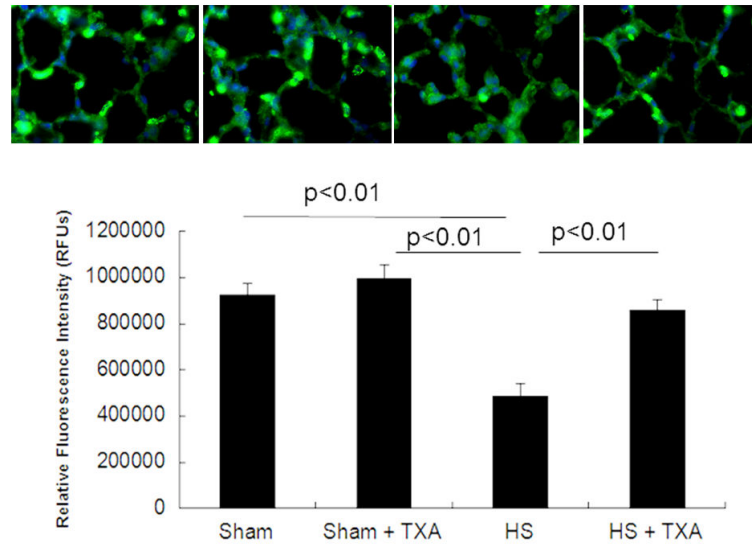


Figure 6. Pulmonary syndecan-1 is maintained by intraluminal tranexamic acid following hemorrhagic shock

C57BL/6J mice underwent 90 minutes of hemorrhagic shock followed by the intraluminal injection of tranexamic acid into the small intestine. Three hours later, lungs were analyzed for pulmonary syndecan-1. Shown are immunostained images (100x magnification) and the corresponding quantitation. Results are expressed as mean \pm SE; n=8/group. Abbreviations: HS= hemorrhagic shock; TXA= tranexamic acid; RFU= relative fluorescent units.

Table 1

Systemic parameters

	Sham-vehicle	Sham-TXA	HS	HS-TXA
ADAM17 (pg/ml)	1.97±0.10	1.40±0.16 [#]	4.75±0.48 ^{#*}	2.59±0.25 ^{#*^}
ADAM17 (%)	100%	49±5 [#]	446±22 ^{#*}	237±13 ^{#*^}
TNFα (pg/ml)	161±17	121±5 [#]	489±44 ^{#*}	321±48 ^{#*^}
Syndecan-1 (pg/ml)	204±13	211±12	321±29 ^{#*}	244±17 [^]

[#] p<0.05 vs sham-vehicle^{*} p<0.05 vs sham-TXA[^] p<0.05 vs HS; % indicates ADAM-17 activity as a % of sham-vehicles. Results are presented as mean±SEM.