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Mesenteric lymph diversion abrogates 5-lipoxygenase activation in the kidney following trauma and hemorrhagic shock

John R. Stringham, MD, Ernest E. Moore, MD, Fabia Gamboni, PhD, Jeffrey N. Harr, MD, MPH, Miguel Fragoso, DVM, Theresa L. Chin, MD, Caitlin E. Carr, Christopher C. Silliman, MD, PhD, and Anirban Banerjee, PhD

Department of Surgery (J.R.S., E.E.M., F.G., J.N.H., T.L.C., A.B.), University of Colorado–Denver; Denver Health Medical Center (J.R.S., E.E.M., J.N.H., M.F., T.L.C.); and Bonfils Blood Center (C.C.S.), Denver Colorado

Abstract

BACKGROUND—Early acute kidney injury (AKI) following trauma is associated with multiorgan failure and mortality. Leukotrienes have been implicated both in AKI and in acute lung injury. Activated 5-lipoxygenase (5-LO) colocalizes with 5-LO-activating protein (FLAP) in the first step of leukotriene production following trauma and hemorrhagic shock (T/HS). Diversion of postshock mesenteric lymph, which is rich in the 5-LO substrate of arachidonate, attenuates lung injury and decreases 5-LO/FLAP associations in the lung after T/HS. We hypothesized that mesenteric lymph diversion (MLD) will also attenuate postshock 5-LO-mediated AKI.

METHODS—Rats underwent T/HS (laparotomy, hemorrhagic shock to a mean arterial pressure of 30 mm Hg for 45 minutes, and resuscitation), and MLD was accomplished via cannulation of the mesenteric duct. Extent of kidney injury was determined via histology score and verified by urinary neutrophil gelatinase-associated lipocalin assay. Kidney sections were immunostained for 5-LO and FLAP, and colocalization was determined by fluorescence resonance energy transfer signal intensity. The end leukotriene products of 5-LO were determined in urine.

RESULTS—AKI was evident in the T/HS group by derangement in kidney tubule architecture and confirmed by neutrophil gelatinase-associated lipocalin assay, whereas MLD during T/HS preserved renal tubule morphology at a sham level. MLD during T/HS decreased the associations

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Address for reprints: John R. Stringham, MD, University of Colorado–Denver, Department of Surgery–Trauma Research Center, 12700 E 19th Ave, Room 6102, Mail Stop C320, Aurora, CO 80045; john.stringham@ucdenver.edu; Ernest E. Moore, MD, Denver Health Medical Center–Department of Surgery, 777 Bannock St, Denver, CO 80204; ernest.moore@dhha.org.

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AUTHORSHIP

A.B., E.E.M., C.C.S. and J.R.S. designed this study. J.R.S., J.N.H., and M.F. performed all animal experiments. F.G. and J.R.S. performed all microscopy. T.L.C. and J.R.S. performed the data analysis. E.E.M., A.B., C.C.S., J.R.S., and T.L.C. performed the data interpretation. J.R.S., E.E.M., C.C.S., A.B., and T.L.C. prepared the manuscript and figures.

DISCLOSURE

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between 5-LO and FLAP demonstrated by fluorescence resonance energy transfer microscopy and decreased leukotriene production in urine.

CONCLUSION—5-LO and FLAP colocalize in the interstitium of the renal medulla following T/HS. MLD attenuates this phenomenon, which coincides with pathologic changes seen in tubules during kidney injury and biochemical evidence of AKI. These data suggest that gut-derived leukotriene substrate predisposes the kidney and the lung to subsequent injury.

Keywords

Acute kidney injury; trauma; hemorrhagic shock; mesenteric lymph diversion; leukotrienes; rats

Recent data indicate that patients with evidence of acute kidney injury (AKI) within 2 days following trauma had a 19-fold increased risk of developing multiorgan failure and a 6-fold increase in mortality.¹ Despite improvements in the management of AKI during the last half century, including the common use of renal replacement therapy, AKI-associated mortality rates approach 60%.² There has been considerable research in organ cross-talk during the past decade with a clear link between gut ischemia-reperfusion (I/R) and acute lung injury (ALI)³ and new evidence relating AKI to ALI. Kidney I/R promotes adaptive and innate immune responses, and has been found to increase apoptosis in the lung.⁴ While some evidence exists for intestinal changes following kidney injury,⁵ there has been little investigation into the direct mechanistic link of gut I/R and resultant AKI.

It is postulated that AKI following traumatic injury is driven by an I/R injury and is mediated by polymorphonuclear cells (PMNs).⁶ Leukotrienes, most notably leukotriene B₄ (LTB₄), are potent PMN chemoattractants and priming agents and have been implicated in the derangement in kidney function following renal I/R.⁷ Leukotrienes have also been shown to be a key mediator in the development of ALI following the global ischemic state that exists after hemorrhagic shock.^{8–11} Intestinal I/R plays a central role in the development of ALI because leukotrienes are synthesized from arachidonate, which has been found in abundance in postshock mesenteric lymph (PSML).¹² The first step in the conversion of arachidonate into bioactive eicosanoids is performed by the enzyme 5-lipoxygenase (5-LO); this process requires the association of 5-LO to the cofactor 5-LO-activating protein (FLAP).¹³ Diversion of mesenteric lymph arrests this rich flow of substrate to 5-LO in the lung, preventing leukotriene synthesis and thus attenuating the lung injury seen after injury.

To visualize when the association between FLAP and 5-LO occurs, fluorescence resonance energy transfer (FRET) microscopy can be used. A FRET signal will occur when a wavelength of light excites a donor fluorescently tagged antibody, causing it to emit a second wavelength of light. If this donor fluorophore is near a second acceptor fluorophore, the emitted wavelength of light can excite the acceptor, which will emit a third wavelength. This last wavelength is the FRET signal; this signal can only occur when the two fluorescently marked antibodies are closely associated with each other. We have previously demonstrated the close association of FLAP and 5-LO in lung tissue following trauma and hemorrhagic shock (T/HS). Since products of 5-LO have been implicated in AKI following I/R,¹⁴ these same associations between FLAP and 5-LO may be seen in the kidney, further demonstrating the deleterious effects of leukotrienes in early AKI.

While the role of mesenteric lymph in the progression of ALI and the production of 5-LO products has been investigated previously, the effect of mesenteric lymph diversion (MLD) on leukotriene production in the kidney has yet to be elucidated. Therefore, we hypothesize that diversion of mesenteric lymph before T/HS will abrogate the colocalization of 5-LO and FLAP in the kidney, thus preventing the production of PMN-priming leukotrienes.

MATERIALS AND METHODS

All animal experiments were performed using the recommendations of the Guide for the Care and Use of Laboratory Animals and in accordance with the guidelines of the University of Colorado-Denver Institutional Animal Care and Use Committee. All animals were purchased from Harlan Laboratories (Indianapolis, IN) and were supplied ad libitum with food and water. Animals were housed with 12-hour light-dark cycles and were acclimated for at least 1 week before experimentation.

T/HS Model

Three male Sprague-Dawley rats weighing from 350 g to 425 g were injected intraperitoneally with 50-mg/kg sodium pentobarbital (Abbott Labs, Chicago, IL). Lidocaine was then injected subcutaneously for local anesthesia. PE-50 polyethylene tubing (Baxter Healthcare, Deerfield, IL) was inserted into the femoral artery and vein, and the arterial line was then used for continual monitoring of blood pressure. Rectal temperature was assessed and animals were kept euthermic throughout the procedure. A tracheostomy was created, and the animals were placed on 30% FIO₂ at a rate of 2 L/min using an air-oxygen mixer (Sechrist, Anaheim, CA). The rats were observed for 45 minutes, before the simulation of a traumatic injury with a midline laparotomy. This was then closed in two layers with a running 3-0 nonabsorbable monofilament suture. Animals then underwent controlled exsanguination of approximately 40% to 50% of estimated blood volume via the arterial line over 10 minutes to achieve a mean arterial blood pressure of 30 mm Hg. Shed blood (SB) was collected with 80-U/kg heparin sulfate. Hypotension was maintained for 45 minutes. To ensure that all animals developed a consistent level of shock, arterial blood gas was taken and base deficit was ensured to be between 18 and 23 at time of end shock. Animals were then resuscitated with a volume of normal saline equal to twice the SB volume over 30 minutes via the venous line, followed by SB resuscitation (half of the SB volume) for 30 minutes. Animals then received another hour of resuscitation with twice the SB volume in normal saline. Following a final hour of resuscitation, animals were sacrificed via pentobarbital overdose. After reopening the laparotomy, kidneys were collected and bisected in the sagittal direction and then embedded in OCT medium before freezing.

MLD

To perform MLD, the same procedure was performed as in the T/HS described earlier. However, following laparotomy, the bowel was rotated to the left and the two mesenteric ducts were identified adjacent to the superior mesenteric artery. One duct was tied off using 7-0 nonabsorbable monofilament suture, while the other was cannulated with PE-10 polyethylene tubing secured with the 7-0 monofilament suture. This cannula was then brought through the skin of the flank, and the lymph was allowed to collect on ice. The

bowel was reapproximated, the laparotomy incision was closed, and the animal then underwent the same hemorrhagic shock and resuscitation as described earlier. A total of three animals underwent MLD followed by hemorrhagic shock.

Control and Sham Animals

Control animals (n = 3) received pentobarbital overdose and were immediately sacrificed. Trauma/sham shock (T/SS) animals (n = 3) underwent anesthesia with pentobarbital, femoral vessel cannulation, and laparotomy but did not undergo hemorrhage and resuscitation. T/SS animals were maintained under anesthesia for 3 hours until sacrifice.

FRET Microscopy

Frozen sections of the kidneys were cut 5 μ m thick and placed on glass slides. These were then permeabilized with a mixture of 70% acetone and 30% methanol for 10 minutes. After drying thoroughly, slides were then washed three times in phosphate-buffered saline (PBS) and then blocked with 10% normal donkey serum in PBS for 1 hour. The slides were then incubated overnight at 4°C with primary antibodies for 5-LO and FLAP and isotype controls. After an additional three washes in PBS, antispecies fluorescent secondary antibodies were then added along with fluorescently conjugated wheat germ agglutinin and then incubated in the dark at room temperature for 1 hour. After another round of three PBS washes, coverslips were mounted using Prolong Gold with DAPI solution (Life Technologies, Carlsbad, CA).

5-LO was labeled with the primary mouse anti-5-LO antibody (BD Biosciences, San Jose, CA). FLAP was labeled with rabbit anti-FLAP antibody (Santa Cruz Biotechnologies, Santa Cruz, CA). Species-specific isotype antibodies (normal mouse IgG, normal rabbit IgG) were obtained from Jackson Immuno Research (West Grove, PA). To secondarily label 5-LO, donkey antimouse Alexa Fluor-555 (red) was used, while to label FLAP, a donkey antirabbit Alexa Fluor-488 (green) was used. Tissue of epidermal origin was stained with Alexa Fluor-633-conjugated wheat germ agglutinin (far red), which stains glycoproteins.

Images of renal tubules were obtained using a 65 $\times$ objective on a Zeiss Axio Observer Z1 microscope using a Chroma Multiple Bandpass filter wheel and Sutter filter that was controlled by Slidebook version 5.0 software (Intelligent Imaging Innovations, Denver, CO). FRET signal was determined for each image. Briefly, the fluorescence energy of a donor dye may be absorbed and then reemitted at a different wavelength by an acceptor fluorophore. This reemitted wavelength can be measured and is the FRET signal. With the use of fluorescently labeled secondary antibodies, the maximal distance when a FRET signal can occur between two proteins of interest is less than 30 nm.¹⁵

Histologic Evaluation

Finely cut (5 μ m thick) frozen kidney sections were stained with hematoxylin and eosin (HE) and then viewed under a light microscope (Eclipse 55i, Nikon, Tokyo, Japan) at 20 $\times$. To score renal tubule histology, the methodology described by Thiemermann et al.¹⁶ was used. In brief, a 25-square graticule was laid over random images of the kidney. Tubules where the lines of the graticule intersected were evaluated and scored on a scale from 0 to 3.

A score of 0 was awarded to normal tubule histology. A score of 1 was given to tubules with nuclear condensation, loss of epithelial brush border, and up to one third of nuclear loss in tubular profile. A score of 2 involved greater than one-third but less than two-thirds nuclear loss, and a 3 was given for greater than two-thirds loss of nuclei in the profile of the tubule. One hundred tubules per kidney were evaluated and scored by a single investigator (J.R.S). The total histology score was obtained by calculating the sum of all tubule scores per kidney. The maximum score that could be obtained by one kidney was thus 300.

Urine Biochemical Parameter Measurement

Urine was obtained at the end of each experiment via aspiration of the bladder with a 25-gauge needle. This was immediately flash frozen until additional analysis could be performed. To determine 5-LO activity in the kidney, urine cysteinyl leukotriene measurements were performed by EIA (GE Healthcare Life Sciences, Piscataway, NJ). Since large amounts of neutrophil gelatinase-associated lipocalin (NGAL) is made in the renal tubules following renal injury in the immediate period,¹⁷ urine NGAL levels were measured as a marker of acute renal injury. NGAL levels were determined using a commercially available colorimetric enzyme-linked immunosorbent assay kit (Abcam, Cambridge, MA).

Statistical Analysis

All data are reported as the mean $\pm$ SEM and compared for significance using one-way analysis of variance with post hoc analysis via the Tukey-Kramer method.

RESULTS

MLD Attenuates AKI Following T/HS

Twelve rats were subjected to control, T/SS, T/HS, or MLD + T/HS ($n = 3$ for each group). Histologic examination of HE-stained kidney sections (Fig. 1) revealed very minimal perturbation in normal kidney histology in control animals. While there were some changes noted in the T/SS animals, including epithelial brush border loss and tubular contraction, the T/HS kidneys showed significant histologic derangement in almost all portions of the kidney. These changes included tubule contraction with almost complete loss of the epithelial brush border, tubule dilation and degeneration, and luminal congestion. The kidneys from animals that underwent MLD + T/HS reverted to a T/SS phenotype. With the use of the histology scoring system described by Thiernemann et al.,¹⁶ the T/HS kidneys displayed a significant increase in histology score over T/SS animals (175.7 ± 19.5 vs. 49.7 ± 6.9 , respectively; $p < 0.001$), supporting the conclusion that our model of hemorrhagic shock and trauma induces significant AKI (Fig. 2). In contrast, MLD + T/HS significantly decreased the extent of renal tubule injury caused by T/HS alone (67.7 ± 4.6 , $p < 0.001$), showing that diversion of postshock lymph could diminish the extent of early AKI following severe injury.

To verify the extent of kidney injury following treatment, urinary NGAL was examined via a commercially available enzyme-linked immunosorbent assay kit (Fig. 3). Serum creatinine was not performed in these animals because the timing from end shock to blood collection

and animal sacrifice was only 3 hours. It was felt that this was not enough time for creatinine to rise to a significant level. A significant rise in urinary NGAL, however, has been found previously after only 2 hours following I/R.¹⁷ In our model, T/HS demonstrated a 4-fold increase over control animals (768.9 ± 103.7 ng/mL vs. 172.4 ± 47.7 ng/mL, $p < 0.001$) and a 2.5-fold increase over T/SS (307.5 ± 62.7 ng/mL, $p = 0.015$), suggesting ischemic injury to the kidney tubules consistent with early AKI. MLD decreased urine NGAL levels 1.5-fold (464.6 ± 24.6 ng/mL), and while this was a sizeable diminution in NGAL levels, this did not reach statistical significance.

5-LO and FLAP Colocalize After T/HS

To determine if 5-LO is active in renal tissue following T/HS, the kidney was examined for colocalization of 5-LO and FLAP via FRET microscopy. While all four groups showed similar intensities of 5-LO and FLAP stain, only the T/HS kidneys demonstrated increased FRET signal intensity (Fig. 4). Individual stains for 5-LO and FLAP can be found in supplemental data online (<http://links.lww.com/TA/A407>). Specifically, intense colocalization signal was seen in the lumen of the kidney and in the interstitial spaces. This increased signal was quenched by diverting lymph before hemorrhagic shock. A quantitative analysis of the total FRET mean intensity (defined as mean intensity $\times$ area) is shown in Figure 5. This demonstrated a greater than 50-fold increase in total FRET intensity in the T/HS animals ($1.05 \times 10^9 \pm 1.87 \times 10^8$) compared with control ($1.45 \times 10^7 \pm 4.94 \times 10^6$, $p < 0.001$) or T/SS ($1.99 \times 10^7 \pm 6.61 \times 10^6$, $p < 0.001$). MLD decreased the total signal intensity by almost eightfold ($1.36 \times 10^8 \pm 3.85 \times 10^7$, $p < 0.001$).

MLD Before T/HS Halts the Urinary Excretion of Leukotrienes

To determine if these 5-LO and FLAP complexes are active in the T/HS group, urine cysteinyl leukotrienes were measured (Fig. 6). T/HS induced a sevenfold increase in leukotriene excretion in the urine versus T/SS (110.5 ± 27.1 pg/mL vs. 15.3 ± 1.1 pg/mL, $p = 0.008$). MLD decreased the amount of cysteinyl leukotrienes measured to below sham levels (7.2 ± 3.1 pg/mL, $p = 0.006$ vs. T/HS), suggesting that the diversion of mesenteric lymph will prevent the production of bioactive lipids made by activated 5-LO.

DISCUSSION

In this study, we demonstrated that immediately following T/HS, 5-LO and FLAP colocalize in the kidney interstitium and tubule lumen. When these two molecules colocalize, they become active and create end products of arachidonate metabolism. Diversion of the PSML from the circulation diminishes the 5-LO and FLAP associations in the kidney and abrogates leukotriene excretion into the urine. Moreover, MLD attenuates the extent of kidney injury that occurs with T/HS, decreasing the urinary AKI-marker NGAL and preventing the derangement in tubule architecture to a morphology seen with trauma alone.

The link between gut ischemia and lung injury following trauma has been intensely investigated but by no means is fully understood. Following ischemia and reperfusion, phospholipase A₂ activity increases in the intestinal mucosa,¹⁸ releasing an increased amount of arachidonate into the gut lymphatics.¹² This free arachidonate can travel to the

lung, providing the substrate for 5-LO to begin the first step in leukotriene synthesis. When PSML cannot reach the lung, markers of ALI are strongly attenuated, and when this toxic lymph is transfused into an uninjured animal, ALI will be seen.³

The mechanistic link between MLD and AKI remains to be elucidated. These data are not the first to suggest that diversion of PSML may provide a protective effect on the kidney.¹⁹ While diversion of PSML will prevent the release of serine proteases,²⁰ cytokines,²¹ and other inflammatory mediators, it will also prevent the release of 5-LO's substrate, arachidonate, which is prevalent in PSML.¹² The data presented here suggest that by not allowing 5-LO to be activated, AKI will be attenuated. Arachidonate will engage the LTB₄ receptor and can cause LTB₄ to be produced in an autocrine manner.²² Thus, the release of increased amounts of arachidonate from PSML may be what is responsible for activating the 5-LO/FLAP complex. Moreover, following MLD, the active complexes of 5-LO and FLAP are not seen in the kidney interstitium and tubule. Thus, a possible mechanism involving leukotriene synthesis is strongly suggested. Alternatively, an unknown factor in lymph may be activating the 5-LO/FLAP system, and the additional arachidonate supplied by PSML is rapidly converted by 5-LO.

The emerging concept of organ cross-talk may be illustrated in this model of AKI. T/HS have been shown to cause a robust ALI, which can be attenuated via diversion of toxic postshock lymph.²³ While some studies have attempted to link ALI to resultant AKI, most have highlighted the problems inherent with mechanical ventilation rather than ALI itself.

No matter the mechanism, the role of MLD and the resultant attenuation of AKI demands that this link be better understood. Because the data in this study suggest that active 5-LO plays a role in the development of AKI and mesenteric lymph causes the colocalization of 5-LO with its activator FLAP, strategies to disable the signal that activates 5-LO could be implemented early following trauma and shock, thus improving outcomes. Early AKI, while rare in patients following severe trauma, has an 82% likelihood of progression to multiorgan failure and an increased likelihood of death occurring from that early renal insult.¹ Mortality in all critically ill patients with severe AKI necessitating renal replacement therapy can have a mortality rate in excess of 60%.²⁴ Further study to better understand the mechanisms of the phenomena highlighted here may offer new therapeutic strategies to improve the outcome of critically injured patients.

References

1. Wohlaer MV, Sauaia A, Moore EE, Burlew CC, Banerjee A, Johnson J. Acute kidney injury and posttrauma multiple organ failure: the canary in the coal mine. *J Trauma Acute Care Surg.* 2012; 72:373–379. [PubMed: 22327979]
2. Uchine S, Kellum JA, Bellomo R, et al. Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA.* 2005; 294:813–818. [PubMed: 16106006]
3. Wohlaer MV, Moore EE, Harr J, Eun J, Fragoso M, Banerjee A, Silliman CC. Cross-transfusion of postshock mesenteric lymph provokes acute lung injury. *J Surg Res.* 2011; 170:314–318. [PubMed: 21550053]
4. White LE, Cui Y, Shelak CM, Lie ML, Hassoun HT. Lung endothelial cell apoptosis during ischemic acute kidney injury. *Shock.* 2012; 38:320–327. [PubMed: 22777112]

5. Rabb H, Wang Z, Postler G, Soleimani M. Possible molecular basis for changes in potassium handling in acute renal failure. *Am J Kidney Dis.* 2000; 35:871–877. [PubMed: 10793021]
6. Awad AS, Ruse M, Huang L, et al. Compartmentalization of neutrophils in the kidney and lung following acute ischemic kidney injury. *Kidney Int.* 2009; 75:689–698. [PubMed: 19129795]
7. Noiri E, Yokomizo T, Nakao A, et al. An in vivo approach showing the chemotactic activity of leukotriene B₄ in acute renal ischemic-reperfusion injury. *Proc Nat Acad Sci U S A.* 2000; 97:823–828.
8. Karasawa A, Guo JP, Ma XL, Tsao PS, Lefer AM. Protective actions of a leukotriene B₄ antagonist in splanchnic ischemia and reperfusion in rats. *Am J Physiol Gastrointest Liver Physiol.* 1991; 261:G191–G198.
9. Kurose I, Argenbright LW, Wolf R, Lianxi L, Granger DN. Ischemia/reperfusion-induced microvascular dysfunction: role of oxidants and lipid mediators. *Am J Physiol Heart Circ Physiol.* 1997; 272:H2976–H2982.
10. Souza DG, Coutinho SF, Silveira MR, Cara DC, Teixeira MM. Effects of a BLT receptor antagonist on local and remote reperfusion injuries after transient ischemia of the superior mesenteric artery in rats. *Eur J Pharmacol.* 2000; 403:121–128. [PubMed: 10969152]
11. Eun JC, Moore EE, Mauchley DC, et al. The 5-lipoxygenase pathway is required for acute lung injury following hemorrhagic shock. *Shock.* 2012; 37:599–604. [PubMed: 22392149]
12. Jordan JR, Moore EE, Sarin EL, Damle SS, Kasuk SB, Silliman CC, Banerjee A. Arachidonic acid in postshock mesenteric lymph induces pulmonary synthesis of leukotriene B₄. *J Appl Physiol.* 2008; 104:1161–1166. [PubMed: 18276905]
13. Murphy RC, Gijon MA. Biosynthesis and metabolism of leukotrienes. *Biochem J.* 2007; 405:379–395. [PubMed: 17623009]
14. Patel NS, Cuzzocrea S, Chatterjee PK, Di Paola R, Sautebin L, Britti D, Thiemermann C. Reduction of renal ischemia-reperfusion injury in 5-lipoxygenase knockout mice and by the 5-lipoxygenase inhibitor zileuton. *Mol Pharmacol.* 2004; 66:220–227. [PubMed: 15266012]
15. Scholes GD. Long-range resonance energy transfer in molecular systems. *Ann Rev Phys Chem.* 2003; 54:57–87. [PubMed: 12471171]
16. Thiemermann C, Patel NSA, Kvale EO, Cockerill GW, Browman PAJ, Stewart KN, Cuzzocrea S, Britti D, Mota-Philipe H, Chatterjee PK. High density lipoprotein (HDL) reduces renal ischemia/reperfusion injury. *J Am Soc Nephrol.* 2003; 14:1833–1843. [PubMed: 12819243]
17. Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol.* 2003; 14:2534–2543. [PubMed: 14514731]
18. Otamiri T, Franzén L, Lindmark D, Tagesson C. Increased phospholipase A2 and decreased lysophospholipase activity in the small intestinal mucosa after ischaemia and revascularisation. *Gut.* 1987; 28:1445–1453. [PubMed: 3428670]
19. Niu CY, Zhao ZG, Ye YL, Hou YL, Zhang YP. Mesenteric lymph duct ligation against renal injury in rats after hemorrhagic shock. *Ren Fail.* 2010; 32:584–591. [PubMed: 20486842]
20. Deitch EA, Shi HP, Lu Q, Feketeova E, Xu DZ. Serine proteases are involved in the pathogenesis of trauma-hemorrhagic shock-induced gut and lung injury. *Shock.* 2003; 19:452–456. [PubMed: 12744489]
21. Cavriani G, Domingos HV, Oliveira-Filho RM, Sudo-Hayashi LS, Vargaftig BB, de Lima WT. Lymphatic thoracic duct ligation modulates the serum levels of IL-1 β and IL-10 after intestinal ischemia/reperfusion in rats with the involvement of tumor necrosis factor α and nitric oxide. *Shock.* 2007; 27:209–213. [PubMed: 17224798]
22. Surette ME, Krump E, Picard S, Borgeat P. Activation of leukotriene synthesis in human neutrophils by exogenous arachidonic acid: inhibition by adenosine A_{2a} receptor antagonists and crucial role of autocrine activation by leukotriene B₄. *Mol Pharmacol.* 1999; 56:1055–1062. [PubMed: 10531413]
23. Deitch EA, Adams C, Lu Q, Xu DZ. A time course study of the protective effect of mesenteric lymph duct ligation on hemorrhagic shock-induced pulmonary injury and the toxic effects of shocked rats on endothelial cell monolayer permeability. *Surgery.* 2001; 129:39–47. [PubMed: 11150032]

24. Metnitz PG, Krenn CG, Steltzer H, Lang T, Ploder J, Lenz K, Le Gall JR, Druml W. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Crit Care Med.* 2002; 30:2051–2058. [PubMed: 12352040]

Figure 1.

Representative images of renal tubule histology in control, T/SS, T/HS, and MLD + T/HS rats. Normal renal tubule histology in control rat, with minimal loss of epithelial brush border, noncondensed nuclei, and minimal to no tubule collapse. T/SS kidney tubule histology demonstrating little brush border loss and some tubule collapse but with noncondensed nuclei. T/HS kidney shows severe tubular dilation (*TD*), tubular degradation, loss of all epithelial brush borders, nuclear collapse and nuclear loss, luminal congestion (*LC*), and tubular collapse (*TC*). MLD + T/HS kidney histology shows reversion to T/SS morphology, with none of the markers of tissue derangement seen in T/HS alone.

Figure 2.

Histology score in control, T/SS, T/HS, and MLD +T/HS. Renal tubules (n = 100) from random HE kidney sections of the four groups were scored for tubule morphology on a scale from 0 to 3, with a maximum score generated for each kidney of 300; see Materials and Methods section). T/HS demonstrated global tubular damage, while MLD was protective. * $p < 0.001$.

Figure 3.

NGAL measured in urine of control, T/SS, T/HS and MLD + T/HS rats. T/HS induces a significant increase in the AKI marker NGAL within 3 hours of end shock. MLD before hemorrhagic shock will reduce urinary NGAL levels; however, this did not reach statistical significance. * $p < 0.05$ compared with T/HS.

Figure 4.

5-LO, FLAP and 5-LO/FLAP colocalization (FRET) in kidney. In all images, glycoprotein stain is gray. These images demonstrate FRET signal in a pseudocolor intensity scale. Increasing intensity of FRET signal results in a color spectrum change, with the most intense FRET signal displayed in red. This demonstrates the close associations between 5-LO and its cofactor FLAP in the T/HS group, which is not seen in any other cohort. The images also display total fluorescent intensities.

Figure 5.

Total 5-LO/FLAP colocalization (FRET) intensity in control, T/SS, T/HS and MLD + T/HS, kidney images. FRET microscopy displayed in Figure 3. Total FRET signal (mean signal intensity × area) displayed in this figure. T/HS causes a significant increase in FRET signal versus control or T/SS, showing that FLAP and 5-LO are associated following T/HS. This signal is abrogated when mesenteric lymph is diverted before T/HS. *ALUFI*, arbitrary linear units of fluorescence intensity. * $p < 0.001$ versus all other groups.

Figure 6.

Urinary cysteinyl leukotrienes in control, T/SS, T/HS, and MLD + T/HS. T/HS induces the increased excretion of 5-LO products in the urine. MLD will prevent this phenomenon from occurring. * $p < 0.05$ versus all other groups.