Journal of Trauma and Acute Care Surgery, Publish Ahead of Print
DOI: 10.1097/TA.0000000000002868

Impact of uric acid on liver injury and intestinal permeability following resuscitated hemorrhagic shock in rats

François Khazoom MD[1], Sydnée L'Écuyer MSc[1,2], Kim Gilbert PhD[3], Marc-André Gagné MSc[1,3], Caroline Bouchard[3], Christopher F. Rose PhD[1,2], Guy Rousseau PhD[1,3] and Emmanuel Charbonney MD PhD[1,2,3]

[1]Medicine Faculty, Université de Montréal**;** [2]Hepato-Neuro Laboratory, Centre Hospitalier Universitaire de Montréal Research Center, Université de Montréal**;** [3]Hôpital du Sacré-Cœur de Montréal Research Center

E-mail addresses for all authors: Francois Khazoom (francois.khazoom@umontreal.ca); Sydnée L'Écuyer (sydnee.lecuyer@hotmail.com); Kim Gilbert (kim.gilbert3@gmail.com); Marc-André Gagné (marc_andre_96@hotmail.com); Caroline Bouchard (c-bouchard@crhsc.rtss.qc.ca); Christopher F. Rose (christopher.rose@umontreal.ca); Guy Rousseau (guy.rousseau@umontreal.ca); Emmanuel Charbonney (echarbonney@gmail.com)

Corresponding author:

Francois Khazoom

Phone: 438-391-9644

5400 Boul. Gouin Ouest

H4J 1C5, Montréal, Quebec, Canada

francois.khazoom@umontreal.ca

Address for reprints: Same as corresponding author

Support/Conflict of interest: This research was funded locally by the research center, the department of critical care and trauma of the Hôpital du Sacré-Cœur de Montréal. E. Charbonney received financial support from Sanofi Genzyme Canada for his research program.

ABSTRACT

Background: Multi-organ failure is a consequence of severe ischemia-reperfusion injury after traumatic hemorrhagic shock, a major cause of mortality in trauma patients. Circulating uric acid, released from cell lysis, is known to activate pro-inflammatory and pro-apoptotic pathways and has been associated with poor clinical outcomes among critically ill patients. Our group has recently shown a mediator role for uric acid in kidney and lung injury, but its role in liver and enteric damage after hemorrhagic shock remains undefined. Therefore, the objective of this study was to evaluate the role of uric acid on liver and enteric injury after resuscitated hemorrhagic shock.

Methods: A murine model of resuscitated hemorrhagic shock was treated during resuscitation with a recombinant uricase, a urate oxidase enzyme (rasburicase, Sanofi), to metabolize and reduce circulating uric acid. Biochemical analyses (liver enzymes, liver apoptotic and inflammatory markers) were performed at 24h and 72h after hemorrhagic shock. Physiological testing for enteric permeability and gut bacterial product translocation measurement (plasma endotoxin) were performed 72h after hemorrhagic shock. *In vitro,* HT-29 cells were exposed to UA, and the expression of intercellular adhesion proteins (ZO-1, e-cadherin) was measured to evaluate the influence of uric acid on enteric permeability.

Results: The addition of Uricase to resuscitation significantly reduced circulating and liver uric acid levels after hemorrhagic shock. It also prevented hemorrhagic shock-induced hepatolysis and liver apoptotic/inflammatory mediators at 24h and 72h. Hemorrhagic shock-induced enteric hyperpermeability and endotoxemia were prevented with uricase.

Conclusions: After resuscitated hemorrhagic shock, uric acid is an important mediator in liver and enteric injury. Uric acid represents a therapeutic target to minimize organ damage in polytrauma patients sustaining hemorrhagic shock.

Level of evidence: Foundational research

Study type: Animal study

Keywords: Hemorrhagic shock, organ failure, danger-associated molecular patterns, uric acid.

BACKGROUND

Hemorrhagic shock (HS) is a leading cause of severe systemic ischemia-reperfusion in critically injured patients[1]. While HS leads to early death, multi-organ dysfunction and sepsis are responsible for late mortality and morbidity among resuscitated survivors[2, 3]. Aside from organ support and treatment of infections, no interventions have demonstrated to be beneficial in preventing organ dysfunction in injured patients sustaining HS. This unmet clinical need warrants new therapeutic approaches. While wide suppression of the immune system is potentially hazardous in the context of ongoing tissue repair and increased risk of sepsis[4, 5], targeting danger associated molecular patterns (DAMPS), key upstream initiators of the sterile inflammatory response observed in injured patients, may prove beneficial[6, 7].

Direct tissue damage and ischemia-reperfusion injury lead to uric acid (UA) release from cell death, a molecule resulting from DNA and purine metabolism through the xanthine oxidase (XO) pathway[8-10]. Animal models have demonstrated a peak of circulating UA following HS and these studies aimed at evaluating the potentially protective role of XO inhibition on oxidative stress, microcirculatory dysfunction and ATP depletion[11-13]. However, the direct impact of UA on organ damage has not been investigated after HS. UA has been well established as a pro-inflammatory and pro-apoptotic molecule, both as a crystal and a soluble molecule[8, 14]. *In-vitro* studies have shown that soluble UA could induce a pro-inflammatory phenotype through interaction with Toll-like receptor-4 (TLR4), promoting inflammasome activation (NLRP-3, caspase-1), pro-inflammatory cytokine release (IL-1β, IL-18) and enhanced endothelial immune cells adhesion proteins (ICAM-1, VCAM)[14, 15]. UA has also been shown to induce the release of High Mobility Group Box Protein 1 (HMGB1) from endothelial cells, another DAMP associated with distant

organ injury, alteration of mucosal barrier, organ failure and mortality in animal and clinical studies[16-19].

Our group has recently shown that UA metabolization after resuscitated HS, using a recombinant urate oxidase enzyme (uricase) specifically targeting UA, prevented apoptosis and inflammation in lungs and kidneys, as well as TNF-α circulation[20]. However, the role of UA in liver damage after HS remains undefined. While some animal studies have suggested a protective role for XO inhibition, a short-term model of HS showed a benefit of UA pre-treatment on hepatocellular injury, presumably through antioxidant pathways[11, 21, 22].

Furthermore, there is accumulating evidence that the gut represents a driver of systemic inflammation and organ dysfunction via endotoxemia as part of systemic ischemia-reperfusion injury[23-26]. This argument, with the direct anatomical relationship between the gut and the liver through the portal circulation, makes the evaluation of intestinal damage of paramount importance. We hypothesized that UA liberation after HS could directly impact liver and enteric injury following ischemia-reperfusion. The main objective of this study was to characterize the role of UA in HS-induced liver damage through evaluation of inflammatory and cell death/survival pathways. The secondary objective was to investigate the role of UA on enteric injury after HS and provide some insight into a potential relationship between liver damage and HS-induced intestinal permeability.

METHODS

Animals handling and group allocation

Male Wistar rats weighing 350-450g were used for experiments following approval by the local animal ethics committee. Rats were allowed a 3-days period of acclimatization and managed according to the Canadian Council on Animal Care Guidelines (2020).

Animals were randomly assigned to Sham, hemorrhagic shock (HS) or hemorrhagic shock+uricase (HS+U) the day before the experiments (n=6/group). All three groups underwent general anesthesia, endotracheal intubation and femoral vessels cannulation. Sacrifice was performed at 24h and 72h for a total of 36 animals distributed in 6 groups.

Interventions, HS induction and experimental design

General anesthesia was induced with ketamine and xylazine (60 and 10 mg/kg, respectively) and maintained with isoflurane 1-2%. After intubation with a 16-gauge angio-catheter, animals were ventilated under volume-controlled mode (6ml/kg). Following a 1cm inguinal incision, the femoral artery and vein were dissected and cannulated. Arterial access allowed invasive blood pressure monitoring and blood withdrawal was done through the vein. Pulse oximetry, rectal temperature and electrocardiogram were displayed in real time.

HS was induced as previously described, with blood withdrawal until a mean arterial pressure (MAP) of 30 mmHg was reached, which was maintained for 1 hour[20]. Animals were then resuscitated to maintain a MAP above 60 mmHg for 1h with a 1:1 mix of Ringer's Lactate and shed blood (kept at 37°C with 0.2ml citrate-dextrose solution, Sigma-Aldrich). In the HS+U group,

uricase (Rasburicase, Sanofi-Aventis) was administered IP (1.5mg/kg) at the beginning of resuscitation[20]. In the HS+vehicle group, an equal volume of saline 0.9% was administered IP as a placebo. The Sham group underwent all procedures, including general anesthesia for 45 minutes, without HS. After resuscitation, femoral vessels were decannulated and ligated. The skin was closed with absorbable suture. The saturation of the cannulated limb was verified. Animals were weaned from isoflurane, extubated and observed over 24h or 72h, depending on group allocation. Analgesia was provided with subcutaneous buprenorphine (0.05 mg/kg). Among the entire protocol, two rats died due to protocol deviations regarding tight MAP maintenance during shock.

Sacrifice, blood sampling and organ harvesting

Animals were sacrificed by decapitation under sedation with ketamine/xylazine injection IP (60 and 10mg/kg, respectively). Blood was sampled simultaneously and centrifuged at 3000g for 15 minutes. The plasma was aliquoted, frozen in liquid nitrogen and stored at -80°C. Organs were harvested on ice, frozen in liquid nitrogen and stored at -80°C. A 5cm segment of jejunum was harvested 10cm from the pylorus for *ex-vivo* permeability assessment (see below).

Plasma analysis/tissue analysis

Uric acid concentration was measured in plasma and liver parenchyma at 24h and 72h using a fluorescent assay (STA-375 400 assays, Cell Biolabs). Liver tissue homogenization was performed the same way as for western blot (see below). The protocol of the manufacturer was followed as described previously[20].

Hepatocellular injury was assessed with duplicated measurement of liver enzymes (ALT, AST) in 200 μL of plasma at 24h and 72h, using the COBAS c111 automated analyzer system (Roche Diagnostics).

Plasma endotoxin (LPS) levels were measured in duplicate at 72h, using an enzymatic assay according the manufacturer protocol (MyBioSource, MBS268498).

Plasma HMGB1 was measured in duplicate at 24h and 72h by ELISA according to the protocol provided by the manufacturer (Elabscience, E-EL-R0505).

Liver Caspases activities

Caspase-1, -3 and -8 activities were measured in duplicate. Liver samples were suspended in lysis buffer and homogenized with sonication. Tissue homogenates were processed as previously described in detail [20]. Fluorescence was quantified with spectrofluorometry (Photon Technology International, Lawrenceville, NJ, USA) at appropriate excitation/emission wavelengths (340/435, 365/465 and 365/430nm for caspase-1, -3 and -8, respectively).

Western blot (WB)

Protein markers of apoptosis (Bax), cell survival (Bcl-2, phosphorylated-AKT), inflammation (ICAM-1) and epithelial junctional proteins (ZO-1, E-cadherin) were assessed by WB. Samples (liver or HT-29 cells) were handled according to standard technique as previously described[28]. Protein expression, probed with primary antibodies for ICAM-1 (Invitrogen, MA5407), E-cadherin (Abcam, ab1416) and Zonula-occludens (Proteintech, 21773-1-AP), was normalized with Beta-Actin (Sigma, A2066-2ML). Proteins such as Bax (Abcam, ab182733), Bcl-2 (R&D systems, MAB8272), phosphorylated-AKT (Cell signalling technology, 4060S) and total AKT (Cell signalling technology, 9272S) were reported as ratios (Bax/Bcl-2; phospho-AKT/AKT).

Myeloperoxidase activity

Myeloperoxidase (MPO) activity was used to quantify neutrophil accumulation in the liver. Tissues were weighted and handled as described elsewhere[29]. MPO activity was measured using the same method as previously published[20]. MPO activity was calculated as follows:

$$\text{MPO activity } \left(\frac{\text{IU}}{\text{mg} \times \text{min}}\right) = \frac{\text{Absorbance}_{\text{max}} - \text{Absorbance}_{\text{min}}\ (\text{IU})}{\text{weight of sample (mg)} \times 5\ (\text{minutes})}$$

Liver TNF-α and IL-1β concentrations

Liver pro-inflammatory cytokines (TNF-α, IL-1β) were measured in duplicate at 24h and 72h using the ELISA technique. Liver samples were homogenized with the same technique as WB. Using the Lowry method, 200μg of proteins was used for measurement of cytokine concentration according to the protocol provided by the manufacturer (Rat IL-1β/TNF-α, DuoSet Elisa, R&D systems).

Ex-vivo intestinal permeability and intestinal resistance

Small bowel permeability was assessed *ex-vivo* using FITC-dextran permeability with a Ussing Chamber. Using a 5cm segment of jejunum, the mucosa was bluntly stripped from the seromuscular layer and a $1cm^2$ of mucosa was placed in the cassette so that the luminal side of the mucosa faced the chamber with the FITC-dextran solution (4mg/ml). Fluorescence was measured at 30, 60 and 90 minutes in the chamber facing the basolateral side of the mucosa using spectrofluorometry (Cary Elipse). Alternatively, intestinal transepithelial resistance was measured continuously during the 90 minutes. Chambers were continuously oxygenated to prevent cell death. Results at 90 minutes were reported for comparisons.

In vitro experiments using human intestinal cells (HT-29)

HT-29 cells were cultured between passage 5 and passage 15 in McCoys medium (Wisent) and 5% Fetal Bovine Serum (FBS, Wisent) and maintained on room air at 37°C with 5% CO_2. A 0.2M solution of soluble UA was prepared with UA sodium salt (SIGMA, U2875-25G) dissolved in 0.2% NaOH medium and filtered with a sterile 0.22µm filter to ensure the absence of crystals. HT-29 cells were divided into 4 groups: 1-Vehicle (NaOH) alone as a control group, 2-UA exposure (UA $5x10^{-4}$M for 18h), 3-UA exposure + Uricase (UA $5x10^{-4}$M for 4h, then Uricase 0.5µg/ml for 14h) and 4-Uricase 0.5µg/ml for 18h alone. Microscopy was performed at the beginning and the end of treatments to confirm the absence of crystals. After three washings with PBS, cells were isolated with centrifugation and adhesion protein expression (ZO-1, E-Cadherin) was assessed by western blot.

Statistical analyses

Based on an effect size of 0.75 (0.6-0.9) from previous data, an alpha error of 0.05 and a power of 0.9, 36 (30-54) animals were needed for the 6 groups (3 experimental groups with two time points). All data were normally distributed and expressed as Mean± Standard Deviation (SD). A one-way ANOVA with Bonferroni post-hoc analysis was performed for homogeneous variance. For heterogeneous variances, a Brown-Forsythe correction with a Games-Howell post-hoc analysis was performed. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Hemodynamic parameters and UA circulation

Physiological variables, including heart rate, MAP, oxygen saturation and temperature are presented in a Table (Supplemental Digital Content 1, Table 1, http://links.lww.com/TA/B735). Both the HS and HS+U groups had similar MAP patterns, weights, total volumes of withdrawn blood to induce shock and reinfused resuscitation volumes (Fig 1A, B).

Circulating UA levels were significantly induced by HS compared to sham, which was prevented following uricase administration at 24h and 72h (Fig 2A). Similar patterns of UA levels were observed in the liver at 24h without detectable difference at 72h (Fig 2B).

Impact of UA modulation on the liver

The intervention with uricase blunted the HS-induced increase in ALT levels at ~~both at 24h and~~ 72h (Fig 3A). For AST, the effect was marginal in the HS+U group compared to HS at 72h (Fig 3A). At 24h, the same pattern was observed for ALT levels, with no difference for AST levels (Supplemental Digital Content 2, Figs 3 and 4, http://links.lww.com/TA/B736).

Apoptosis indicators were assessed using caspase-3 (common pathway effector caspase) and caspase-8 (extrinsic pathway initiator caspase) activities. Other components involved in survival program signalling pathways were Bax/Bcl-2 and pAKT/AKT ratios, respectively. At 72h after HS, uricase prevented a nearly two-fold increase in liver caspase-3 and -8 activities (Fig 3B). Increase in liver Bax/Bcl-2 ratio after HS was also prevented (Fig 3C). HS lead to a significant decrease in liver phosphorylated-AKT/AKT ratio, a protein that promotes cell survival upon

phosphorylation; lowering UA with uricase prevented this phenomenon (Fig 3C). The same patterns were observed 24h after HS (Supplemental Digital Content 2, Figs 3 and 4, http://links.lww.com/TA/B736).

Regarding inflammation in the liver, MPO enhancement at 24h after HS was completely prevented with uricase (Fig 4A), whereas no difference was observed at 72h (Supplemental Digital Content 2, Figs 3 and 4, http://links.lww.com/TA/B736). The adhesion protein ICAM-1 was significantly enhanced by HS at both timepoints, and also prevented by uricase administration (Supplemental Digital Content 2, Fig 4A, http://links.lww.com/TA/B736). Furthermore, uricase completely prevented increases in liver TNF-α and IL-1β 24h after HS (Fig 4B). Although IL-1β was not measured at 72h due to its early kinetics[30], liver TNF-α showed no difference at 72h (Supplemental Digital Content 2, Figs 3 and 4, http://links.lww.com/TA/B736). Caspase-1 activity, part of the inflammasome complex[31], was significantly induced both at 24h and 72h after HS and completely abolished with UA metabolism by uricase (Supplemental Digital Content 2, Fig 4B, http://links.lww.com/TA/B736).

Impact of UA modulation on intestinal permeability and epithelial adhesion proteins

HS lead to significantly increased plasma endotoxin (LPS) circulation at 72h compared to sham, a phenomenon completely prevented by the administration of uricase during resuscitation (Fig 5A). In line with this finding, both *ex-vivo* intestinal transepithelial resistance and dextran permeability were altered 72h after HS (Fig 5B). The drop in transepithelial resistance, as well as intestinal hyperpermeability following HS, were prevented with uricase. HS-induced circulating HMGB1 was persistently abolished at both timepoints following uricase treatment (Fig 5C).

Additional *in vitro* experiments were performed to investigate how UA could alter intestinal permeability. HT-29 cells treatment with UA decreased junctional protein expression (E-cadherin and ZO-1) by almost half and UA metabolism with subsequent addition of uricase to culture media prevented this phenomenon (Fig 6A). As control measures, no difference was observed between control and uricase alone regarding the expression of both proteins (Fig 6A). Uricase was confirmed to completely metabolize UA in the culture media, with a final medium UA concentration identical to control (Fig 6B).

DISCUSSION

We have previously shown that UA, released in the circulation as part of HS, contributes to lung and kidney injury in association with systemic inflammation[20]. This study demonstrates that targeting one specific DAMP during resuscitation, namely UA, directly impacts on enteric organs after HS. We show that UA plays a key role in liver enzyme release (ALT), liver inflammation, regulatory apoptotic and survival signaling pathways, as well as increased enteric permeability following HS. We specifically evaluate the role of circulating UA as a direct mediator for hepatic and enteric injury after HS, given controversial previous experimental data[11, 21, 32-34]. In the past, most studies pertaining to the UA/XO pathway were performed using XO inhibitors with the theory that decreasing XO activity would decrease oxidative stress[35, 36]. However, UA levels were either not measured[34] or measured not as a direct mediator but rather as a marker of XO inhibition[11, 35, 36]. In addition, organ injury was assessed relatively early after HS[11, 21], and some studies tested pre-treatment strategies[21, 34, 35]. Although this is relevant to planned procedures such as organ transplantation, this is of questionable relevance to the trauma population, knowing that post-traumatic organ failure is a late phenomenon and that it is impossible to pre-treat a trauma

patient before injury occurs[2, 3]. With time, UA has been well established as a DAMP and has been implicated in numerous inflammatory conditions such as the tumor lysis syndrome, coronary artery disease, gouty arthritis, and morbidity in ICU populations[37-40]. Our pharmaceutical strategy (uricase), used as an adjunct to resuscitation, metabolizes UA downstream of XO and therefore differentiates whether organ damage is related to XO-induced oxidative stress or UA itself. Furthermore, our model was designed to evaluate organ damage with a temporal resolution that is clinically relevant to the trauma population, as we used a rat model of HS with longer survival (24-72h).

Our study shows that HS-induced liver ischemia-reperfusion injury is related to UA circulation as evidenced by decreased ALT levels at both 24h and 72h timepoints in the uricase treated groups. This phenomenon was less clear with AST levels, presumably due to non-hepatic AST sources such as skeletal muscle. Further experiments showed that UA plays a role in hepatocellular regulation of apoptotic pathways through activation of caspases and increased Bax/Bcl-2 ratio. Such phenomena have also been observed kidney tubular cell line after UA exposure[41]. Uricase treatment prevented the decreased AKT phosphorylation (pAKT) induced by HS, a marker of one signaling pathway involved in cell survival. This contrasts with the survival benefit and pro-inflammatory profile induced by UA in human monocytes[42]. At least, in the liver parenchyma, our findings are consistent with our previous study on lung and kidney caspases activation[20]. Although mechanisms remain incomplete, these results suggest either multiple distinct pro-apoptotic effects of UA, or significant cross-talk between the intrinsic and extrinsic apoptotic pathways.

UA also seems to be involved in liver inflammation following ischemia-reperfusion injury. Decreased liver pro-inflammatory cytokines (TNF-α, IL-1β), ICAM-1 expression and neutrophilic infiltration were observed at 24h when UA was targeted during resuscitation. The fact that MPO and TNF-α were not increased in the parenchyma 72h after HS, despite enhanced ICAM-1 expression, points to a possible bimodal systemic effect. The ischemia effect present in the early phase of inflammation might decrease with time, while the persistent circulating UA might enhance ICMA-1 endothelial expression. The effect of persistent activation at the level of the immune system or hepatocytes by circulating UA is suggested through caspase-1 activation, a key downstream effector of the NLRP-3 inflammasome complex[43]. Hence, UA has been shown to promote IL-1β expression and ICAM-1 expression on renal tubular cells[14]. The fact that IL-1β levels and caspase-1 activities were significantly blunted with uricase suggests that UA contributes to liver ischemia-reperfusion injury through an inflammasome-mediated process. It is possible that the phenomenon is related to local TNF-α activated by inflammation and apoptosis, particularly through the extrinsic apoptotic pathway[44].

One of the most interesting findings of the present study is the impact on intestinal permeability and resistance. The clinical relevance of intestinal permeability was assessed with measurement of endotoxemia, with the assumption of a digestive tract source in absence of gram-negative sepsis. Consistent with the prevention of HS-induced intestinal hyperpermeability, the intervention on UA prevented the development of endotoxemia 72h after HS, which has been shown to correlate with organ dysfunction in populations sustaining severe ischemia-reperfusion injury such as severe trauma[23, 45] and cardiac surgery patients[46,47]. The mechanism by which UA alters intestinal permeability may be related to decreased expression of epithelial contact proteins (E-cadherin and

ZO-1) as demonstrated *in-vitro*; indeed, other have demonstrated its impact on the intestinal barrier in hyperuricemic mouses[48]. An alternative mechanism may be through promotion of HMGB1 circulation, a DAMP known to cause distant organ damage and to alter mucosal barrier function[17-19, 49]. The fact that our *in-vivo* intervention on UA persistently prevented HMGB1 circulation after HS supports this mechanism and is consistent with previous *in-vitro* studies showing that soluble UA can induce HMGB1 release[15].

Whether altered intestinal permeability is a driver of systemic inflammation and distant organ damage or merely an injured organ among others is currently undefined. The fact that multiple studies have shown an association between endotoxemia and distant organ failure suggests that increased intestinal permeability, with concomitant endotoxemia, may be a major factor in distant organ failure[23,45]. Alternatively, one could postulate that gut failure is solely a marker of hypoperfusion severity and may not mechanistically contribute to distant organ injury. Although the direct anatomical relationship between the gut and the liver provides insight into a potential causal relationship, our model cannot answer this question.

We have to acknowledge several limitations of our study. First, our model is limited to a controlled HS survival model with measures up to 72h, without direct tissue injury as sustained by polytrauma patients. Secondly, although our model does present significant hypoperfusion (Delta lactate) and transient renal dysfunction as previously reported[20, 28], liver dysfunction *per se* was not measured. In addition, histologic assessment of intestine and liver injury, including cellular apoptosis, were not performed. Finally, despite our *ex-vivo* and *in-vitro* approach for the evaluation of intestinal

permeability and epithelial junctional protein expression, these findings remain consistent with the *in-vivo* measures of endotoxemia.

Even though targeting UA with uricase consistently prevented the consequences of ischemia-reperfusion injury on the liver and the gut after HS, whether uricase can prevent long-term organ failure and sepsis remains to be explore. Although our findings point to an overall deleterious effect of UA as part of systemic ischemia-reperfusion injury, caution remains important as beneficial anti-oxidant effects have been suggested in local neuronal ischemia[50, 51]. Notwithstanding these findings, they do not necessarily apply to injury sustained in HS, as different mechanisms involving other DAMPs or immune mediators could induce distant organ injury.

CONCLUSION

UA released from systemic ischemia-reperfusion injury after HS impacts liver and enteric function, leading to injury through activation of inflammatory and apoptotic pathways and alteration of intestinal permeability. Despite limited knowledge of underlying mechanisms, UA appears to be a key upstream mediator in HS-induced organ injury and could represent a potential therapeutic target in adjunction to standard resuscitation.

AUTHOR CONTRIBUTION

F. K. and EC contributed to the design, the analysis and interpretation of the results. They drafted the manuscript and revised the final version.

S. L., K.G., M.A.G. and C. B. participated in the animal/biochemical procedures and to the revision of the manuscript

C. F. R. and G. R. contributed to the interpretation of the results and reviewed the scientific content of the final manuscript

ACKNOWLEDGEMENTS

We acknowledge the support of the research center of the Hôpital du Sacré-Coeur and particularly Dr. François Madore, as a former acting director.

We are thankful to the Fonds de Recherche en Santé du Québec, the Clinician-Scientist program and the General Surgery program from the Université de Montréal for allowing Dr. François Khazoom to complete his research training.

We are also thankful to Mélanie Tremblay for her contribution in the measurement of liver enzymes.

CONFLICT OF INTEREST

E. C. received financial support from Sanofi Genzyme Canada for his research program.

FUNDING

This research was funded locally by Hôpital du Sacré-Coeur de Montréal Research Center and the department of critical care and trauma of the Hôpital du Sacré-Cœur de Montréal.

REFERENCES

1. Sobrino J, Shafi S. Timing and causes of death after injuries. *Proc* (Bayl Univ Med Cent). 2013;26(2):120-3.

2. Ulvik A, Kvale R, Wentzel-Larsen T, Flaatten H. Multiple organ failure after trauma affects even long-term survival and functional status. *Crit Care* (London, England). 2007;11(5):R95.

3. Pfeifer R, Tarkin IS, Rocos B, Pape HC. Patterns of mortality and causes of death in polytrauma patients--has anything changed? *Injury*. 2009;40(9):907-11.

4. Tschoeke SK, Ertel W. Immunoparalysis after multiple trauma. *Injury*. 2007;38(12):1346-57.

5. Vanzant EL, Lopez CM, Ozrazgat-Baslanti T, Ungaro R, Davis R, Cuenca AG, Gentile LF, Nacionales DC, Cuenca AL, Bihorac A, et al. Persistent inflammation, immunosuppression, and catabolism syndrome after severe blunt trauma. *J Trauma Acute Care Surg*. 2014;76(1):21-9; discussion 9-30.

6. Pugin J. How tissue injury alarms the immune system and causes a systemic inflammatory response syndrome. *Ann Intensive Care*. 2012;2(1):27.

7. Manson J, Thiemermann C, Brohi K. Trauma alarmins as activators of damage-induced inflammation. *Br J Surg*. 2012;99 Suppl 1:12-20.

8. El Ridi R, Tallima H. Physiological functions and pathogenic potential of uric acid: A review. *J Adv Res*. 2017;8(5):487-93.

9. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*. 2003;425(6957):516-21.

10. Kono H, Chen CJ, Ontiveros F, Rock KL. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest*. 2010;120(6):1939-49.

11. Flynn WJ, Jr., Hoover EL. Allopurinol plus standard resuscitation preserves hepatic blood flow and function following hemorrhagic shock. *J Trauma*. 1994;37(6):956-61.

12. Shadid M, Van Bel F, Steendijk P, Dorrepaal CA, Moison R, Van der Velde ET, Baan J. Pretreatment with allopurinol in cardiac hypoxic-ischemic reperfusion injury in newborn lambs exerts its beneficial effect through afterload reduction. *Basic Res Cardiol*. 1999;94(1):23-30.

13. Hopkins RW, Abraham J, Simeone FA, Damewood CA. Effects of allopurinol on hepatic adenosine nucleotides in hemorrhagic shock. *J Surg Res*. 1975;19(6):381-90.

14. Xiao J, Zhang XL, Fu C, Han R, Chen W, Lu Y, Ye Z. Soluble uric acid increases NALP3 inflammasome and interleukin-1beta expression in human primary renal proximal tubule epithelial cells through the Toll-like receptor 4-mediated pathway. *Int J Mol Cell Med*. 2015;35(5):1347-54.

15. Cai W, Duan XM, Liu Y, Yu J, Tang YL, Liu ZL, Jiang S, Zhang CP, Liu JY, Xu JX. Uric Acid Induces Endothelial Dysfunction by Activating the HMGB1/RAGE Signaling Pathway. *Biomed Res Int*. 2017;2017:4391920.

16. Cohen MJ, Brohi K, Calfee CS, Rahn P, Chesebro BB, Christiaans SC, Carles M, Howard M, Pittet JF. Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. *Crit Care* (London, England). 2009;13(6):R174.

17. Yang R, Miki K, Oksala N, Nakao A, Lindgren L, Killeen ME, Mennander A, Fink MP, Tenhunen J. Bile high-mobility group box 1 contributes to gut barrier dysfunction in experimental endotoxemia. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(2):R362-9.

18. Yang R, Harada T, Mollen KP, Prince JM, Levy RM, Englert JA, Gallowitsch-Puerta M, Yang L, Yang H, Tracey KJ, et al. Anti-HMGB1 neutralizing antibody ameliorates gut barrier dysfunction and improves survival after hemorrhagic shock. *Mol Med*. 2006;12(4-6):105-14.

19. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu Rev Immunol*. 2011;29:139-62.

20. Gilbert K, Rousseau G, Bouchard C, Dunberry-Poissant S, Baril F, Cardinal AM, Khazoom F, Vega MA, Brochiero E, Charbonney E. Caspase-(8/3) activation and organ inflammation in a rat model of resuscitated hemorrhagic shock: A role for uric acid. *J Trauma Acute Care Surg*. 2019;86(3):431-9.

21. Tsukada K, Hasegawa T, Tsutsumi S, Katoh H, Kuwano H, Miyazaki T, Yamamoto Y. Effect of uric acid on liver injury during hemorrhagic shock. *Surgery*. 2000;127(4):439-46.

22. Matsumura F, Yamaguchi Y, Goto M, Ichiguchi O, Akizuki E, Matsuda T, Okabe K, Liang J, Ohshiro H, Iwamoto T, et al. Xanthine oxidase inhibition attenuates kupffer cell production of neutrophil chemoattractant following ischemia-reperfusion in rat liver. *Hepatology* (Baltimore, Md). 1998;28(6):1578-87.

23. Charbonney E, Tsang JY, Li Y, Klein D, Duque P, Romaschin A, Marshall JC. Endotoxemia Following Multiple Trauma: Risk Factors and Prognostic Implications. *Crit Care Med*. 2016;44(2):335-41.

24. Doig CJ, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. *Am J Respir Crit Care Med*. 1998;158(2):444-51.

25. Klingensmith NJ, Coopersmith CM. The Gut as the Motor of Multiple Organ Dysfunction in Critical Illness. *Crit Care Clin*. 2016;32(2):203-12.

26. Zhou Q, Verne GN. Intestinal hyperpermeability: a gateway to multi-organ failure? *J Clin Invest*. 2018;128(11):4764-6.

27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248-54.

28. Dunberry-Poissant S, Gilbert K, Bouchard C, Baril F, Cardinal AM, L'Ecuyer S, Hylands M, Lamontagne F, Rousseau G, Charbonney E. Fluid sparing and norepinephrine use in a rat model of resuscitated haemorrhagic shock: end-organ impact. *Intensive Care Med Exp*. 2018;6(1):47.

29. Duval DL, Howard D, McCalden TA, Billings RE. The determination of myeloperoxidase activity in liver. *Life sciences*. 1990;47(24):Pl145-50.

30. Abraham E, Richmond NJ, Chang YH. Effects of hemorrhage on interleukin-1 production. *Circ Shock*. 1988;25(1):33-40.

31. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int J Mol Sci*. 2019;20(13).

32. Flynn WJ, Jr., Pilati D, Hoover EL. Effect of allopurinol on venous endothelial dysfunction after resuscitated hemorrhagic shock. *Int J Surg Investig*. 1999;1(1):11-8.

33. Flynn WJ, Jr., Pilati D, Hoover EL. Xanthine oxidase inhibition prevents mesenteric blood flow deficits after resuscitated hemorrhagic shock by preserving endothelial function. *J Surg Res*. 1997;68(2):175-80.

34. Matsumura F, Yamaguchi Y, Goto M, Ichiguchi O, Akizuki E, Matsuda T, Okabe K, Liang J, Ohshiro H, Iwamoto T, et al. Xanthine oxidase inhibition attenuates kupffer cell production of neutrophil chemoattractant following ischemia-reperfusion in rat liver. *Hepatology* (Baltimore, Md). 1998;28(6):1578-87.

35. Crowell JW, Jones CE, Smith EE. Effect of allopurinol on hemorrhagic shock. *Am J Physiol*. 1969;216(4):744-8.

36. Cowsert MK, Jr., Carrier O, Jr., Crowell JW. The effect of hemorrhagic shock on blood uric acid level. *Can J Physiol Pharmacol*. 1966;44(5):861-4.

37. Akbar SR, Long DM, Hussain K, Alhajhusain A, Ahmed US, Iqbal HI, Ali AW, Leonard R, Dalton C. Hyperuricemia: An Early Marker for Severity of Illness in Sepsis. *Int J Nephrol.* 2015;2015:301021.

38. Cortese F, Giordano P, Scicchitano P, Faienza MF, De Pergola G, Calculli G, Meliota G, Ciccone MM. Uric acid: from a biological advantage to a potential danger. A focus on cardiovascular effects. *Vascul Pharmacol*. 2019;120:106565.

39. Howard SC, Jones DP, Pui CH. The tumor lysis syndrome. *N Engl J Med*. 2011;364(19):1844-54.

40. Lee HW, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK, Lee SM. Serum Uric Acid Level as a Prognostic Marker in Patients With Acute Respiratory Distress Syndrome. *J Intensive Care Med*. 2019;34(5):404-10.

41. Verzola D, Ratto E, Villaggio B, Parodi EL, Pontremoli R, Garibotto G, Viazzi F. Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4. *PloS One*. 2014;9(12):e115210.

42. Crisan TO, Cleophas MCP, Novakovic B, Erler K, van de Veerdonk FL, Stunnenberg HG, Netea MG, Dinarello CA, Joosten LAB. Uric acid priming in human monocytes is driven by the AKT-PRAS40 autophagy pathway. *Proc Natl Acad Sci U S A*. 2017;114(21):5485-90.

43. Braga TT, Forni MF, Correa-Costa M, Ramos RN, Barbuto JA, Branco P, Castoldi A, Hiyane MI, Davanso MR, Latz E, et al. Soluble Uric Acid Activates the NLRP3 Inflammasome. *Sci Rep*. 2017;7:39884.

44. Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. *Cell*. 2008;133(4):693-703.

45. Pfeiffer L, Ehrhardt N, Kretzschmar M, Urbaschek R, Schubert K, Schirrmeister W. [Endotoxinemia and multiple organ failure after polytrauma]. *Anaesthesiol Reanim*. 1996;21(4):91-6.

46. Braun JP, Buhner S, Kastrup M, Dietz E, Langer K, Dohmen PM, Lochs H, Spies C. Barrier function of the gut and multiple organ dysfunction after cardiac surgery. *J Int Med Res*. 2007;35(1):72-83.

47. Pathan N, Burmester M, Adamovic T, Berk M, Ng KW, Betts H, Macrae D, Waddell S, Paul-Clark M, Nuamah R, et al. Intestinal injury and endotoxemia in children undergoing surgery for congenital heart disease. *Am J Respir Crit Care Med*. 2011;184(11):1261-9.

48. Guo Y, Li H, Liu Z, Li C, Chen Y, Jiang C, Yu Y, Tian Z. Impaired intestinal barrier function in a mouse model of hyperuricemia. *Mol Med Rep*. 2019;20(4):3292-300.

49. Sodhi CP, Jia H, Yamaguchi Y, Lu P, Good M, Egan C, Ozolek J, Zhu X, Billiar TR, Hackam DJ. Intestinal Epithelial TLR-4 Activation Is Required for the Development of Acute Lung Injury after Trauma/Hemorrhagic Shock via the Release of HMGB1 from the Gut. *J Immunol*. 2015;194(10):4931-9.

50. Yu ZF, Bruce-Keller AJ, Goodman Y, Mattson MP. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *J Neurosci Res*. 1998;53(5):613-25.

51. Sautin YY, Johnson RJ. Uric acid: the oxidant-antioxidant paradox. *Nucleosides Nucleotides Nucleic Acids*. 2008;27(6):608-19.

FIGURE'S LEGENDS

Figure 1: Mean arterial pressure (A), weight (B), Shed blood volume (C) and resuscitation volume (D) among HS and HS + Uricase groups. Expressed as mmHg, grams or ml ± SD.

Figure 2: Circulating (A) and liver (B) uric acid concentration 24h and 72h after hemorrhagic shock. Values expressed as fold change ± SD. * indicates $p<0.05$ between HS and the two other groups.

Figure 3: Plasma ALT and AST (A), Caspase-3 and -8 activation (B), Bax/Bcl-2 ratio and pAKT/AKT ratio (C) and representative Western Blot bands (D) at 24 and 72h after HS. Values expressed as IU/L or fold change ± SD. * indicates $p<0.05$ between HS and the two other groups. # indicates $p<0.05$ between HS+U and HS group.

Figure 4: Liver neutrophil infiltration, ICAM-1 adhesion protein expression with representative bands (A), pro-inflammatory cytokine expression related to inflammasome activation and caspase-1 activation (B). Values expressed in fold change or pg/ml ± SD. * indicates $p<0.05$ between HS and the two other groups.

Figure 5: Plasma LPS (A), ex-vivo intestinal permeability studies (B) and plasma HMGB-1 (C). Expressed in ng/ml (A), fold change and Ohms (B) and pg/ml (C) ± SD. * indicates $p<0.05$ between HS and the two other groups.

Figure 6. In-vitro HT-29 junctional protein expression (A) and UA supernatants concentrations (B) after UA exposure. Expressed in fold change and μM ± SD. n=6/group. * indicates $p<0.05$ between the UA group and all other groups.

Figure 1

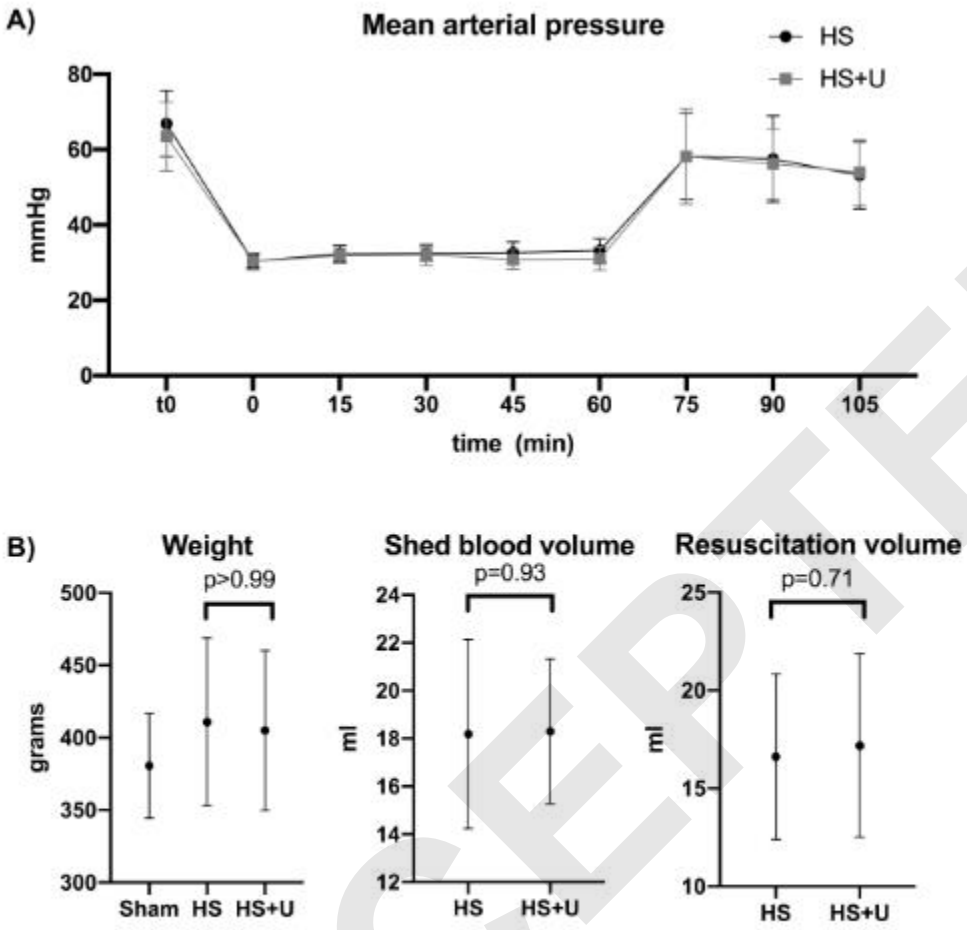


Figure 1: Mean arterial pressure (A), weight (B), Shed blood volume (C) and resuscitation volume (D) among HS and HS + Uricase groups. Expressed as mmHg, grams or ml ± SEM.

Figure 2

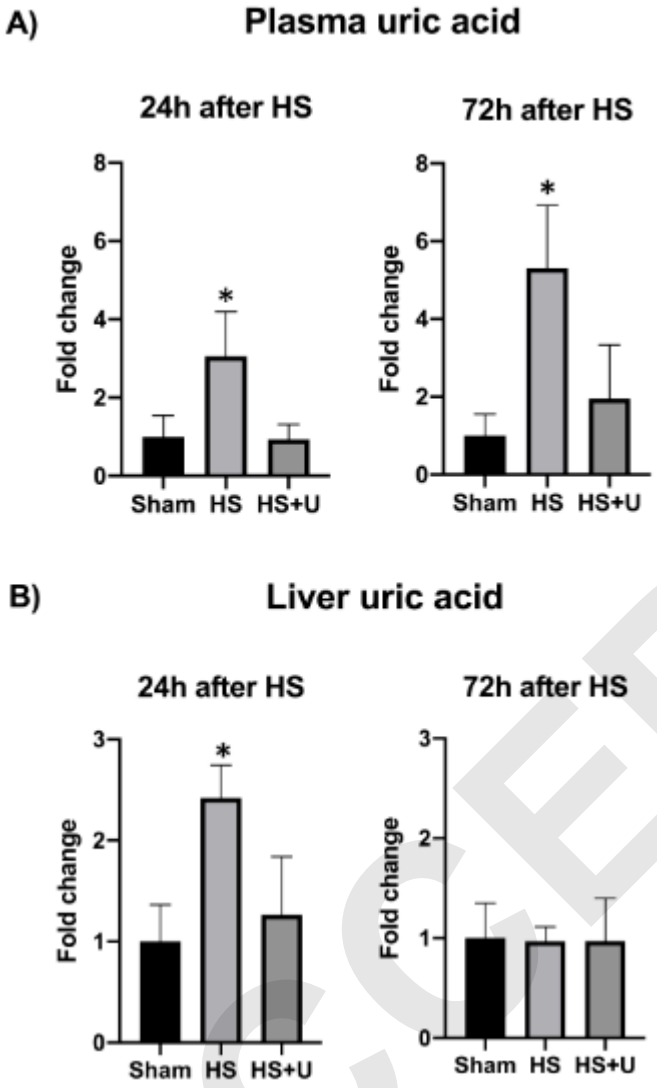


Fig 3

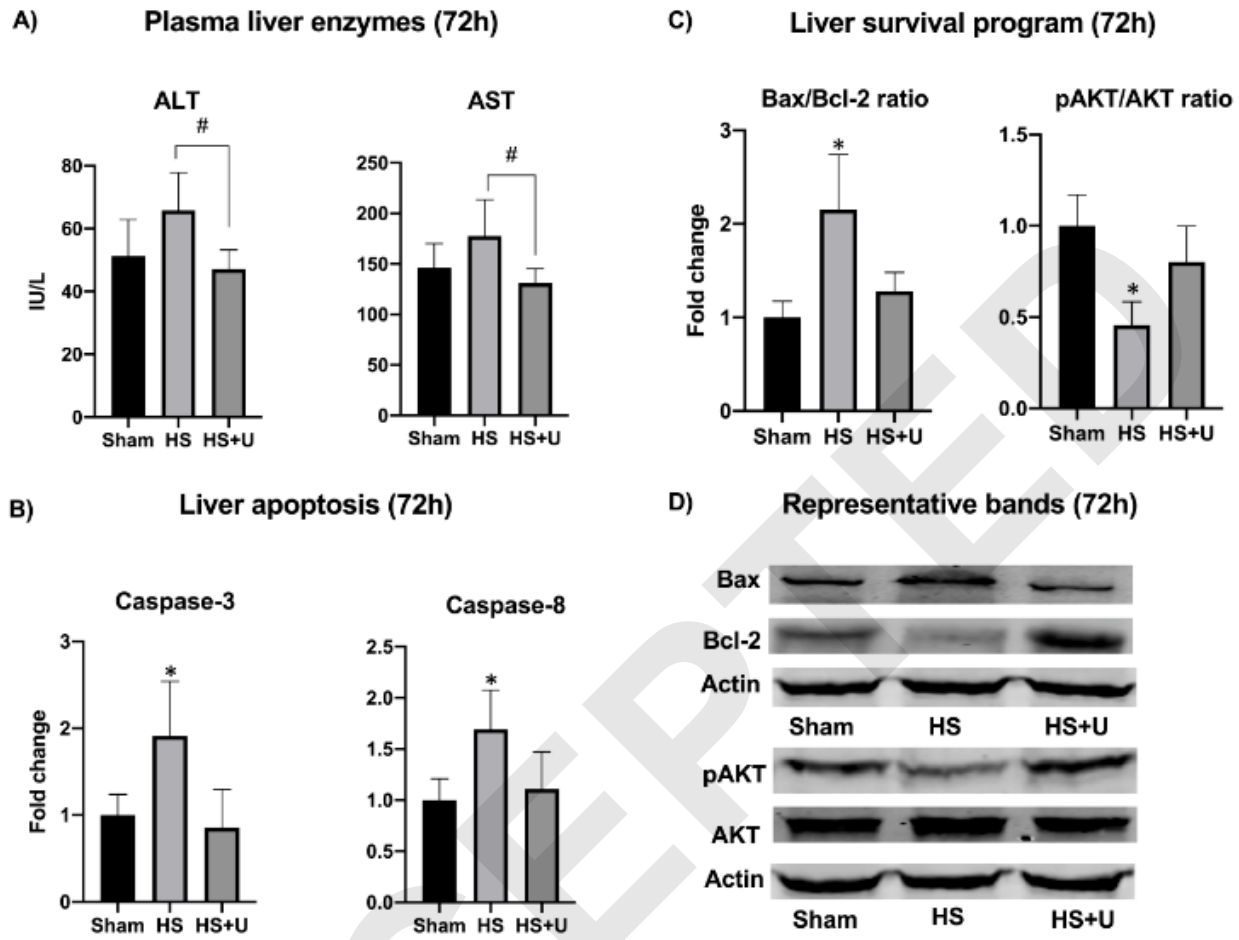


Figure 4

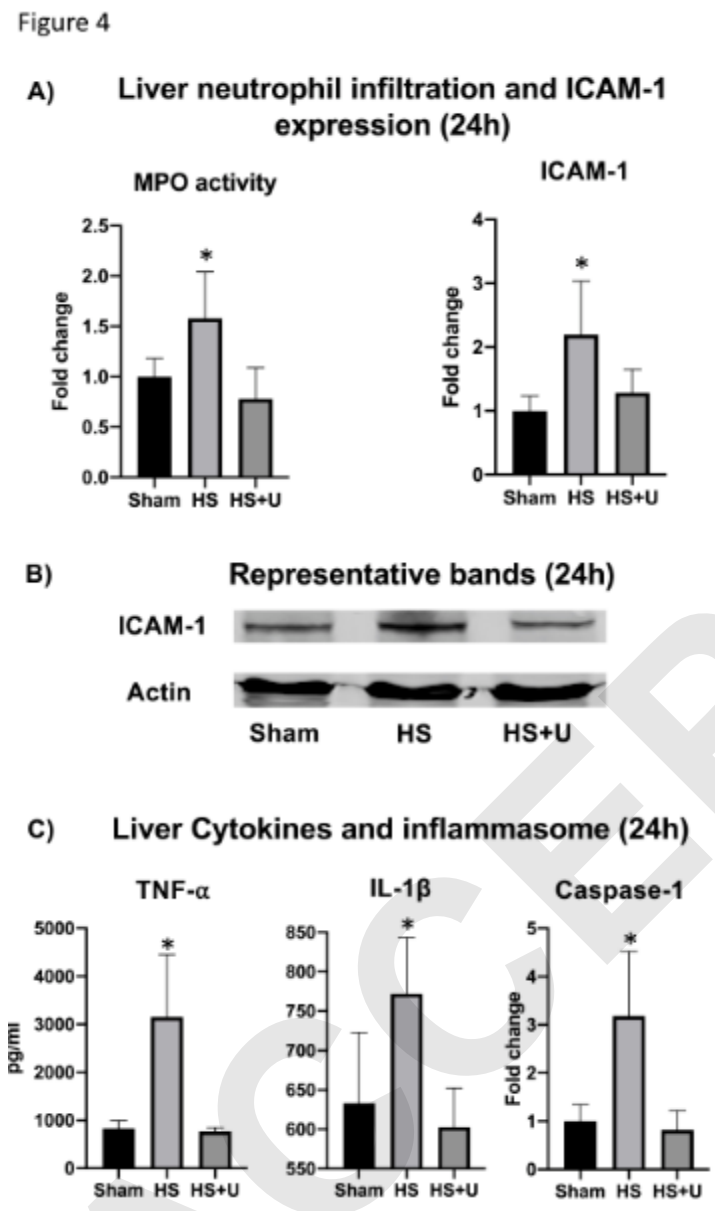


Figure 5

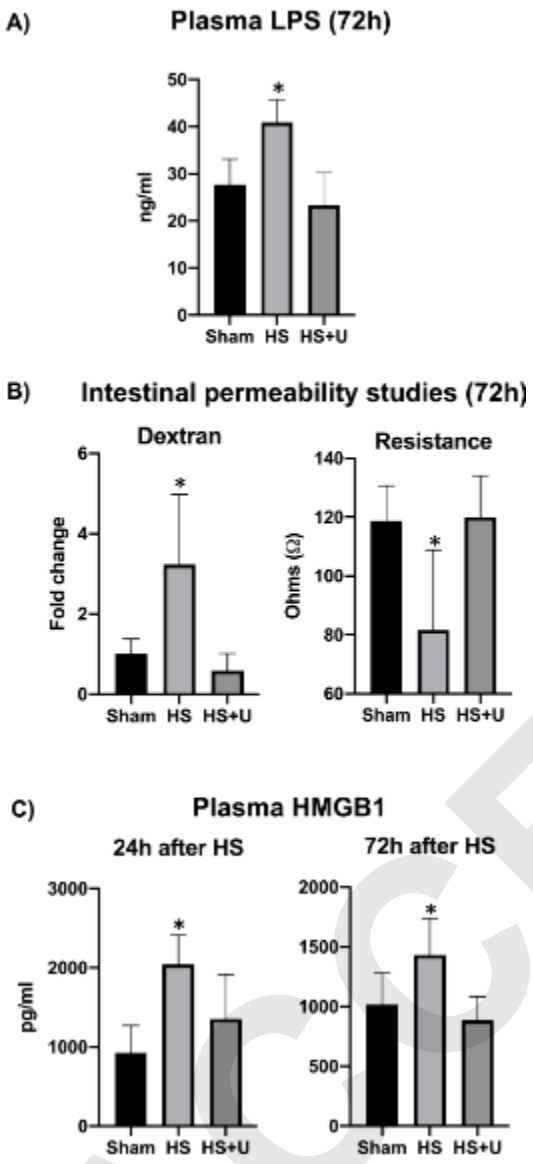


Figure 6

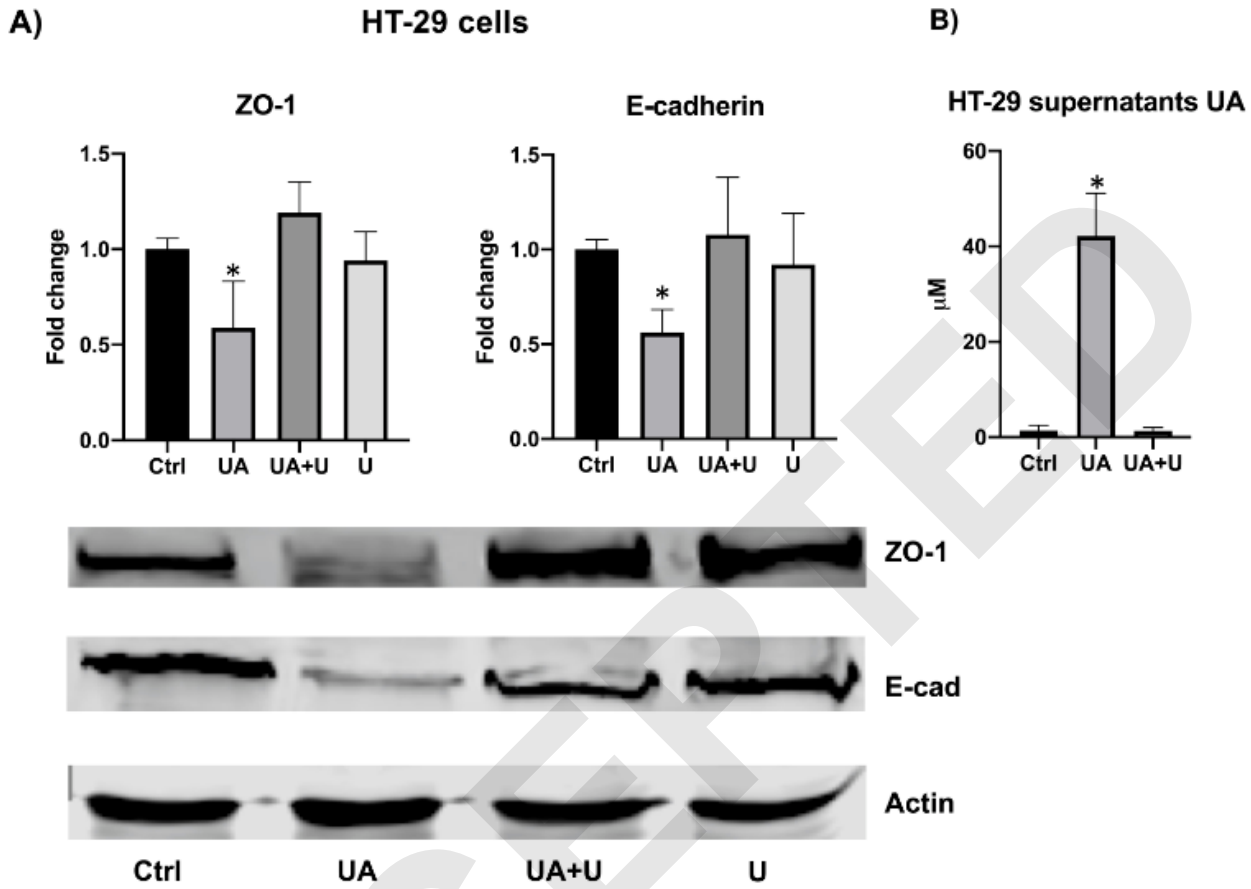


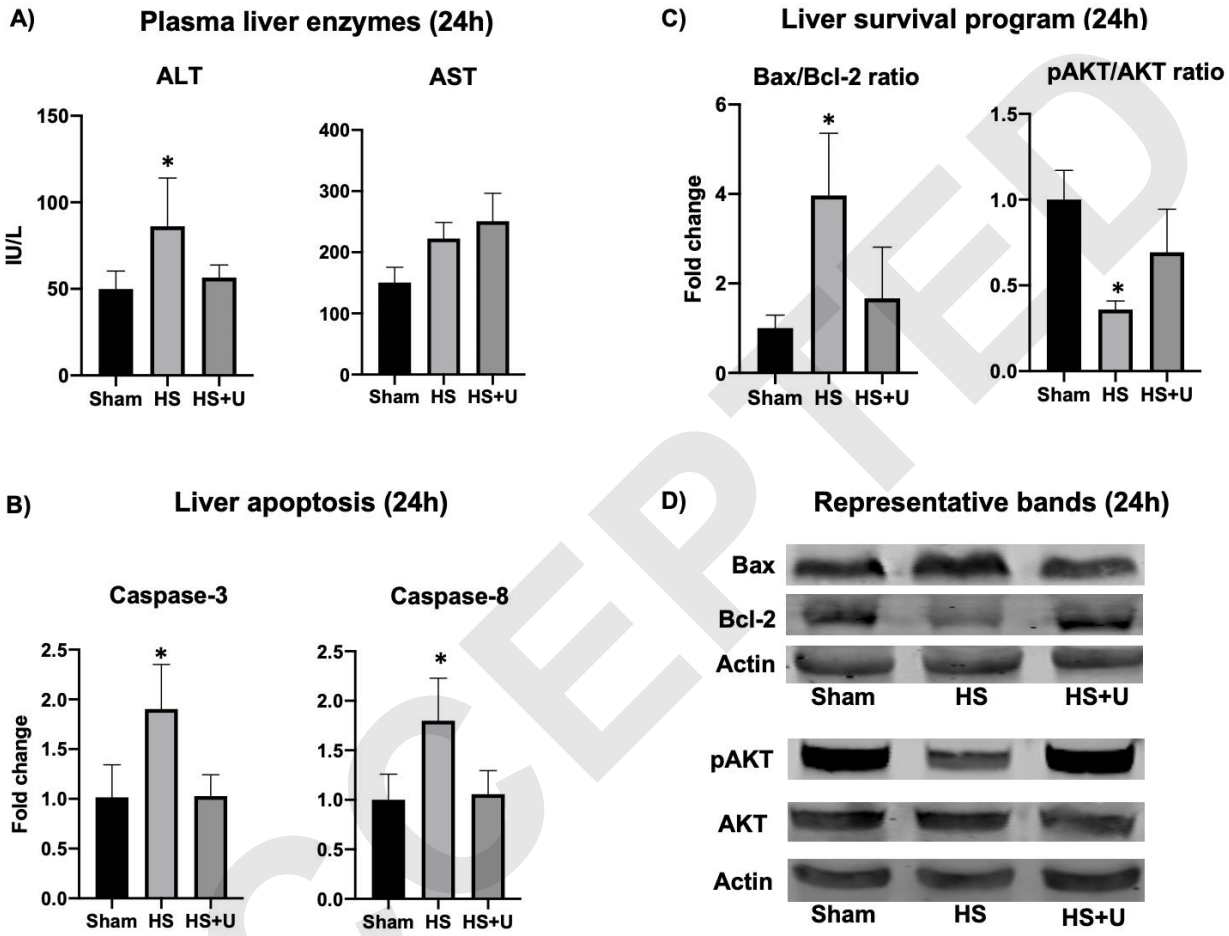
Table: Physiological variables of experimental groups.

	Baseline	Shock	Reperfusion
Sham (n=12)			
MAP, mmHg	65.2 (7.6)	64.7 (7.8)	64.7 (7.8)
HR, bpm	238 (19.6)	243 (19.6)	243 (19.5)
Saturation, %	98 (1.2)	98 (1)	98 (1)
Temperature, (°C)	33.8 (1)	34.1 (1)	34.1 (1)
HS (n=12)			
MAP, mmHg	66.9 (6.1)	32.2 (1.8)	56.3 (7.6)
HR, bpm	241 (14.7)	223 (22)	250 (19.6)
Saturation, %	98 (0.7)	97 (1.5)	98 (0.7)
Temperature, (°C)	33.8 (1)	33.4 (1)	33.5 (1.2)
HS + Uricase (n=12)			
MAP, mmHg	63.5 (6.4)	31.2 (1.7)	56.1 (7.4)
HR, bpm	243 (22)	235 (27)	252 (29.4)
Saturation, %	98 (0.7)	98 (1)	98 (1.2)
Temperature, (°C)	34.4 (1)	33.9 (1)	33.9 (1)

All values are represented as means (SD). MAP: mean arterial pressure; HR: heart rate; bpm: beats per minutes.

Average values are reported at baseline, during hemorrhagic shock, then during reperfusion until extubation.

Supplementary figure 3



A. At 24h, the intervention with uricase (U) blunt HS-induced increase in ALT levels; increased AST levels after HS compared to Sham is not attenuated following uricase intervention.

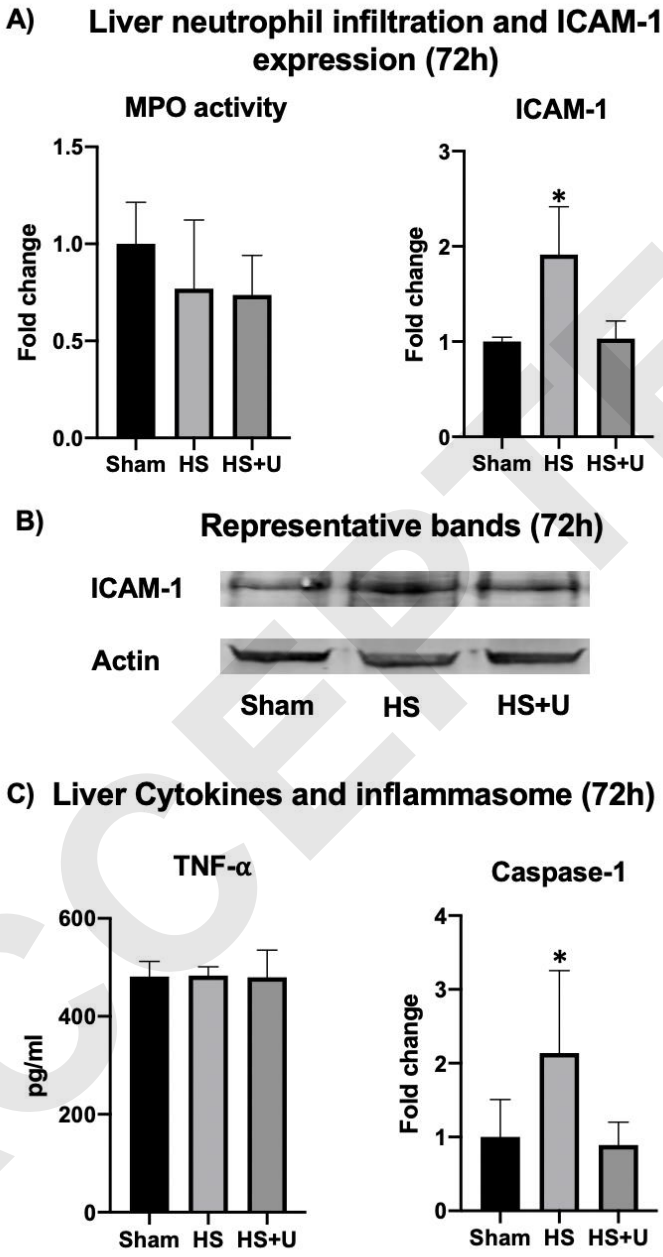
B. At the 24h after HS, uricase prevents a nearly two-fold increase in liver caspase-3 and -8 activities.

C. Increase in liver Bax/Bcl-2 ratio after HS is prevented. HS leads to a significant decrease in liver phosphorylated-AKT/AKT ratio; lowering UA with uricase prevented this phenomenon.

D. Representative Western Blot bands.

Values expressed as IU/L or fold change ± SD. * indicates $p<0.05$ between HS and the two other groups.

Supplementary figure 4



A. No differences are observed at 72h for Myeloperoxidase (MPO). The adhesion protein ICAM-1 is significantly enhanced by HS and prevented by uricase administration.

B. Representative Western Blot bands of ICAM-1

C. liver TNF-α showed no difference at 72h; Caspase-1 activity, is significantly induced till 72h after HS and completely abolished with UA metabolism by uricase.

Values expressed in fold change or pg/ml ± SD. * indicates $p<0.05$ between HS and the two other groups.