Original Article

Soluble *p*-selectin Rescues Mice from a "2-hit" Model of Hemorrhagic Shock and Reperfusion Injury

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Objectives: Binding of *p*-selectin to PSGL-1 induces leukocyte-endothelial adhesion and disruption of capillary barriers during major trauma and results in multiple organ failure. However, the soluble form *p*-selectin (sP-sel) has been recently found to possess characteristic properties that mediate cell survival. This study aimed to determine the rescue potentials of sP-sel in a "2-hit" model of hemorrhagic shock and reperfusion injury.

Methods: Hemorrhagic shock resuscitation (HSR) was induced by direct withdrawing blood (0.3 mL) from the femoral artery of anesthetized mice and resuscitated with crystalloid 30 min later. Ischemia to the mid-jejunum was achieved by cross-clamping of the superior mesenteric artery for 10 min. Time of survival after mesenteric reperfusion was recorded. In other quantitative studies, ischemic jejunal tissues were obtained at 6-h after reperfusion for analysis.

Results: Intraperitoneal injection of recombinant P-sel IgG-Fc fusion protein (rP-sel-Fc) before release of mesenteric blood flow significantly improved the overall survival rate of the mice at 15 h after reperfusion (80% vs. 40%, $p = 0.047$). rP-sel-Fc did not affect the infiltration of inflammatory cells or the activity of myeloperoxidase in the ischemic intestine, but restored the Bcl-2/BAX ratio, and enhanced the expression of superoxide dismutases in the injured jejunal tissue. Treatment with rP-sel-Fc also suppressed the formation of malondialdehyde and attenuated the intestine injury score.

Conclusions: sP-sel attenuated tissue injury during ischemia-reperfusion insult and improved the survival of mice with HSR. The protective effect of sP-sel may be mediated by the potent antioxidative reaction through restoring the endogenous activities of superoxide dismutases and attenuating mitochondrial damage.

Key words: trauma, hemorrhagic shock, ischemia-reperfusion injury, *p*-selectin

Introduction

Hemorrhagic shock associated with major trauma remains one of top ten lead-

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ing causes of death globally. According to the World Health Organization updates, trauma killed more than 1.3 million people in 2015, mainly due to central nervous injury and multiple systemic organ failure (MSOF).[1] Hypoperfusion in the splanchnic circulation secondary to hemorrhagic shock and/or abdominal compartment syndrome (ACS) results in non-occlusive intestinal ischemia and mucosal damage[2-4], which in turn causes translocation of inflammatory mediators and microbial pathogen. The reperfusion of splanchnic circulation after resuscitation may also precipitate the hemodynamic instability and systemic acidosis. Therefore, reperfusion injury following hemorrhagic shock resuscitation (HSR) has been shown to be associated with complicated detrimental physiological effects and increased major morbidity and mortality.[5,6]

The interaction between leukocytes and endothelial cells promotes the migration of leukocytes through "outside-in" signals from adhesion molecules on the endothelial cells, leading to increased tissue permeability and transmigration of inflammatory cells into the tissue shortly after major trauma that leads to MSOF.[7,8] Selectins are important adhesion molecules responsible for the initial step of trauma-induced systemic vasculopathy, including the rolling and attachment of the activated circulating inflammatory cells and platelets on the luminal side of the endothelium.[9] Among the selectins, *p*-selectin expressed on the endothelial cells has been recognized as a potential therapeutic target because of its role in the regulation of acute inflammation in situations such as trauma resuscitation.[8] The interactions between *p*-selectin and the leukocytes/megakaryocytes are mediated through the *p*-selectin glycoprotein legend (PSGL)-1 that is expressed on the surface of these circulating cells and supports their recruitment onto the endothelium.[10,11] On the other hand, soluble *p*-selectin (sP-Sel) is released from the activated platelets and endothelial cells.[12] Increased serum sP-sel level was reported in coagulation disorders, infectious diseases, thrombosis, systemic inflammation, vascular damage and trauma.[13] Contrast to the pro-inflammatory property of the membrane-bound form, most recent studies have shown that soluble form of *p*-selectin inhibits the adhesion of leukocytes onto the inflamed tissues, and mediates anti-inflammation effect, serving as a self-rescue response that is beneficial for host recovery from lethal hypocoagulation and pro-inflammatory reactions.[14,15]

The aims of this study were to develop a "2-hit" model of hemorrhagic shock and bowel ischemia reperfusion injury that mimics hemodynamic changes during trauma resuscitation, and evaluate the rescue property of sP-Sel in this "2-hit" experimental model.

Materials and Methods

Murine model of hemorrhagic shock resuscitation (HSR) and bowel ischemia

The animal studies were conducted in compliance with the Animal Center of the E-Da Cancer Hospital and approved by the Institutional of Animal Care and Use Committee (Approval #EDCH 106006, E-Da Cancer Hospital, Kaoshiung, Taiwan). Mice (C57BL/6) were anesthetized with intraperitoneal injection of cocktail anesthetics Zoletil® (50 mg/kg, i.p.). Femoral artery was cannulated with a 30G polyethylene catheter (PE10, I.D. 0.28 mm) connecting to a pressure transducing system (Kent Scientific Corporation, Torrington, CT, USA) for blood pressure monitoring. A total of 300 μL blood was drew slowly from a 3-way stopcock in the transducing system over 10 min and maintain a low mean arterial pressure of approximately 40 mmHg for 30 min (hypovolemic shock status), which was known to induce systemic proinflammatory cytokine production.[16] Same amount of normal saline was infused through the arterial cannulation for resuscitation at the end of experiment.

Ten minutes before the end of experiment, the SMA was identified and cross-clamped using a vascular clip following midline laparotomy. Immediately after the administration of resuscitation fluids, the vascular clip was released to restore the splanchnic circulation supplied by SMA. The abdomen was then closed in layers and the animals were allowed to recover spontaneously from anesthesia in a heated incubator.

Treatment protocols

Mice were randomly allocated into two groups, receiving normal saline (placebo, n = 36) or recombinant rP-sel-Fc groups (n = 32). Placebo or rP-sel-Fc (1.5 mg/kg, R&D Systems, MN) was injected into peritoneal cavity 30 min before reperfusion of SMA. Animals were observed up to 15 h after reperfusion in the survival study. In other quantitative experiments, animals were sacrificed and tissue was harvested at 6 h after reperfusion.

Tissue myeloperoxidase (MPO) activity and malondialdehyde (MDA) formation

The enzymatic activity of myeloperoxidase in the ischemic jejunum was measured by the MPO assay as previously described. Briefly, tissues were homogenized and the resulting pellets were resuspended in hexadecyltrimethylammonium bromide. The samples were thaw and frozen twice before mixing with O-dianisidine dihydrochloride and hydrogen peroxide. The solutions were then subject to detection of absorbance at 460 nm at regular intervals for two minutes. Lipid peroxidation in the jejunum was determined by analyzing tissue content of MDA with a commercially available kit (TBARS kit, Cayman Chemical, MI).

Western blot

Soluble protein extracts of jejunal tissue were loaded into polyacrylamide gels and transferred onto nitrocellulose membranes. Anti-MPO, iNOS, CD11b, TNF-α, IL-1β, IL-6, CuZnSOD, MnSOD, HO-1, caspase-3, BAX, Bcl-2 antibodies were used. All primary antibodies were purchased from BD Pharmingen or R&D Systems. After incubation with HRP-linked secondary antibodies, bands were visualized by enhanced chemiluminescence and quantified using the ImageJ (1.48v, NIH).

Histological examination and intestinal injury scale

Bowel tissues were immersed in 4% formaldehyde for 24 hours and the paraffin-embedded biopsies were sectioned. Tissues were processed for hematoxylin and eosin (HE) stain and analyzed under microscopy. Degrees of intestinal injury were independently assessed by a pathologist, who was blinded to the treatment group. The degree of tissue injury was graded 0 to 5, according to the grading system by Chiu et al;[19] grade 0: normal intestinal mucosa; grade 1: presence of subepithelial space in the villi; grade 2: presence of extended subepithelial space; grade 3: significant epithelial lifting in the villi; grade 4: denudation of epithelial layer; grade 5: loss of villi structure. Each tissue slide was graded over the four quadrates of the tissue section, and a median value was obtained for each slide.

Statistical analysis

Results are presented as mean ± SD. Differences in survival rates were analyzed using the log-rank tests for Kaplan-Meier curves. Data were compared by unpaired t-test, ANOVA or non-parametric test, as appropriate. Statistical significance was defined as a level of $p < 0.05$.

Results

Soluble form *p*-selectin (rP-sel-Fc) improved survival rate

Hemorrhagic shock was induced by

drawing 300 μL blood over 10 min from the femoral artery to maintain a mean arterial pressure (MAP) below 40 mmHg.[16] The animals were then resuscitated with same volume of normal saline at the end of experiment. Compared with animals treated with HSR-only, those received 2-hit injury with HSR and ischemic bowel were associated with significantly higher mortality up to 15 h after operation (0 vs. 80% mortality rate, $p < 0.01$; Fig. 1A). At autopsy, segmental ischemic changes were observed in the proximal small bowel (Fig. 1B). Histological section revealed transmural necrosis of the intestinal wall consistent with acute mesenteric ischemia (Fig. 1C). Figure 1A shows that pre-treatment with recombinant P-sel IgG-Fc fusion protein (rP-sel-Fc) before reperfusion significantly improved the overall survival rate in mice subject to 2-hit injury at 15 h after reperfusion (80% vs 40%, $p = 0.047$). Since significantly increased mortality was observed 6 h after reperfusion injury in the control group, quantitative analysis of tissue samples were performed at 6 h after reperfusion.

Soluble form *p*-selectin (rP-sel-Fc) did not affect regional inflammatory response

Infiltration of acute phase inflammatory cells was determined by tissue expression of CD11b (i.e., a cell surface marker of activated leukocytes and macrophages), iNOS and myeloperoxidase (MPO).[17] The protein expressions of these cell markers of inflammatory cells and the enzymatic activity of MPO were up-regulated in the ischemic bowel tissue following HSR and superior mesenteric artery (SMA) occlusion, indicating a profound acute inflammation response in the intestinal tissue (Fig. 2A and 2B). Treatment with rP-sel-Fc did not affect the regional inflammation response

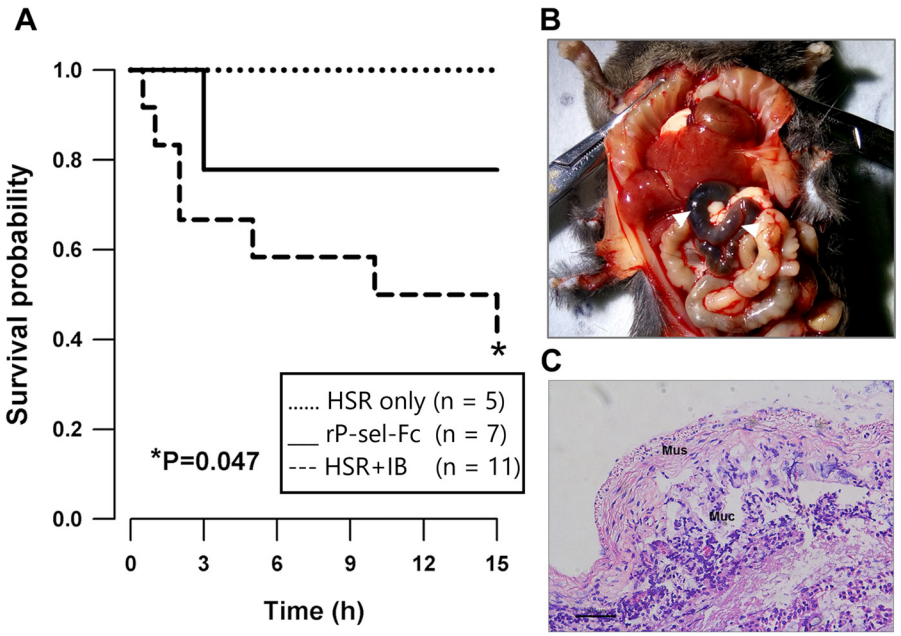

*Fig. 1 (A) High mortality in mice subject to HSR+IB. Treatment with rP-sel-Fc significantly enhanced the overall survival at 15 h after reperfusion mesenteric. *p = 0.047 HSR+IB vs. HSR+IB+rP-sel-Fc using the Kaplar Meier Survival analysis. (B) Representative photograph of a non-survived animal following laparotomy autopsy. Arrowheads indicate segments of ischemic jejunum following a 10-min occlusion of superior mesenteric artery. (C) Representative histologic section of the ischemic bowel. HE staining section showing transmural necrosis of intestinal wall. Muc: mucosal layer, Mus: muscularis propria. Presentations of the photos and histological images in compliance with the digital imaging and integrity policies defined by Nature Research. HSR: hemorrhagic shock and resuscitation, IB: ischemic bowel, rP-sel-Fc: recombinant P-sel IgG-Fc fusion protein*

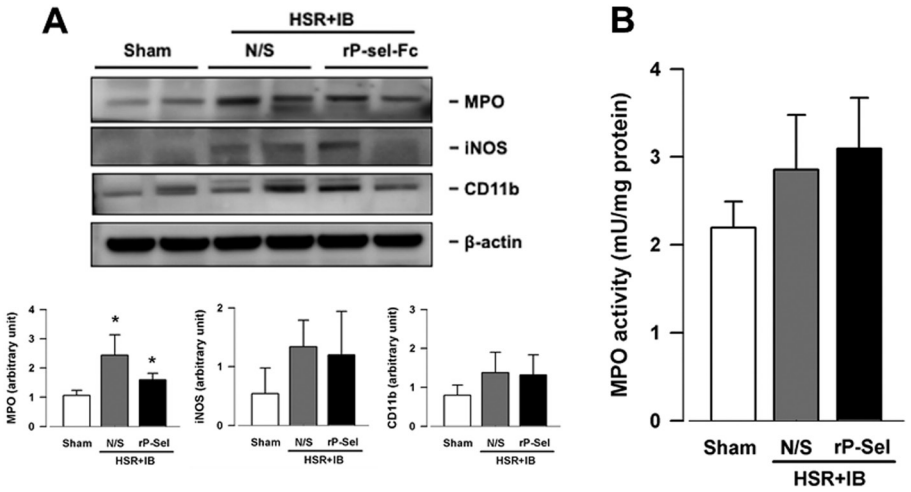

*Fig. 2 Profiles of infiltration of inflammatory cells in the ischemic intestinal tissue. Expression of myeloperoxidase (MPO) was significantly enhanced in the jejunal biopsies of animals exposed to HSR+IB (*p < 0.05 vs sham). Administration of rP-sel-Fc did not significantly affect the infiltration of inflammatory cells or their enzymatic activity after ischemia-reperfusion injury. (A): each group contained 5 different animals; (B): each group contained 5-7 animals. Data were analyzed using one-way ANOVA. All protein expressions presented as the original blots in compliance with the digital imaging and integrity policies defined by Nature Research. HSR: hemorrhagic shock and resuscitation, IB: ischemic bowel, rP-sel-Fc: recombinant P-sel IgG-Fc fusion protein, N/S: normal saline*

in the injured intestine (Fig. 2A and 2B). Tissue levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) were also not different following rP-sel-Fc treatment (data not shown).

Soluble form *p*-selectin (rP-sel-Fc) suppressed pro-apoptotic reaction

Two-hit injury promoted cell apoptosis in the ischemic bowel, as evidenced by the enhanced expression of caspase-3 (Fig. 3A). Administration of rP-sel-Fc up-regulated the tissue expressions of bcl-2 (anti-apoptosis) and BAX (pro-apoptosis) in the isolated jejunum (Fig. 3A), and significantly increased the bcl-2/BAX ratio (Fig. 3B), suggesting a higher pro-surviving capacity in the injured tissue.[18]

Soluble form *p*-selectin (rP-sel-Fc) restored antioxidant defense system

Significantly higher oxidative stress in the ischemic bowel was shown by the enhanced expression of inducible heme oxygenase (HO)-1 (Fig. 4A) and tissue concentration of malondialdehyde (MDA) (Fig. 4B). On the other hand, the expressions of endogenous antioxidant defense enzymes, namely, CuZnSOD and MnSOD, were suppressed in the bowel tissues of mice subject to HSR and bowel ischemia (Fig. 4A). Treatment with rP-sel-Fc not only restored the protein levels of CuZnSOD and MnSOD but also significantly attenuated the formation of MDA in the ischemic bowel (Fig. 4A and 4B).

Soluble form *p*-selectin (rP-sel-Fc) attenuated bowel ischemic injury

Two-hit model of HSR and bowel ischemia resulted in different degrees of injury, ranging from apical villous destruction to transmural injury in the bowel wall (grades 3 to 5)[19], and significantly increased infiltration of polymorphonuclear leukocytes in the epithelial layer (Fig. 5A and 5B). The degrees of ischemic injury in the reperfused intestine were significantly attenuated in the animals receiving rP-sel-Fc treatment (Fig. 5A and 5B).

Discussion

There are few clinically relevant experimental models of HSR in traumatology research.[20] In major trauma, isolated hem-

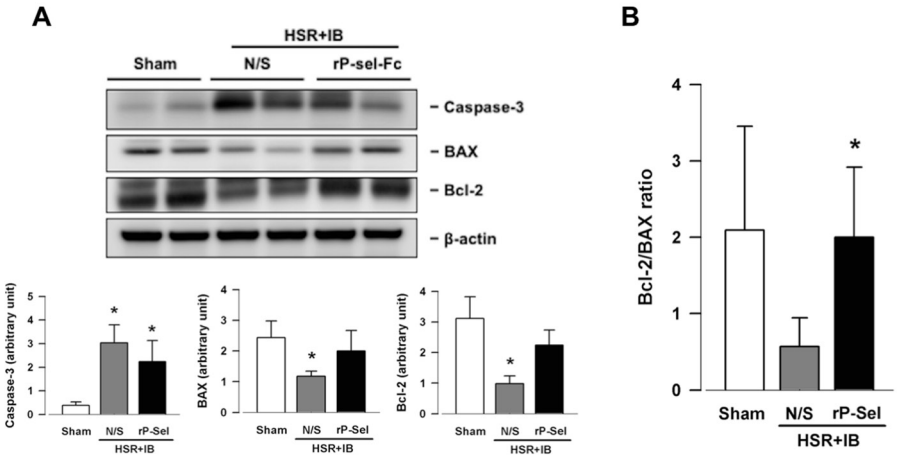

*Fig. 3 Profile of mitochondrial function in the ischemic intestinal tissue. (A) HSR and IB significantly enhanced the expression of caspase-3, indicating the presence of apoptotic reaction following reperfusion insult. The expression of bcl-2 was significantly suppressed in the IB jejunum, and treatment with rP-sel-Fc restored the expression of this cell surviving protein. (B) The increased bcl-2-to-BAX protein ratio suggested an anti-apoptotic effect of rP-sel-Fc treatment. *p < 0.05 vs. shams (n = 5 in each group). All protein expressions presented as original blots in compliance with the digital imaging and integrity policies defined by Nature Research.*

orrhagic shock without any organ injury is uncommon in the clinical setting. In fact, both hemorrhagic shock and traumatic tissue injury can independently trigger cascades of posttraumatic systemic inflammatory and immune responses, leading to MOSF and death.[20,21] In this study, we characterized a "2-hit" model of HSR and bowel ischemia injury that mimics mesenteric hypoperfusion or "no reflow" phenomenon followed by reperfusion after resuscitation. Reperfusion injury results in systemic inflammatory response syndrome (SIRS), metabolic acidosis, electrolyte derangement and formation of toxic reactive oxygen species, which accounts for 30 – 40% mortality during the post-reperfusion care.[8] Compared with isolated HSR, this 2-hit model significantly increased the mortality of experimental mice from 0% to 80% at 15 h after resuscitation and exacerbated transmural necrosis of the ischemic intestine.

At 6 h after HSR and mesenteric artery occlusion, the segment of ischemic intestine showed evidence of significant inflammatory reaction, including the increased expressions of iNOS, MPO activity and infiltration of polymorphonunclear leukocytes. Both hemorrhagic shock and ischemia-reperfusion injury may induce a surge of pro-inflammatory cytokines (i.e., systemic inflammatory response syndrome, SIRS) and a suppression of adaptive immunity (i.e., compensatory anti-inflammatory response syndrome, CARS).[8,22] The transmigration of leukocytes and platelets in the blood circulation following the 2-hit injury is initiated by rolling of these activated circulating cells on the luminal side of the endothelium mediated by the receptors on the adhesion molecules, such as selectins, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and platelet/endothelial cell adhesion molecule (PECAM)-1.[23,24] During ischemia-reperfusion injury, the generation of reactive oxygen species (ROS) and intracellular calcium overload lead to the opening of the mitochondrial transition pore (mPTP) and the generation of endoplasmic reticulum stress, thereby causing apoptosis and cell death.[25] In this study, animals exposed to 2-hit ischemia-reperfusion injury expressed significantly higher oxidative stress in the endoplasmic reticulum and cell apoptosis, evidenced by reversed Bcl-2/BAX ratio and enhanced expression of caspase-3 in the ischemic bowel tissue, which is consistent with characteristic mitochondrial

dysfunction-related cell apoptosis.[19] Furthermore, high regional oxidative stress in the injured bowel was also demonstrated by the significant induction of HO-1 and increased tissue lipid peroxidase activity, as measured by the formation of MDA. The profound oxidative stress generated by the 2-hit injury model resulted in suppression of cytosolic CuZnSOD, suggesting irreversible injury[26]; while the mitochondria-associated MnSOD may be upregulated or remain unchanged.[27]

The role of *p*-selectin in hemorrhagic shock was reported by Scalia et al. in 1999.[28] Using a mouse model of hemorrhagic shock, the authors characterized the increased number of rolling and adherent leukocytes in the splanchnic microcirculation under intravital microscopy. That study demonstrated that leukocyte adhesion was significantly reduced in *p*-selectin knockout mice and wild-type mice treated with anti-*p*-selectin monoclonal antibody or a recombinant soluble PSGL-1 immunoglobulin. They also demonstrated that the infiltrations of leukocytes in the lungs, liver and intestines were also attenuated in *p*-selectin deficiency mice. Although that elegant study underscored the essential role of *p*-selectin in the recruitment of leukocytes in the microcirculation during hemorrhagic shock, the phenotypes of *p*-selectin were not addressed. The phenotypes of soluble and membrane-bound forms of *p*-selectin could be distinct; plasma soluble *p*-selectin has been shown to mediate cell surviving response.[14,15] Based on these previous studies demonstrating the pro-inflammatory and pro-coagulation properties of *p*-selectin bound to PSGL-1, the present study took a further step to examine the potential rescue effect of soluble *p*-selectin on animals subject to HSR and reperfusion injury.

Previous studies have reported the administration of recombinant P-sel IgG-Fc as a soluble form of *p*-selectin to rescue the wild-type mice from 2-hit injury.[14,15] Administration of rP-sel-Fc in the current study failed to alleviate the regional inflammatory reaction, measured by tissue levels of iNOS, CD11b myeloperoxidase activity and inflammatory cytokines, supporting the hypothesis that the profound recruitment of leukocytes during HSR and ischemia-reperfusion injury is mediated by the membrane-bound *p*-selectin, and is not reversible by soluble *p*-selectin. However, our study found that lipid peroxidation was

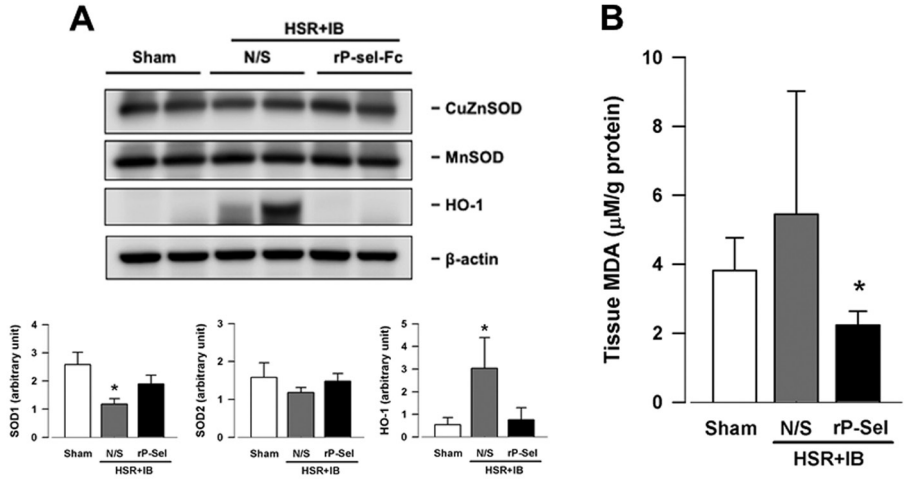

Fig. 4 Profile of oxidative stress in the ischemic intestinal tissue. Increased tissue oxidative stress was detected in the ischemic jejunum as reflected by (A) expression of heme oxygenase-1(n = 5 in each group), and (B) MDA activity (n = 6-7 in each group). rP-sel-Fc restored the expressions of CuZnSOD (SOD1) and MnSOD (SOD2) and suppressed tissue levels of MDA, suggesting a reduction in ischemia-reperfusion-induced regional oxidative stress in the bowel by the soluble form of P-sel. Data were analyzed using one-way ANOVA. All protein expressions presented as the original blots in compliance with the digital imaging and integrity policies defined by Nature Research.

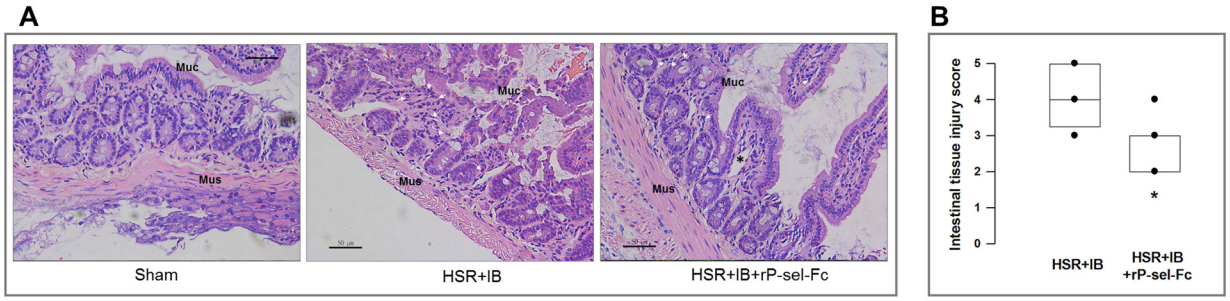

*Fig. 5 Histological analysis of jejunum exposed to HSR and IB. (A) (Sham) Normal intestinal histology with typical villi and crypts in the mucosa layer (Muc), and underneath muscularis externa (Mus). (HSR+IB) Extensive mucosal ulceration and necrosis with crypt layer infarction (Chiu's intestinal injury score Grade IV). (HSR+IB+rP-sel-Fc) Epithelial lifting and formation of Gruenhagen's space (*) in the lamina propria (Chiu's intestinal injury score Grade III). Infiltration of polymorphonuclear cells (white arrows) was observed in the mucosal layer of the ischemic jejunum. (B) Quantitative analysis of intestinal tissue injury using the Chiu's injury scoring system.[19] Four quadrants of each section were assessed under light microscopy and a median score was obtained from the four readings (*p = 0.03 analyzed by Wilconxin rank-sum test, n = 8 in each group). The presentations of all histological images are in compliance with the digital imaging and integrity policies defined by Nature Research.*

significantly suppressed and the expression of CuZnSOD was restored in the reperfused intestine of rP-sel-Fc-treated animals. Treatment with rP-sel-Fc also significantly enhanced regional Bcl-2 expression and reversed Bcl-2-to-BAX ratio in the intestine. Bcl-2 family members have different roles to play in eliciting MOMP during cell apoptosis; while Bcl-2 acts as an anti-apoptotic protein, BAX is pro-apoptotic through inducing mitochondrial pore formation and cytochrome c release.[19,29] Collectively, we showed that supplement of soluble *p*-selectin attenuates the oxidative stress in injured intestine by enhancing endogenous antioxidant capacity and mitochondrial homeostasis. These beneficial cellular effects led to morphological improvement in the ischemic intestine and, most importantly, the reduction in overall mortality at 15 h after 2-hit injury.

There were a number of limitations in this study. First, gene modulation animals were not employed to differentiate the effects of *p*-selectin bound to PSGL-1 from those of its soluble form. However, a body of evidence support the pro-inflammatory property of membrane bound form *p*-selectin including induction of vasculopathy and entrapment of acute inflammatory cells as in the hemorrhagic shock model reported by Scalia et al.[28] Our study provides new information endorsing the anti-oxidant and anti-apoptotic properties of soluble *p*-selectin in hemorrhagic shock. Second, a clinical study has recently demonstrated that serum level of soluble *p*-selectin was positively correlated with the development of MSOF and mortality in trauma patients presenting with hemorrhagic shock in the emergency room.[30] Our results seem to be contradictory to such clinical observation. Nonetheless, causal relationship could not be established from an observational study, as higher levels of serum *p*-selectin may simply be the result of a compensatory increase in response to more severe traumatic injuries. Third, the effect of rP-sel-Fc on survival outcome was only observed up to 15 h after reperfusion injury. Therefore, the effect of soluble form *p*-selectin on experimental outcomes after a longer duration remains unclear.

Conclusion

Using a 2-hit experimental model of hemorrhagic shock resuscitation and ischemia-reperfusion injury, our results demonstrated that exogenous soluble *p*-selectin could offer potent antioxidant and anti-apoptotic effects against reperfusion injury in the ischemic in-

testine, thereby improving the overall survival in wild-type mice.

Acknowledgments

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References

1. The top 10 causes of death. WHO, 2017. http://www.who.int/mediacentre/factsheets/fs310/en/. Accessed July 25, 2019.
2. Sonnier DI, Makley AT, Friend LA, et al: Hemorrhagic shock induces a proinflammatory milieu in the gut lumen. J Surg Res 2011;170:272-9.
3. Ameno H, Tani T, Hanasawa K, et al: New method for the detection of bacterial translocation using intestinal permeability with polyethylene glycol 4000. Eur Surg Res 2000;32:23-9.
4. Strang SG, Van Lieshout EM, Van Waes OJ, et al: Prevalence and mortality of abdominal compartment syndrome in severely injured patients: A systematic review. J Trauma Acute Care Surg 2016;81:585-92.
5. Beishuizen A, Girbes AR: Hemorrhagic shock and reperfusion injury: the critical interplay of fibrin fragments, leukocytes, and vascular endothelial-cadherin. Crit Care Med 2009;37:771-2.
6. Rushing GD, Britt LD: Reperfusion injury after hemorrhage: a collective review. Ann Surg 2008;247:929-37.
7. Cook-Mills JM, Deem TL: Active participation of endothelial cells in inflammation. J Leukoc Biol 2005;77:487-95.
8. Collard CD, Gelman S: Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology 2001;94:1133-8.
9. McEver RP: Selectins: initiators of leucocyte adhesion and signaling at the vascular wall. Cardiovasc Res 2015;107:331-9.
10. Patel MS, Miranda-Nieves D, Chen J, et al: Targeting *p*-selectin glycoprotein ligand-1/*p*-selectin interactions as a novel therapy for metabolic syndrome. Transl Res 2017;183:1-13.
11. Mangell P, Mihaescu A, Wang Y, et al: Critical role of *p*-selectin-dependent leukocyte recruitment in endotoxin-induced intestinal barrier dysfunction in mice. Inflamm Res 2007;56:189-94.
12. Semenov AV, Romanov YA, Loktionova SA, et al: Production of soluble *p*-selectin by platelets and endothelial cells. Biochemistry (Mosc) 1999;64:1326-35.
13. Barron E, Lara J, White M, et al: Blood-borne biomarkers of mortality risk: systematic review of cohort studies. PLoS One 2015;10:e0127550.
14. Sun DS, Chang YW, Kau JH, et al: Soluble *p*-selectin rescues mice from anthrax lethal toxin-induced mortality through PSGL-1 pathway-mediated correction of hemostasis. Virulence 2017;8:1216-28.
15. Sun DS, Ho PH, Chang HH: Soluble *p*-selectin rescues viper venom-induced mortality through anti-inflammatory properties and PSGL-1 pathway-mediated correction of hemostasis. Sci Rep 2016;6:35868.
16. Kao MC, Yang CH, Sheu JR, et al: Cepharanthine mitigates pro-inflammatory cytokine response in lung injury induced by hemorrhagic shock/resuscitation in rats. Cytokine 2015;76:442-8.
17. Su MW, Chang SS, Chen CH, et al: Preconditioning renoprotective effect of isoflurane in a rat model of virtual renal transplant. J Surg Res 2014;189:135-42.
18. Wu XD, Zhang ZY, Sun S, et al: Hypoxic preconditioning protects microvascular endothelial cells against hypoxia/reoxygenation injury by attenuating endoplasmic reticulum stress. Apoptosis 2013;18:85-98.
19. Chiu CJ, McArdle AH, Brown R, et al: Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970;101:478-83.
20. Fülöp A, Turóczi Z, Garbaisz D, et al: Experimental models of hemorrhagic shock: a review. Eur Surg Res 2013;50:57-70.
21. Liu H, Xiao X, Sun C, et al: Systemic inflammation and multiple organ injury in traumatic hemorrhagic shock. Front Biosci (Landmark Ed) 2015;20:927-33.
22. Haberstroh J, Gilleland HE Jr, von Specht BU: Effect of anti-OprF-OprI immunoglobulin on APACHE II score in a porcine two-hit model of hemorrhagic shock/resuscitation and pseudomonas aeruginosa sepsis. Eur Surg Res 2005;37:265-73.
23. Coenen DM, Mastenbroek TG, Cosemans J: Platelet interaction with activated endothelium: mechanistic insights from microfluidics. Blood 2017;130:2819-28.
24. McEver RP: Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol 2002;14:581-6.
25. Salvadori M, Rosso G, Bertoni E: Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. World J Transplant 2015;5:52-67.
26. Singh I, Gulati S, Orak JK, et al: Expression of antioxidant enzymes in rat kidney during ischemia-reperfusion injury. Mol Cell Biochem 1993;125:97-104.
27. Dobashi K, Ghosh B, Orak JK, et al: Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol Cell Biochem 2000;205:1-11.
28. Scalia R, Armstead VE, Minchenko AG, et al: Essential role of *p*-selectin in the initiation of the inflammatory response induced by hemorrhage and

reinfusion. J Exp Med 1999;189:931-8.
29. Iurlaro R, Muñoz-Pinedo C: Cell death induced by endoplasmic reticulum stress. FEBS J 2016;283:2640-52.
30. Furmaga W, Cohn S, Prihoda TJ, et al: Novel markers predict death and organ failure following hemorrhagic shock. Clin Chim Acta 2015;440:87-92.