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Research Article

Plasma-Derived C1 Esterase Inhibitor Treatment to Switch the Injury Phenotype to an Organ-Protective Phenotype after Traumatic Hemorrhage in Rats

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Abstract

Background: Hemorrhage is a major preventable cause of death in patients with trauma. Dysregulated complement activation constitutes part of the "second hit" of trauma, resulting in end-organ dysfunction. This study aimed to evaluate the effect of plasma-derived C1 inhibitor (pdC1-INH) on multi-organ damage in a rat model of combined blast injury and volume-controlled hemorrhage.

Methods: Anesthetized rats underwent moderate blast overpressure and severe bleeding (52%). The injured animals were randomized to two groups: 1) Injury + Placebo (n=10), and 2) Injury + pdC1-INH (200U/kg, i.v, n=10). After 30 min of the shock phase, all the animals received 2x the shed blood volume of Plasma-Lyte A and were monitored up to 24 hours after the shock.

Results: Pharmacokinetic measurement revealed C_{max} (2.69 ± 0.2U/ml) by 45min, clearance (1.29 ± 0.75 ml/min), volume distribution (14.2 ± 0.45 ml), and half-life (8.27 ± 1.96h). The pharmacodynamic assessment showed that pdC1-INH treatment significantly inhibited the terminal complement activity by 75% at 11h and remained at 50% inhibition until the end of the experiment. Administration of pdC1-INH significantly alleviated tissue inflammation and organ damage in the brain and liver but not in the lung tissue compared to the placebo. However, there was no significant difference in mortality between pdC1-INH-treated and placebo-treated animals.

Conclusion: pdC1-INH switched the injury phenotype to an organ-protective phenotype after blast injury and hemorrhage by mitigating tissue inflammation. The utilization of pdC1-INH in prehospital settings may lead to a significant reduction in morbidity and mortality of military and civilian trauma patients.

Keywords: Coagulopathy; Damage control resuscitation; Immunopathy; MOF; Mortality; Plasma-derived C1-inhibitor; Rats; Traumatic hemorrhage

Introduction

Almost all trauma deaths happen in the first few hours after injury, often before the injured patient gets to a medical facility. Hemorrhagic shock is invariably the second-leading cause of early death among trauma patients, with most traumatic brain injuries more deadly. However, hemorrhage and hemorrhagic shock are more manageable for treatments to reduce morbidity and mortality secondary to hemorrhage [1,2]. Ischemia/reperfusion injury following hemorrhagic shock resuscitation could be amplified by traumatic brain injury (TBI)-induced cut-off of cerebral autoregulation [3]. In the pre-hospital trauma situation, the golden-hour rule always evokes providers. Therefore, prompt transportation may be beneficial for patients with neurotrauma and penetrating injury with unstable hemodynamic features [4]. Most injuries in trauma patients require major surgery, as it is reiterated a decade and a half ago. Major surgery causes critical inflammatory, immune, neuroendocrine, and metabolic changes, broadly accounting for the "stress response" [5]. Complement activation is a component of the stress response to traumatic injury, crisscrossing and amplifying the coagulation activation pathways [6]. We showed that rhC1-inhibitor reduced organ injury and extended survival in porcine combat simulated injury [7]. Human plasma-derived C1-inhibitor (pdC1-INH) indications are used to prevent and treat hereditary angioedema (HAE) [8,9]. We assumed that this off-label use would be effective in the early treatment of traumatic hemorrhage. This study aimed to 1) assess systemic complement activation in rats subjected to the blast wave and controlled bleeding, 2) assess pharmacokinetic parameters of pdC1-INH in rats, and 3) test the therapeutic effects of pdC1-INH on traumatic hemorrhage.

Materials and Methods

Animal Study

The study complied with the Animal Welfare Act, the corresponding Animal Welfare regulations, the principles of the Guide for the Care and Use of Laboratory Animals, and National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC International.

General Procedures, Surgical Preparations, and Experimental Design

As the timeline shown in Figure 1, male Sprague-Dawley rats (350-475g; 10-12 weeks of age; Charles River Laboratories, Wilmington, MA) were randomly assigned to two groups: rats subjected to the blast wave and hemorrhagic shock (Injury + placebo/saline, n = 10) and injured rats treated with pdC1-INH (Injury + pdC1-INH, n = 10). The rats underwent anesthesia (1-3 % isoflurane) with suspension and the cannulation of the left carotid artery and jugular vein by cut-down. After recovery over 5-7 days, the rats were anesthetized (60/5 mg/kg of ketamine/xylazine, intraperitoneally) and exposed to the moderate blast wave (Applied Research Associates, Littleton, CO). A rat was placed in a prone position, allowing the head to face the blast front, on a holder of a flat plastic mesh suspended between two stainless steel rods running horizontally alongside the animal. A second plastic mesh was placed over the top of the rat and secured to the rods to prevent the rat from falling off the holder. The holder was attached to a swing mechanism to let the rat partially recoil against the blast. The animal was transferred to the surgical room, anesthesia continued maintained with isoflurane1-2.5%), and 15 minutes after the blast overpressure and immediately before controlled hemorrhage [52% of the Estimated Total Blood Volume (ETBV)] was started, an intravenous bolus of pdC1-INH (200IU/kg in 0.5ml saline, C1-esterase inhibitor (human), Berinert, CSL Behring GmbH, Marburg, Germany) was injected. The hemorrhage was completed in 15 minutes, followed by a 30-minute shock phase, which ended with the infusion of Plasma-Lyte A (PLA) at two times of Shed Blood Volume (SBV) via the jugular vein line at a rate of 0.5 ml/min to create hypotensive resuscitation. Three hours after the shock, animals were allowed to recover from anesthesia, return to their cages, and be observed for 25 hours. The arterial blood pressures of animals were consistently monitored and recorded with the BIOPAC MP160 Data Acquisition and Analysis Systems via the carotid arterial catheter.

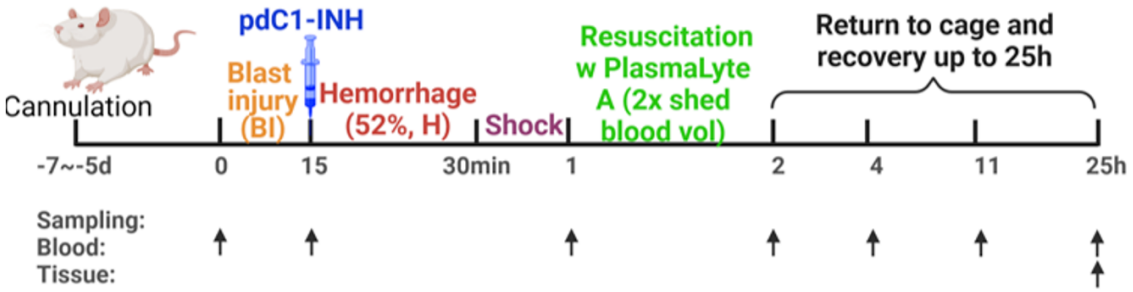

Figure 1: Schematic showing the time frame of traumatic hemorrhage with the various post-injury phases. pdC1-INH, plasma-derived C1 inhibitor; vol, volume.

Biological Samples

At the end of the total procedure and observation time of 25 hours, the animals were euthanized with Fatal Plus at 150 mg/kg. Necropsies and tissue harvests were carried out. Tissue samples were fixed with 10% formalin for subsequent histological analyses. Blood samplings were performed at 7 time points as shown in Figure 1. Serums and plasmas were prepared by centrifuging at 4000 rpm for 10 minutes. Aliquots of serum and plasma samples were stored at – 80°C for subsequent analyses.

Assays

Pharmacokinetic Measurement: The plasma level of functional C1-INH was determined using the MicroVue™ C1-Inhibitor Plus EIA kit (Quidel, San Diego, CA) following the manufacturer's instructions. The maximum concentration (C_{max}) above the baseline was derived directly from the concentration-time profiles. The pharmacokinetic parameters of functional C1-INH were determined using a 1-compartment pharmacokinetic model. Calculations were performed using linear model (first-order kinetics) estimation.

Blood chemistry: Blood lactate, base excess/deficit, and potassium were analyzed by i-STAT using CG4+ and CG8+ cartridges (Abbott Laboratories, Green Oaks, IL),

Hemolytic Complement Activity Assay: Serum complement hemolytic 50% activity (CH50) assay was performed to determine the function of the complement classical pathway, as previously described [10]. Briefly, antibody-sensitized chicken red blood cells (Colorado Serum Company, Denver, CO) were incubated for 30 min at 37 ℃ with serial dilutions of rat serum samples in gelatin-veronal buffer (Complement Technology, Tyler, TX). After centrifugation, the supernatant was transferred to a new plate, and the absorbance of the supernatant was determined at 405 nm by SpectraMax microplate reader (Molecular Devices San Jose, CA, USA). The fold serum dilution inducing 50% of complement hemolytic activity was determined and presented as the CH50 value.

Measurement of complement protein, plasminogen, MCP-1, HMGB1 and MPO Levels: Plasma levels of C1q (HycultBiotech, Plymouth Meeting, PA), C3 (Abcam, Cambridge, MA), plasminogen C3 (Abcam, Cambridge, MA), MCP-1 C3 (Abcam, Cambridge, MA), HMGB1 (IBL International Morrisville, NC), and myeloperoxidase (MPO) (HycultBiotech, Plymouth Meeting, PA) were measured using ELISA kits according to the manufacturer's instructions.

Histological Examination and Tissue Injury Scoring: Ten percent of formalin-fixed tissues were embedded in paraffin. Coronal sections were then stained with hematoxylin-eosin (H&E). Five random histologic images were recorded at ×2, ×100 and ×400 magnifications under a slide scanner (Axio Scan.Z1, Carl Zeiss, Thornwood, NY), evaluated, and scored by a pathologist blinded to the treatment group using the tissue scoring protocols as previously described [10,11].

Statistical Analysis

All data were presented as means ± SEM. The responses in inflammation and organ injury parameters were compared between groups using Mann-Whitney U test or unpaired t-test with Welch's correction. Two-way ANOVA was performed to compare the animal groups on particular variables. Bonferroni post hoc tests were conducted only if the F value in ANOVA achieved $p < 0.05$. Survival was analyzed by the Kaplan-Meier method. Statistical significance was accepted at $p < 0.05$.

Results

Blast Wave Parameters, Mean Arterial Pressure, and Blood Chemistry after Combined Injury

As shown in Table 1, the shock tube produced typical open-field blast parameters. There was no significant difference in blast wave parameters between injured/untreated and injured/treated groups. The mean arterial pressure (MAP) was significantly decreased in 30 minutes and the first 11 hours after the blast and hemorrhage

(Figure 2). Injured/treated animals tended to increase MAP in the first 40 min of the resuscitation phase compared with injured/untreated rats but did not reach a significant difference. Base excess/base deficit significantly decreased (Figure 3A), unlike plasma lactate (Figure 3B) and potassium (Figure 3C) concentrations, which significantly increased immediately after hemorrhagic shock. However, there was no significant difference in these parameters between the injured and treated rats.

Group	Reference			Overpressure			Reflected		
	P0 (kPa)	t+ (ms)	I (kPa-ms)	P0 (kPa)	t+ (ms)	I (kPa-ms)	P0 (kPa)	t+ (ms)	I (kPa-ms)
Injury	109.3 ± 1.2	3.3 ± 0.01	140.4 ± 0.7	117.2 ± 1.2	3.3 ± 0.01	141.5 ± 0.7	162 ± 1.6	3.5 ± 0.03	178.8 ± 1.1
pdC1-INH	107 ± 1.4	3.3 ± 0.02	141.3 ± 0.3	114.7 ± 1.4	3.2 ± 0.02	142.4 ± 0.3	161.7 ± 1.3	3.5 ± 0.03	179.7 ± 0.4

Notes: Data were expressed as mean ± SEM. Injury group = blast + hemorrhage;)]; I = impulse; pd-C1-INH group = Injury + pdC1-INH; P0 = peak pressure; t+ = the positive-pressure phase duration in milliseconds.

Table 1: Blast wave parameters.

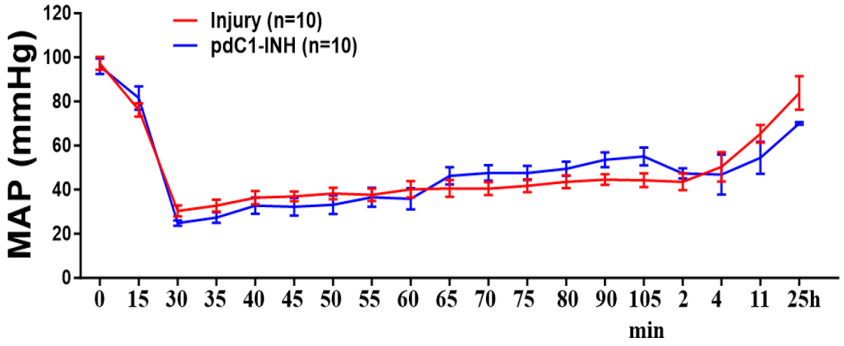

Figure 2: Effects of pdC1-INH on mean arterial pressure (MAP) in a rat blast injury and hemorrhage model. Changes in MAP were monitored via the carotid artery using the BIOPAC system. During the shock and fluid resuscitation period, the MAP was recorded every 5 min. Data are expressed as mean ± SEM. n = 10.

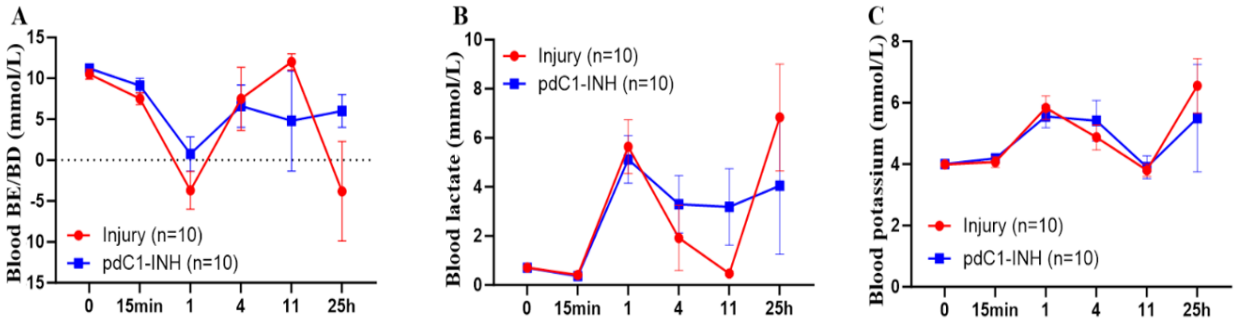

Figure 3: Effects of pdC1-INH on blood chemistry changes in a rat blast injury and hemorrhage model. Rat blood levels of BE/BD (A), lactate (B), and potassium (C) were measured by i-Stat. Data are expressed as mean ± SEM. n = 10. BE, base access; BD, base deficit.

Effect of pdC1-INH on Systemic Inflammatory Response

The HMGB1 plasma concentrations significantly increased 3 hours after hemorrhagic shock, but no significant difference existed between the treated and non-treated rats (Figure 4A). Plasma myeloperoxidase (MPO; Figure 4B) and monocyte chemoattractant protein [MCP-1, also referred to as the chemokine ligand 2 (CCL2); Figure 4C] concentrations were not affected by the combined injury and no significant difference in these parameters between treated and non-treated rats.

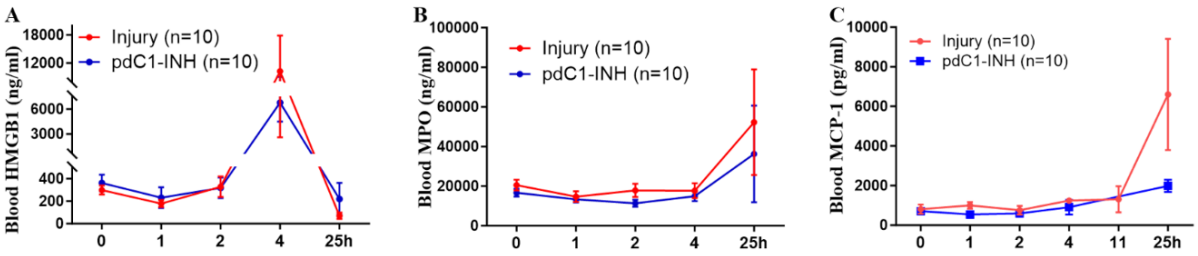

Figure 4: Effects of pdC1-INH on systemic inflammatory response in a rat blast injury and hemorrhage model. Rat blood levels of HMGB1 (A), MPO (B), and MCP-1 (C) were measured by ELISA. Data are expressed as mean ± SEM. n = 10. HMGB1, high mobility group box 1 protein; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase.

Effect of pdC1-INH on Survival

As shown in Figure 5, the injured/untreated rats had a 70% mortality rate, and the treatment with an IV bolus of pdC1-INH 15 minutes after the blast but immediately before hemorrhage did not improve survival compared to the vehicle control.

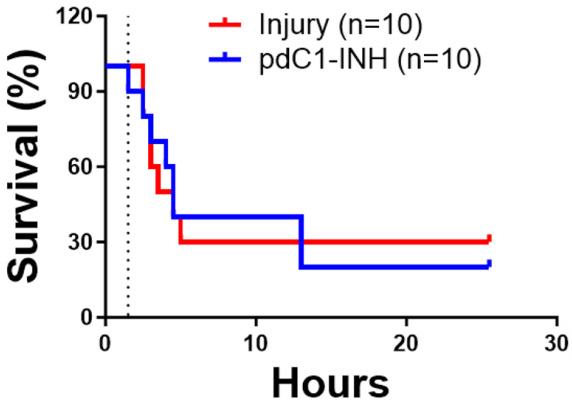

Figure 5: Effect of pdC1-INH on injury-induced mortality. All rats were subjected to blast and severe hemorrhage, treated with vehicle control (saline, Injury) or pdC1-INH, and monitored for survival up to 25 h after blast injury. The survival distribution of these two groups was determined using the log-rank Mantel-Cox test.

Effect of pdC1-INH on Organ Histopathological Changes

Exposure to the blast wave and hemorrhagic shock elicited pathological alterations characterized by neuroinflammation, neuronal apoptosis, neuronal death, and cerebral cytotoxic and vasogenic edema in the cortex (Figure 6A & B) and hippocampus (Figure 6C & D). The injured rats treated with pdC1-INH significantly improved the cerebral pathological changes (Figure 6A-D). Figure 6E & F illustrate hepatic ischemic infarct injury around the portal vein featured by foci of moderate coagulative necrosis with pyknosis, karyolysis, nuclear absence, significant neutrophil infiltration, and the sharp border between normal and ischemic tissue in injured/untreated animals, while pdC1-INH significantly attenuated the liver injury. Traumatic hemorrhage-induced severe pulmonary damage manifested as a septal thickening, inflammatory cell infiltration, alveolar hemorrhage, and edema (Figure 6G & H). The lung injury score showed no significant improvement in the tissue alterations in the injured animals treated with pdC1-INH (Figure 6H).

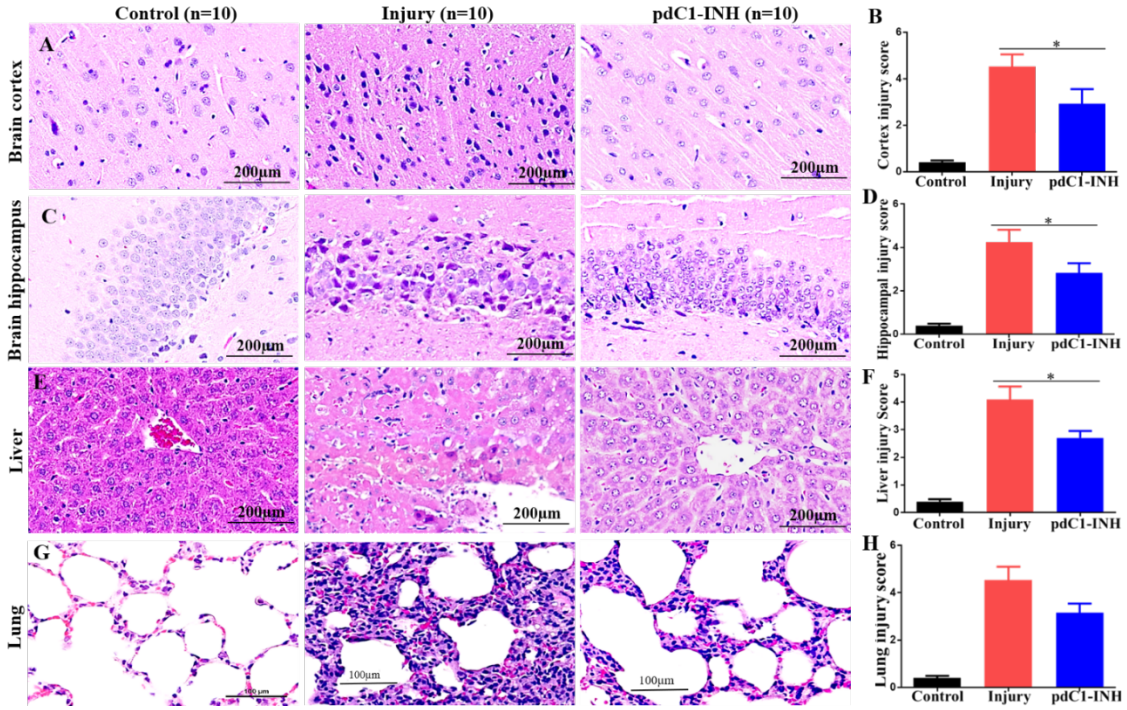

Figure 6: Effect of pdC1-INH treatment on pathological changes in rats after blast injury and hemorrhage. Representative H&E photomicrographs and injury scores of brain cortex (A & B, scale bar = 200 μm), hippocampus (C & D, scale bar = 200 μm), liver (E & F, scale bar = 200 μm), and lung (G & H, scale bar = 100 μm). The data were presented as means ± SEM. * $p < 0.05$, Injury vs. pdC1-INH (Mann–Whitney U test).

Pharmacokinetic Activities

Plasma-derived C1-inhibitor concentrations in the blood were measured by MicroVue™ C1-INH Plus EIA (Figure 7 and Table 2). Functional pdC1-INH peaked by 1 h after administration and remained ≥ 1 U/ml up to 20h (Figure 7A). The elimination curve (semi-log plot, Figure 7B) showed a single straight line and a steady decrease in concentration as the pdC1-INH was eliminated (Figure 7C), suggesting one compartment pharmacokinetic model and first-order kinetics of pdC1-INH in this study. Pharmacokinetic parameters of functional pdC1-INH showed C_{max} (2.69 ± 0.20 U/ml), dose normal Cmax (0.01 ± 0.00 U/ml/kg), clearance (1.29 ± 0.75 ml/min), volume distribution (14.20 ± 0.45 ml), and half-life (8.27 ± 1.96 h) (Table 2).

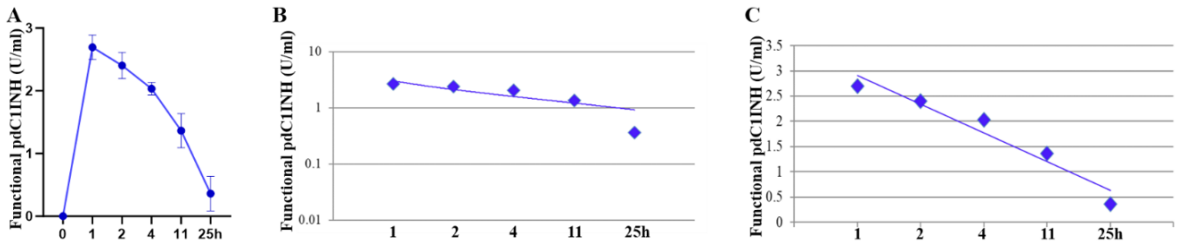

Figure 7: Pharmacokinetic measurement from initiation to the end of treatment in rats subjected to blast injury and hemorrhage. Rat plasma levels of functional pdC1-INH were determined by a modified Microvue™ C1-INH Plus EIA kit (A). Elimination curves of semi-log plot (B) and linear plot (C). Group data are expressed as mean ± SEM. n = 3.

Dose (U/kg, iv)	n	Baseline (U/ml)	C_{max} (U/ml)	Dose normal C_{max} (U/ml/kg)	Clearance (ml/min)	Volume distribution (ml)	Half-life (h)
200U/kg	3	0.00 ± 0.00	2.69 ± 0.20	0.01 ± 0.00	1.29 ± 0.75	14.20 ± 0.45	8.27 ± 1.96

Notes: Data were expressed as mean ± SEM. C_{max}: the peak plasma concentration of a drug after administration.

Table 2: Summary of pharmacokinetic parameters of functional pdC1-INH in rats subjected to blast injury and hemorrhage.

Pharmacodynamic Activities

CH50 in the injured rats started to decrease 15 min, reached its lowest level at 2h, normalized to baseline level at 11 h, and exceeded the baseline (150%) at 25h after blast injury, whereas CH50 values in the injured/treated rats were significantly lower at 11h and 25h than in the injured/untreated group (Figure 8A). Administration of pdC1-INH did not significantly affect C1q, C3, and plasminogen circulating levels compared with the injured/untreated groups (Figure 8B-D).

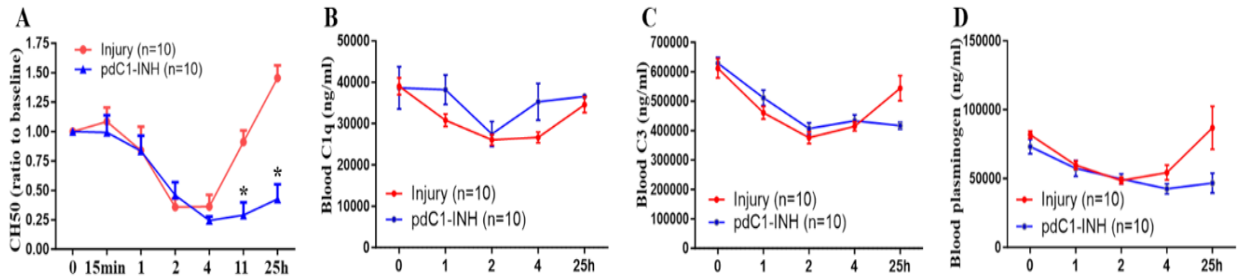

Figure 8: Pharmacodynamic measurements from initiation to the end of treatment in rats subjected to blast injury and hemorrhage. Rat blood levels of terminal complement activation (A), C1q (B), C3 (C), and plasminogen (D) were assessed by CH50 and ELISA. Data are expressed as mean ± SEM. n = 10. Injury = blast injury + hemorrhage; pdC1-INH = Injury + pdC1-INH.

Discussion

Traumatic hemorrhage is a leading cause of potentially preventable death in military and civilian ambiance. Tissue damage after traumatic hemorrhage and post-traumatic hemorrhage therapeutic interventions (e.g., transfusion, surgery, instrumentation) trigger the extracellular release of damage-associated molecular patterns (DAMPs) that stimulate innate immune system (e.g., complement, neutrophils, monocytes, macrophages) through the interaction of DAMPs and pattern recognition receptors (e.g., complement receptors, C1q, mannan-binding lectin, ficolins), leading to "cytokine" storm, organ inflammatory syndrome, and Multiple-Organ Failure (MOF) [12-15]. We and others previously demonstrated that 1) the classical (CP)/lectin (LP) pathways initiate complement activation [16,17], 2) the CP and LP appeared to be the predominantly activated complement pathways after trauma [18-20] and 3) early CP/LP activation was associated with alterations of RBCs and platelets, tissue hypoperfusion, systemic inflammatory response syndrome, TBI, massive fresh frozen plasma transfusion, MOF, and mortality following major trauma and TBI [19,21-24].

C1 inhibitor has been named after its inhibitory effect on complement C1; however, it is a crucial regulator involved in the inhibition of several additional plasma cascades (e.g., LP, contact, coagulation, fibrinolytic proteases) as well as in the interactions with leukocytes, platelets, endothelial cells, extracellular matrix components, polyanions, and infectious agents, operating as a significant anti-inflammatory protein in circulation and at local injured sites [25-27]. The therapeutic administration of the C1 inhibitor is clinically applied in hereditary angioedema (HAE). A type of HAE with regular C1-esterase inhibitor activity, linked to mutations in genes associated with kallikrein-kinin system activation, bradykinin formation, or the coagulation factor XII [28], implies how much plasma cascades are intertwined. Potential alternative therapeutic applications of C1 inhibitors are investigated in animal models of traumatic hemorrhage in which complement-mediated inflammatory organ syndrome plays an important pathogenic role. Matsuura et al. reported that lower admission plasma functional C1 inhibitor levels after trauma correlated with fluid infusion requirement and mortality [29]. Early administration of C1 inhibitor reduced leukocyte adhesion and systemic/local inflammation, improved metabolic acidosis, attenuated organ damage, and increased survival in preclinical animal models of hemorrhage and traumatic hemorrhage (≤6h)

[7,30,31]. However, the efficacy of C1 inhibitor has not been examined in animal traumatic hemorrhage models in a prolonged field care setting.

In this study, administration of pdC1-INH significantly alleviated tissue inflammation and organ damage in the brain and liver but not in the lung tissue compared to the placebo 25h post-injury. The effect of pdC1-INH on tissue inflammation in the brain and liver may be due to its regulation of microvasculatures and/or its interaction with extracellular matrix components that may serve to concentrate pdC1-INH at sites of inflammation. In contrast, we did not observe a significant effect of pdC1-INH on systemic inflammatory parameters (e.g., HMGB1, MPO, MCP-1). It remains unclear why pdC1-INH does not effectively modulate systemic inflammatory response compared to our previous observations in porcine models of hemorrhage and traumatic hemorrhage [7,30]. Plausible explanations that the effect of pd-C1-INH on systemic inflammation does not depend on its capacity to regulate the complement cascade [25,32] and/or the formation of pdC1-INH-plasma protease complexes impair its function and clearance from circulation that is unable to inhibit systemic inflammation. Although this provides no likely explanation, there are also some differences in methodology between porcine and rat studies: 1) we used different brands of C1 inhibitors in porcine (Ruconest, a recombinant C1 inhibitor, Pharming NV) and rat (Berinert, a pdC1-INH, CSL Behring GmbH) studies; 2) different models of traumatic hemorrhage were used in porcine (femur fracture + soft tissue injury + controlled/uncontrolled hemorrhage) and rat (blast injury + controlled hemorrhage) studies; and 3) the animals in this study received a lower dose (200 U/kg) compared to the swine model (500 U/kg).

The blast wave creates rapid cycles of compression and decompression that can result in air emboli in the tissue or within the vasculature, leading to vascular occlusion, organ infarction, inflammation, MOF, and death. Pathological evaluation frequently observes air embolism in abdominal solid organs (e.g, liver, spleen, kidney). Liver damage was ~40% after blast injury, and the prone position often causes more severe lesions in abdominal organs than a supine position [33]. Consistent with these findings, this study also demonstrated that pathological alterations in the liver were characterized by ischemic infarction and inflammatory cell accumulation next to a distended and hemorrhaged portal vein in rats exposed to blast overpressure in a prone position. Administration of pdC1-INH diminished those changes. Air emboli can activate complement [34] and platelets [35] and trigger C3-dependent thromboinflammation. However, our results in the current study showed that tissue thromboinflammation was C3-independent, probably through the coagulation-C5 axis.

However, there was no significant difference in lung injury between pdC1-INH-treated and placebo-treated injured rats. In a recently published negative rat study, repetitive nebulization with a pdC1-INH could not alleviate pneumonia-related pulmonary complement activation. The rats with pneumonia had lower C1-inhibitor concentrations in BALF compared to healthy control, possibly caused by persistent but low tidal breathing. The authors speculated that pdC1-INH level was affected by the breathing pattern because of pneumonia and scavenging C1-inhibitor by mucus or increased consumption of C1-inhibitor because of enhanced inflammation. An increased neutrophil influx is associated with inflammation, and increased proteolytic activity by elastase could also inactivate C1-inhibitor [36]. Such a proteolytic environment may induce further pulmonary complement activation and lung injury [37]. Early administration of pdC1-INH significantly inhibited TCA but did not significantly affect plasma levels of C1q and C3 in this study. The existence of a cross-talk between the complement and coagulation cascades has been broadly discussed. Huber-Lang et al. discovered that thrombin in a proteolytic pattern activates C5 to generate C5a without C3 [38]. Amara et al. found that coagulation/fibrinolytic factors activate complement C5, which subsequently activates the complement pathway [39]. It is more likely that the effect of pdC1-INH on TCA in this study may be through inhibiting coagulation and/or fibrinolytic cascades.

Our study has certain limitations. The current study only measured a few inflammatory parameters. Future studies should assess whether pdC1-INH affects systemic/local "cytokine" storms. Additionally, the C1 inhibitor has "moonlighting" functions on other cascades, such as coagulation, kinin, and fibrinolytic cascades, in addition to the complement cascade. Further analyses of the effects of pd-C1INH on those cascades are warranted. Finally, to achieve optimal therapeutic efficacy, optimization of pdC1-INH treatment regimen may be required and will be further evaluated in this traumatic hemorrhage model.

Conclusion

Plasma-derived C1-INH shifts the injury phenotype to an organ-protective phenotype after the blast injury and hemorrhage by alleviating tissue inflammation. The utilization of pdC1-INH in prehospital settings may lead to a significant reduction in morbidity and mortality of trauma patients.

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References

1. Eastridge BJ, Holcomb JB, Shackelford S (2019) Outcomes of traumatic hemorrhagic shock and the epidemiology of preventable death from injury. Transfusion 59: 1423-1428.

2. Kauvar DS, Lefering R, Wade CE (2006) Impact of hemorrhage on

trauma outcome: An overview of epidemiology, clinical presentations, and therapeutic considerations. The Journal of Trauma 60: S3-11.

3. Muller CR, Courelli V, Lucas A, Williams AT, Li JB, et al. (2021) Resuscitation from hemorrhagic shock after traumatic brain injury with polymerized hemoglobin. Scientific Reports 11: 2509.
4. Harmsen AMK, Giannakopoulos GF, Moerbeek PR, Jansma EP, Bonjer HJ, et al. (2015) The influence of prehospital time on trauma patients outcome: A systematic review. Injury 46: 602-609.
5. Dobson GP (2015) Addressing the Global Burden of Trauma in Major Surgery. Frontiers in Surgery 2: 43.
6. Delvaeye M, Conway, E. M. (2009) Coagulation and innate immune responses: Can we view them separately? Blood 114: 2367-2374.
7. Campbell JC, Li Y, van Amersfoort E, Relan A, Dubick M, et al. (2016) C1 Inhibitor Limits Organ Injury and Prolongs Survival in Swine Subjected to Battlefield Simulated Injury. Shock 46: 177-188.
8. Farkas H, Kőhalmi KV, Visy B, Veszeli N, Varga L (2020) Clinical Characteristics and Safety of Plasma-Derived C1-Inhibitor Therapy in Children and Adolescents with Hereditary Angioedema-A Long-Term Survey. The Journal of Allergy and Clinical Immunology: In Practice 8: 2379-2383.
9. Martinez-Saguer I, Bork K, Latysheva T, Zabrodska L, Chopyak V, et al. (2024) Plasma-derived C1 esterase inhibitor pharmacokinetics and safety in patients with hereditary angioedema. Journal of Allergy and Clinical Immunology: Global 3: 100178.
10. Yang Z, Nunn MA, Le TD, Simovic MO, Edsall PR, et al. (2023) Immunopathology of terminal complement activation and complement C5 blockade creating a pro-survival and organ-protective phenotype in trauma. British Journal of Pharmacology 180: 422-440.
11. Yang Z, Simovic MO, Edsall PR, Liu B, Cancio TS, et al. (2022) HMGB1 Inhibition to Ameliorate Organ Failure and Increase Survival in Trauma. Biomolecules 12: 101.
12. Cohen MJ, Brohi K, Calfee CS, Rahn P, Chesebro BB, et al. (2009) Early release of high mobility group box nuclear protein 1 after severe trauma in humans: Role of injury severity and tissue hypoperfusion. Critical Care (London, England) 13: R174.
13. Dobson GP, Morris JL, Letson HL (2022) Immune dysfunction following severe trauma: A systems failure from the central nervous system to mitochondria. Frontiers in Medicine 9.
14. Huber-Lang MS, Ignatius A, Köhl J, Mannes M, Braun CK (2021) Complement in trauma-Traumatised complement? British Journal of Pharmacology 178: 2863-2879.
15. Lord JM, Midwinter MJ, Chen Y-F, Belli A, Brohi K, et al. (2014) The systemic immune response to trauma: An overview of pathophysiology and treatment. Lancet (London, England) 384: 1455-1465.
16. Ciechanowska A, Ciapała K, Pawlik K, Oggioni M, Mercurio D, et al. (2020) Initiators of Classical and Lectin Complement Pathways Are Differently Engaged after Traumatic Brain Injury-Time-Dependent Changes in the Cortex, Striatum, Thalamus and Hippocampus in a Mouse Model. International Journal of Molecular Sciences 22.
17. Ganter MT, Brohi K, Cohen MJ, Shaffer LA, Walsh MC, et al. (2007) Role of the alternative pathway in the early complement activation following major trauma. Shock (Augusta, Ga.) 28: 29-34.
18. Burk A-M, Martin M, Flierl MA, Rittirsch D, Helm M, et al. (2012) Early complementopathy after multiple injuries in humans. Shock 37: 348-354.
19. Li Y, Zhao Q, Liu B, Dixon A, Cancio L, Dubick M, et al. (2019) Early complementopathy predicts the outcomes of patients with trauma. Trauma Surgery & Acute Care Open 4.
20. Wallis R, Mitchell DA, Schmid R, Schwaeble WJ, Keeble AH (2010) Paths reunited: Initiation of the classical and lectin pathways of complement activation. Immunobiology 215: 1-11.
21. Atefi G, Aisiku O, Shapiro N, Hauser C, Dalle Lucca J, et al. (2016) Complement Activation in Trauma Patients Alters Platelet Function. Shock 46: 83-88.
22. Muroya T, Kannan L, Ghiran IC, Shevkoplyas SS, Paz Z, et al. (2014) C4d Deposits on the Surface of RBCs in Trauma Patients and Interferes With Their Function*: Critical Care Medicine 42: e364-e372.
23. Satyam A, Andreo K, Lapcha, PH, Dalle Lucca JJ, Davis RB, et al. (2020) Complement Deposition on the Surface of RBC After Trauma Serves a Biomarker of Moderate Trauma Severity: A Prospective Study. Shock 53: 16-23.
24. Schaid TR, Hansen KC, Sauaia A, Moore EE, DeBot M, et al. (2022) Postinjury complement C4 activation is associated with adverse outcomes and is potentially influenced by plasma resuscitation. Journal of Trauma and Acute Care Surgery 93: 588-596.
25. Davis AE, Cai S, Liu D (2007) C1 inhibitor: Biologic activities that are independent of protease inhibition. Immunobiology 212: 313-323.
26. Davis A, Lu F, Mejia P (2010) C1 inhibitor, a multi-functional serine protease inhibitor. Thrombosis and Haemostasis 104: 886-893.
27. Karnaukhova E (2022) C1-Inhibitor: Structure, Functional Diversity and Therapeutic Development. Current Medicinal Chemistry 29: 467-488.
28. Bork K, Machnig T, Wulff K, Witzke G, Prusty S, et al. (2020) Clinical features of genetically characterized types of hereditary angioedema with normal C1 inhibitor: A systematic review of qualitative evidence. Orphanet Journal of Rare Diseases 15: 289.
29. Matsuura H, Osuka A, Hirose T, Ogura H, Ueyama M, et al. (2019) C1 Esterase Inhibitor Activity Is Reduced in the Acute Phase Following Burn Injury: A Prospective Observational Study. Journal of Burn Care & Research 40: 893-899.
30. Dalle Lucca JJ, Li Y, Simovic M, Pusateri AE, Falabella M, et al. (2012) Effects of C1 inhibitor on tissue damage in a porcine model of controlled hemorrhage. Shock (Augusta, Ga.) 38: 82-91.
31. Horstick G, Kempf T, Lauterbach M, Bhakdi S, Kopacz L, et al. (2001) C1-esterase-inhibitor treatment at early reperfusion of hemorrhagic shock reduces mesentery leukocyte adhesion and rolling. Microcirculation 8: 427-433.
32. Zeerleder S (2011) C1-Inhibitor: More Than a Serine Protease Inhibitor. Seminars in Thrombosis and Hemostasis 37: 362-374.
33. Committee on Gulf War and Health: Long-Term Effects of BlastExposures, Board on the Health of Select Populations, & Institute of Medicine. (2014) Gulf War and Health, Volume 9: Long-Term Effects of Blast Exposures. National Academies Press 2014.
34. Storm BS, Christiansen D, Fure H, Ludviksen JK, Lau C, et al. (2021)

Air Bubbles Activate Complement and Trigger Hemostasis and C3-Dependent Cytokine Release Ex Vivo in Human Whole Blood. Journal of Immunology 207: 2828-2840.

35. Schäfer ST, Neumann A, Lindemann J, Görlinger K, Peters J (2009) Venous air embolism induces both platelet dysfunction and thrombocytopenia. Acta Anaesthesiologica Scandinavica 53: 736-741.

36. De Beer F, Lagrand W, Glas GJ, Beurskens CJP, Van Mierlo G, et al. (2016) Nebulized C1-Esterase Inhibitor does not Reduce Pulmonary Complement Activation in Rats with Severe Streptococcus Pneumoniae Pneumonia. Cell Biochemistry and Biophysics 74: 545-552.

37. McGuire WW, Spragg RG, Cohen AB, Cochrane CG (1982) Studies on the pathogenesis of the adult respiratory distress syndrome. The Journal of Clinical Investigation 69: 543-553.

38. Huber-Lang M, Sarma JV, Zetoune FS, Rittirsch D, Neff TA, et al. (2006) Generation of C5a in the absence of C3: A new complement activation pathway. Nature Medicine 12: 682-687.

39. Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, et al. (2010) Molecular Intercommunication between the Complement and Coagulation Systems. The Journal of Immunology 185: 5628-5636.