EFFICACY OF AN FDA-APPROVED C1 COMPLEMENT INHIBITOR IN A PRE/EARLY HOSPITAL MODEL OF TRAUMATIC HEMORRHAGE IN SWINE

Alexander H. Penn, Ph.D., Orlando Hernandez, B.S., Antonio Sanchez, B.S., Michael Falabella, Jack Hutcheson, Ph.D.
United States Air Force, 59MDW/ST

Abstract

Background: The leading cause of death in military casualties is hemorrhage and most of these deaths occur prior to arrival at a medical treatment facility. Coagulopathy due to trauma and hemorrhage contributes to mortality by making control of hemorrhage more difficult, while inflammatory processes contribute to later mortality and morbidity. The complement cascade is activated by trauma/hemorrhage and is known to interact with both inflammatory and coagulation cascades, making it a target for intervention. Methods: We tested the effect of an FDA-approved complement C1 inhibitor (C1INH) on survival, hemodynamics, coagulation, inflammation and tissue damage in a swine polytrauma model consisting of lung contusion, laparotomy and liver laceration (~5% uncontrolled hemorrhage), and 40% controlled hemorrhage. After 90 minutes of shock, animals were given a bolus of C1INH (500 UI/kg; N=12) or Lactated Ringer's (LR; N=11), then resuscitated with up to 3x shed volume of LR over 1 h and observed for an additional 12 h. Results: We found a non-significant trend towards improved survival with C1INH (67% vs 45% with LR) and significantly improved hemodynamics (mean, systolic, and diastolic blood pressures) early after treatment. However, re-bleeding at the liver laceration was similar between groups with no consistent differences in coagulopathy measured by thromboelastography. Likewise, we found no improvement in plasma pro-inflammatory cytokines. Though there were no differences in hematocrit between groups, lymphocytes and monocytes were elevated by C1INH later in the observation period. Histological organ damage was minimal to moderate on average across the tissues examined, with only the spleen showing significant differences (both positive and negative) between groups. Liver damage as measured by plasma alkaline phosphatase and total bilirubin was worse in the C1INH group. Conclusions: These results suggest that C1INH may be beneficial after trauma/hemorrhage, but the mechanism may not be via the expected pathways. Given the early (but not late) benefit to hemodynamics, repeat dosing with C1INH may be needed to improve survival in cases of prolonged field care.

Background

- Hemorrhage and its sequelae are the leading cause of death in military casualties with most of these deaths occurring prior to arrival at a medical treatment facility (1).
- Severe bleeding can lead to depletion of clotting factors, which may be further diluted when treated with resuscitation fluids. This can lead to coagulopathy.
- Coagulopathy makes controlling hemorrhage more difficult and re-bleeding easier, while initiation of the inflammatory response can lead to organ dysfunction. Both of these pathways contribute to increased mortality and morbidity.
- Hemorrhage and trauma also initiate inflammatory processes, which contribute to organ dysfunction leading to later mortality and morbidity.
- The complement cascade is also activated by trauma and hemorrhage (2) and interacts with both inflammatory and coagulation cascades (3), making it a target for intervention.
- C1 is the complement protein at the start of the classical pathway of the complement cascade.
- An FDA-approved C1 inhibitor (C1INH) is able to block activation of both C1 and the MASP proteins that start the lectin pathway of complement, and may also directly interact with coagulation cascade proteins and the kinin-kallikrein system (e.g. blocks kallikrein, a protein which contributes to fibrinolysis by converting plasminogen to plasmin and contributes to vasodilation by converting kininogen to bradykinin).

Hypothesis and Objectives

Our objective was to test C1INH in a swine model of polytrauma/hemorrhage followed by resuscitation with crystalloid. We hypothesized that early treatment with C1INH would decrease coagulopathy, re-bleeding, inflammation, and organ damage and increase blood pressure compared to vehicle.

Methods

Anesthetized and mechanically ventilated male, Yorkshire swine (40 ± 5 kg) were instrumented for continuous measurement of blood pressure and cardiac output. They were then subjected to blunt injury (lung contusion) followed by laparotomy, liver laceration (~3 ml/kg uncontrolled hemorrhage), and controlled hemorrhage (24 ml/kg). After liver laceration stopped bleeding, the wound was packed with pre-weighed gauze for measurement of re-bleeding at final time point. For the controlled hemorrhage, blood was drawn until mean arterial pressure (MAP) reached 30 mmHg (T=0 h). MAP was kept there by intermittent withdrawal until the full volume was drawn. At T=1.5 h, swine received a bolus of C1INH (500 UI/kg; N=12) or Lactated Ringer's (LR; N=11), followed by resuscitated with up to 3x shed volume of LR over 1 h and observed for an additional 12 h (to T=14.5 h). Blood was sampled regularly for blood gases, hematocrit, cytokines, markers of tissue damage, complete blood cell count, and thromboelastography. Tissues were fixed post-mortem and examined for histological damage by a trained, blinded pathologist.
Data expressed as mean ± SD. * p<0.05. NS = not significant.

Results

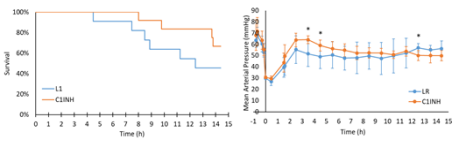

Figure 1 Effects of C1INH on (A) Survival vs. time and (B) blood pressure vs. time. Start of shock at T = 0 h. Treatment and start of resuscitation at T = 1.5 h. Resuscitation completed by T = 2.5 h. Trend for improved survival with C1INH (not significant; p=0.22). MAP increased initially with C1INH, but declined with time (note the improvement in LR MAP was due in part to the death of animals with lower pressures).

Table 1: Re-bleed and TEG data

	Baseline		at T = 1.5		at T = 2.5		at T = 14.5	
	LR	C1INH	LR	C1INH	LR	C1INH	LR	C1INH
Re-bleed (ml/kg)							8.1 ± 3.0	8.8 ± 3.2
SP (min)	2.3 ± 0.6	2.0 ± 0.7	2.1 ± 0.6	2.2 ± 0.8	2.5 ± 0.9	2.4 ± 0.6	2.6 ± 1.3	3.0 ± 0.7
R (min)	2.6 ± 0.7	2.5 ± 0.6	2.4 ± 0.7	2.5 ± 0.8	2.8 ± 1.1	3.2 ± 1.7	2.9 ± 1.4	3.2 ± 0.8
K (min)	0.9 ± 0.2	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.3	1.0 ± 0.4	1.0 ± 0.2	1.1 ± 0.4
Angle (deg)	76.7 ± 2.8	77.9 ± 2.3	76.6 ± 1.9	78.3 ± 2.4	73.3 ± 4.3	76.2 ± 3.1	75.8 ± 3.0	75.6 ± 5.8
MA (mm)	71.7 ± 4.6	75.9 ± 3.1 *	70.0 ± 2.7	71.6 ± 3.6	65.8 ± 3.9	67.4 ± 5.1	71.3 ± 3.6	70.0 ± 7.5
TMA (min)	15.7 ± 1.3	14.0 ± 1.2	16.9 ± 1.7	14.9 ± 1.8	18.4 ± 2.2	17.6 ± 2.7	18.1 ± 2.0	17.7 ± 3.7
LY30 (%)	1.6 ± 1.1	2.0 ± 1.4	1.2 ± 0.9	2.0 ± 1.3	0.8 ± 0.7	1.3 ± 1.2	0.5 ± 0.7	0.9 ± 1.1

No group effect on coagulation parameters or re-bleeding.

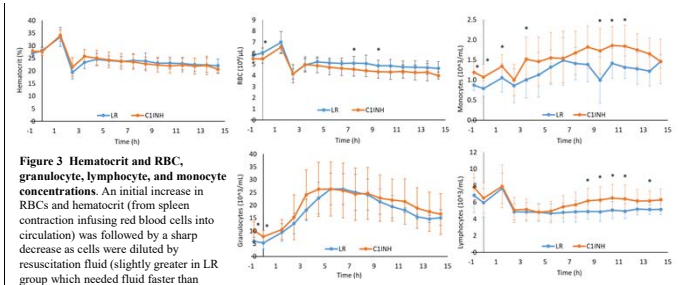

Figure 3 Hematocrit and RBC, granulocyte, lymphocyte, and monocyte concentrations. An initial increase in RBCs and hematocrit (from spleen contraction infusing red blood cells into circulation) was followed by a sharp decrease as cells were diluted by resuscitation fluid (slightly greater in LR group which needed fluid faster than C1INH group, NS). This appears to have been corrected by Starling forces causing fluid to extravasate (see Albumin data), after which the groups closely paralleled each other. Note: Mean RBC Volume was consistently larger in the C1INH group at all time points (not shown), so fewer cells needed to get same hematocrit). There was an influx of granulocytes (even with dilution) early in shock, but no differences between groups after start of shock. Lymphocytes and monocytes were elevated in the C1INH group.

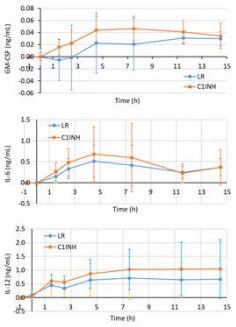

Figure 2 Cytokines. GM-CSF, IL-6, and IL-12 levels (as change from baseline). There was a trend for higher values in the C1INH group (NS). Also measured, but not shown were IFN-gamma, IL-1a, IL-1b, IL-1ra, IL-2, IL-4, IL-8, IL-10, IL-18, and TNF-alpha (NS).

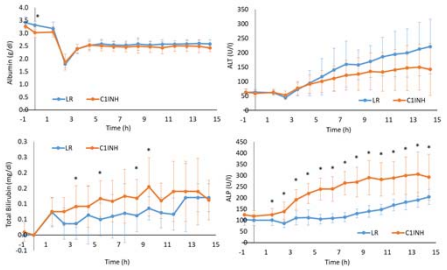

Figure 4 Albumin, Total Bilirubin, Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP) levels. Albumin slightly decreased during hemorrhage (suggesting autotransfusion) then decreased sharply with crystalloid resuscitation. Concentration then rose during (presumed) fluid extravasation, after which it remained constant for the duration (the downward trend in hematocrit during this time suggests albumin was entering the circulation, allowing circulating volume to expand). The group difference at the start of shock suggests autotransfusion was faster in the animals that would later receive C1INH. ALP and Total Bilirubin were worse with C1INH, though ALT tended to be improved (NS). This suggests that the ALP may not be originating from the liver.

Table 2: Tissue Histology

		LR	C1INH	
Left Middle Lung	Hemorrhage	0.0 ± 0.0	0.0 ± 0.0	NS
	Edema	0.1 ± 0.4	0.2 ± 0.4	NS
Right Middle Lung (contusion)	Hemorrhage	1.9 ± 1.7	1.1 ± 1.4	NS
	Edema	0.9 ± 1.1	0.8 ± 1.0	NS
Heart	Necrosis	2.1 ± 0.9	1.3 ± 1.0	NS
Abdominal Muscle	Necrosis	2.1 ± 1.2	1.3 ± 1.1	NS
Jejunum	Crypt Abcess	0.1 ± 0.4	0.5 ± 0.8	NS
Liver	Central Lobular Necrosis	0.7 ± 1.3	1.8 ± 1.4	NS
	Sinusoid Congestion	1.9 ± 0.9	2.4 ± 0.8	NS
Spleen	Decreased Red Pulp	1.6 ± 1.1	0.4 ± 0.7	p=0.03
	Diffuse Congestion	0.0 ± 0.0	0.9 ± 1.2	p=0.02
Kidney	Tubular Necrosis	1.6 ± 1.4	1.1 ± 1.4	NS
Pancreas	Adipocyte Necrosis	0.1 ± 0.4	0.3 ± 0.7	NS

Relatively low levels of tissue injury away from direct sites of polytrauma. No group differences except in spleen.

Discussion

The trend towards fewer deaths and the early improvement to hemodynamics suggests that C1INH may be beneficial after polytrauma and hemorrhage. Given the drop in MAP over time, repeated dosing may be necessary to observe significant improvement in mortality. We have yet to elucidate how C1INH is exerting its benefit, since re-bleeding, coagulopathy, inflammation, organ damage, and hematocrit (which can be used to estimate changes in vascular volume) were mostly unchanged between groups. A more severe hemorrhage, with greater resulting organ dysfunction, may be necessary to see any effect of C1INH. However, the absence of an effect on coagulopathy suggests that the classical and lectin pathways of complement are not critical driving forces in that pathology. Since C1INH does not affect it, it is still possible that the alternative pathway exerts a major effect. The effect of C1INH on blood pressure may be due to its interactions with the kinin system, which can increase vasoconstriction. Those mediators may be measured from banked plasma in a future study.

Conclusions

- C1INH has beneficial effects on hemodynamics, though re-dosing may be necessary to improve mortality.
- C1INH did not demonstrate any effects on coagulopathy or inflammation.

References

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Disclaimers and Support

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