HUMAN RECOMBINANT DECAY-ACCELERATING FACTOR (DAF) INCREASES SURVIVAL AND LIMITS TISSUE INJURY AFTER HEMORRHAGIC SHOCK.

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ABSTRACT

The current wars in Iraq and Afghanistan have resulted in the highest rates of combat casualties experienced by the U.S. military since the Vietnam conflict, and hemorrhage has been indentified as the principal cause of death among potentially salvageable patients. Hemorrhagic blood loss or resuscitation following hemorrhage leads to complement activation, which in turn, plays a key role in the pathogenesis of subsequent shock, tissue inflammation and multiple organ failure. The current study used a porcine model of controlled hemorrhage to determine the effects of early bolus injection of a complement inhibitor, decay-accelerating factor (DAF), administered 20 minutes after the onset of hemorrhagic shock. We report that hemorrhaged animals if untreated die 100 minutes before the procedure endpoint, whereas animals treated with DAF alone or in combination with resuscitation fluids displayed increased survival when compared to controls. Administration of DAF (5 and 25μg/kg) reduces the volume of Hextend required 60 minutes after achieving target blood pressure by approximately 56.9 and 62%, respectively. Furthermore, DAF-treated pigs showed improvement of hemodynamic and metabolic parameters and reduced injury in several organs including the lungs and the intestine. In summary, our data demonstrate that administration of DAF within 20 minutes of hemorrhagic shock may reduce mortality and morbidity of severely injured soldiers. Furthermore, its effect in reducing or eliminating the need for resuscitation fluids should reflect in great logistical and operational improvement during far-forward medical support missions.

1. INTRODUCTION

Hemorrhage and complications after resuscitation are the major cause of mortality and morbidity associated with battlefield wounds. Associated morbidity includes sepsis, ischemia and reperfusion injury, multiple organ failure, and secondary brain and spinal cord injury (Spain, et al., 1999; Yao, et al., 1998; Ummenhofer and Scheidegger, 2002; Soderstrom and Brumback, 1986). Following hemorrhage and/or resuscitation, intestinal injury and the initiation of an inflammatory response play a major role in the complications that ensue. These complications and secondary organ injury following hemorrhage are associated with the redistribution of blood flow away from less vital organs. Following hemorrhage and resuscitation, the microvascular beds in the intestine and lungs are particularly susceptible to injury. The initiation of an inflammatory response appears to play a major role in the complications that occur subsequent to hemorrhage and resuscitation. We and others have previously demonstrated in rodents that complement activation is centrally involved in causing organ injury following hemorrhage and resuscitation (Yao, et al., 1998; Szebeni, et al, 2003; personal communication). In this study we have used recombinant human decay-accelerating factor (DAF) to inhibit complement activation and demonstrated its beneficial effects in a porcine model of tissue injury induced by hemorrhage and resuscitation.

1.1 Hemorrhage as a Cause of Morbidity and Mortality. The current wars in Iraq and Afghanistan have resulted in the highest rates of combat casualties experienced by the U.S. military since the Vietnam conflict. These casualties suffer wounds that have no common civilian equivalent and more frequently require massive transfusion (greater than 10 units of packed red blood cells in less than 24 hrs) than injured civilians. Military surgeons have found that traditional approaches to resuscitation, particularly in terms of the ratio of blood

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DEC 2008	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Human Recombinant Decay-Accelerating Factor (Daf) Increases Survival And Limits Tissue Injury After Hemorrhagic Shoc	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research Silver Springs, MD, 20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002187. Proceedings of the Army Science Conference (26th) Held in Orlando, Florida on 1-4 December 2008, The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**8**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

products to each other and the timing of these products, often fail to effectively treat the coagulopathy that is invariably present on arrival in these soldiers. This observation has been concurrently noted in the civilian trauma literature and has ignited strong interest in an alternative approach to the resuscitation of these most grievously injured patients. These approaches include among others the use of permissive hypotension, the prevention and aggressive treatment of hypothermia, and introduction of new alternatives to fluid infusion resuscitation. This strategy has been called "damage control resuscitation" to emphasize its pairing with damage control surgical techniques (Beekley, 2008).

1.2 Complement (C) activation in hemorrhage. DAF, a ubiquitously expressed intrinsic C regulatory protein, inhibits C activation by interfering with the function of C3/C5 convertases in both of classic and alternative pathways, thereby limiting local C3a/C5a and C5b-9 (MAC) production (Lublin and Atkinson, 1989; Miwa and Song, 2001). In the present study, we investigated potential effects of DAF on resuscitation fluid requirement, hemodynamic responsiveness, tissue damage, and animal survivability after hemorrhagic shock managed with a hypotensive resuscitation strategy.

We have previously demonstrated that C inhibition with DAF results in tissue protection during ischemia/reperfusion in mice (Weeks, et al., 2007). We have also reported previously that bolus injection of antibody against C5 results in significant decrease in resuscitative fluid requirements after hemorrhagic shock in rats (Peckham, et al., 2007). In continuing our efforts to identify the most efficacious C inhibitor to limit tissue injury and minimize resuscitation fluid volumes we investigated the effect of DAF administered 20 minutes after the onset of hemorrhagic shock in a porcine model of hemorrhage/resuscitation. We report that indeed DAF, a human recombinant protein, limits the resuscitation fluid volumes and prevents significantly tissue injury.

2. MATERIALS AND METHODS

The study adhered to the principles stated in the Guide for the care and use of Laboratory Animals was approved by the Institute's Animal Care and Use Committee and was performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. All research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals and experiments involved animals.

2.1 Reagents and antibody: Recombinant human CD55/DAF was obtained from R&D Systems (Minneapolis, MN). 6% Hetastarch in lactated electrolyte injection (Hextend®) was from Hospira Inc. (Berkeley, CA). Polyclonal anti-serum to human C5 antibody was purchased from Quidel Corporation (San Diego, CA).

2.2 Experimental design. The animals were hemorrhaged using controlled, isobaric Wiggers' model of controlled hemorrhagic shock (Figure 1).

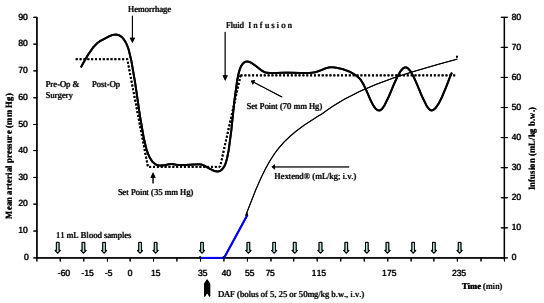

Figure 1. Scheme of the experimental design in pigs. Actual and target mean arterial blood pressure (MAP) are shown as are volume of shed blood and Hextend infused.

The animals (swine, Yorkshire crossbred, 12 weeks old, 30-40 kg) were enrolled in one of seven experimental groups: 1) H, hemorrhage only (n = 9); 2) H + Hex, hemorrhage + Hextend® (n = 9); 3) H + DAF50µg, hemorrhage + DAF (50µg/kg bw, n = 8); 4) H + DAF5µg+Hex, hemorrhage + DAF (5µg/kg bw)+Hextend ® (n=4); 5) H + DAF25µg + Hex, hemorrhage + DAF (25µg/kg bw) + Hextend ® (n=6); 6) H+DAF50µg + Hex, hemorrhage + DAF (50µg/kg bw) + Hextend ® (n=6); and 7) Control, Sham (not hemorrhaged, n = 5). Animals were hemorrhaged to a MAP of 35mmHg over a period of 15 minutes and then held at 35mmHg for 20 minutes. At the end of 20-minute stabilization period animals underwent resuscitation with Hextend® to a target MAP of 70 mmHg over 15 minutes and maintained with a minimum MAP of 70 mmHg for 180 minutes. Recombinant human DAF was given as bolus in 60 ml of saline simultaneously with the start of resuscitation. Arterial blood samples were obtained at the prior to surgery (Pre-Op), prior to hemorrhage (C1, C2), hemorrhage to 35 mmHg (35 mmHg) of MAP, prior to resuscitation, at 70 mmHg of MAP, and then at 20 minute intervals throughout the experimental protocol. Blood samples were

analyzed for complement activation, hematocrit, PO_2, PCO_2, pH, HCO_3, glucose, lactate, Na^+, K^+, Ca^{++}, Cl^-.

2.3 Tissue harvest: The animals were euthanized by lethal bleeding and isoflurane at endpoint. Tissue samples including lung and small intestine were removed, frozen in liquid nitrogen and stored at -80ºC for gene and protein expression, or fixed with 10% formalin or 4% paraformaldehyde for histological and immunohistochemical analysis.

2.4 Tissue protein extraction: Frozen tissue samples were thawed, washed with ice-cold PBS, suspended in RIPA buffer containing protease inhibitors (2μg/ml of aprotinin, 10μM of leupeptin, 1mM of AEBSF), and minced on ice. Then, the samples were sonicated on ice at setting 5, for 4 x 10s at continuous output, with 10s pause in between. The samples were then centrifuged at 13,000 rpm for 10min at 4ºC. The supernatants were frozen and stored at -80C until further processing. Aliquots were used to determine protein concentration.

2.5 Histological examination: 10% formalin-fixed tissues were embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E). Histological evaluation was observed and histological images were recorded under a light microscope (Olympus Leica, AX80,) with ×40/×20 objective by a pathologist blind to the treatment groups. Histological injury scores were graded using the scales as follow:

For lung injury scoring as described previously (Carraway, et al., 2003), four parameters (alveolar fibrin edema, alveolar hemorrhage, septal thickening and intra-alveolar inflammatory cells) were scored on each slide for 1) severity (0: absent; 1, 2 and 3 for more severe changes) and 2) extent of injury (0: absent; 1: <25%; 2: 25-50%; 3: >50%). for the injury score represents the sum of the extent and the severity of injury.

Mucosal damage of small intestine for each slide was graded on the six-tiered scale (Fleming, et al., 2002; Rehrig, et al., 2001). A score of 0 was assigned to a normal villus; villi with tip distortion were scored as 1; villi lacking goblet cells and containing Gugenheims' spaces were score as 2; villi with patch disruption of the epithelial cells were scored as 3; villi with exposed but intact lamina propria and epithelial cell sloughing were assigned a score of 4; villi in which the lamina propria was exuding were scored as 5; villi displaying hemorrhage or denuded were scored as 6.

2.5 Statistical analysis: Data are expressed as Mean ± SEM. The logrank test or one- or two-way ANOVA. P values <0.05 were considered significant.

3. RESULTS

3.1 Baseline characteristics. The experimental groups did not differ significantly in the baseline characteristics including weight, hemoglobin concentration, arterial pH, pO_2, pCO_2, glucose, HCO_3, base excess, potassium, ionized calcium, lactate levels, and MAP. The groups of animals did not differ in the total blood loss during the 35-mmHg ceiling hemorrhagic phase (data not shown).

3.2 Hemodynamic and metabolic changes. At the end of the 35-mmHg ceiling hemorrhage phase ("Decompensation"), mean arterial pressure (MAP) in treated animals except those in the Hemorrhage + DAF25μg + Hextend® group was not different from that in the Hemorrhage group. There were no clear differences in shock index, arterial pH, pCO_2, HCO_3, base excess, lactate, glucose, potassium, or ionized calcium levels between treated and non-treated hemorrhage at the decompensation point. Mean arterial pressure (MAP) in animals treated with the combination of DAF and Hextend® was significantly higher at 120 min from the onset of resuscitation when compared with non-treated hemorrhaged animals or those treated only with a high dose of DAF (Table 1). The administration of a high dose of DAF (50μg/kg b.w.) without Hextend® did not clearly increase the mean blood pressure throughout the observation. Shock index, that is, the ratio of heart rate to systolic blood pressure, in hemorrhaged animals treated with the combination of DAF and Hextend® was significantly lower than in non-treated animals. The high dose of DAF alone also significantly decreased shock index in hemorrhaged animals at 120 min from the onset of resuscitation phase (Table 1). Shock index in animals treated with

Table 1. Effects of DAF on hemodynamics and metabolism in pigs during hemorrhagic shock

		Treatment					
		Hemorrhage (H)	**H+Hex**	**H+DAF*5μg*+Hex**	**H+DAF*25μg*+Hex**	**H+DAF*50μg*+Hex**	**H+DAF*50μg***
MAP (mm Hg)	*Decomp.*	29.41±3.60	33.74±0.58	35.64±1.57	35.44±0.94***	35.09±0.87	35.39±1.14
	120 min	29.58±2.48	68.53±5.82***‡	68.84±0.99***‡	71.06±1.75***¶	69.69±0.50***‡	43.16±3.46
Shock Index	*Decomp.*	2.47±0.20	2.69±0.27	3.00±0.59	2.16±0.22	2.20±.24	2.34±0.17
	120 min	4.48	1.26±0.11***¶	1.41±0.06***¶	1.44±0.13***¶	1.31±0.05***¶	2.49±0.35***
pH	*Decomp.*	7.36±0.06	7.42±0.01	7.43±0.05	7.37±0.03	7.41±0.02	7.36±0.02
	120 min	7.13±0.12	7.43±0.03**	7.50±0.01***	7.45±0.02***	7.50±0.02***	7.40±0.04**
pCO_2 (mm Hg)	*Decomp.*	59.54±11.88	50.05±2.61	45.71±5.22	47.21±2.32	46.11±3.51	56.56±2.67
	120 min	62.30±12.48	45.56±0.75	41.56±1.05*	42.84±1.75*	38.28±1.85*	55.35±6.05
HCO_3 (mmol/L)	*Decomp.*	31.06±1.39	32.03±0.94	29.38±0.71	27.82±1.64	29.04±0.85	31.72±0.80
	120 min	28.83±2.48	30.54±1.46	32.54±0.56	30.48±1.66	29.43±0.51	33.21±0.83
Base Excess (mmol/L)	*Decomp.*	32.50±1.33	33.67±0.97	30.63±0.83	28.93±1.47	30.36±0.94	33.31±0.82
	120 min	30.50±3.00	32.00±1.35***	33.88±0.69***	31.25±1.56***	30.39±0.56***	34.81±0.87***
Lactate (mmol/L)	*Decomp.*	2.81±0.53	2.32±0.43	3.88±1.07	3.74±1.07	2.27±0.21	2.12±0.34
	120 min	6.75±1.28	2.67±1.26**	1.99±0.31***	2.64±1.13***	1.71±0.33***	2.24±0.50***
Glucose (mg/dl)	*Decomp.*	126.33±23.26	112.33±16.55	141.75±3.25	173±30.59	122±6.38	115.38±15.89
	120 min	58±38	93.88±8.73	103.25±4.13	122.8±13.42	92.67±6.98	99.5±24.48
Potassium (mmol/L)	*Decomp.*	5.37± 0.53	4.57±0.12	4.63±0.31	4.83±0.23	4.74±0.11	4.6± 0.08
	120 min	5.8± 2	4.34±0.37*‡	3.93±0.11***¶	4.66±0.09†	4.13±0.27**‡	5.5±0.53
Ionized Calcium (mmol/L)	*Decomp.*	1.24±0.04	1.21±0.04	1.11±0.02	1.32±0.02	1.21±0.02	1.20±0.02
	120 min	1.16±0.02	1.14±0.03	0.95±0.05	1.28±0.02§	1.15±0.04	1.16±0.06#

n =3-9. Decomp.=decompensation; decompensatory shock. 120 min refers to the time after the onset of infusion. Two-way ANOVA (Bonferroni post tests) was performed. *p < 0.05 vs. H; **p < 0.01 vs. H; ***p < 0.001 vs. H; † p < 0.05 vs. H+DAF*50μg*; ‡p < 0.01 vs. H + DAF*50μg*; ¶p < 0.001 vs. H + DAF*50μg*; # p < 0.01 vs. H+DAF*5μg*+Hex; § = p < 0.001 vs. H+DAF*5μg*+Hex.

DAF alone was not different from the index in not-treated hemorrhage (data not shown). Treatment with DAF and/or Hextend® corrected pH levels in hemorrhaged animals at 120 min from the onset of resuscitation. Only the combination of DAF and fluid though reduced pCO_2 in hemorrhaged animals. There were no clear changes in bicarbonate levels between the experimental groups at 120 min (Table 1). Significantly reduced bicarbonate levels and base deficit were observed in the H + DAF25μg + Hextend® group at the resuscitation point of the target MAP. Treatment with DAF alone or followed by fluid infusion significantly improved base deficit in hemorrhage. There were no differences in potassium or ionized calcium concentration between hemorrhaged non-treated animals and those treated with DAF50μg (Table 1). The observed differences in electrolyte concentrations between the Hemorrhage group and animals treated with combined agents may be attributed to diluted blood plasma by infused Hextend®, a plasma expander.

3.3 Fluid requirements. To determine whether treatment with DAF reduces requirement of resuscitation fluid, we calculated the ratio of cumulative fluid infusion over total blood loss (TBL) for each group at 20, 80, 140 and 200 minutes after resuscitation with Hextend® (Figure 2). The combination of smaller doses of DAF (5, 25μg/kg b.w., i.v.) followed by infusion of Hextend® significantly reduced the requirement for resuscitative fluid at 60 min after target blood pressure was achieved (70 mmHg) when compared with the animals treated with Hextend® alone ($p<0.05$). DAF (25μg/kg) in combination with Hextend® significantly reduced the fluid requirement at 1 and 2 hours after the target point of resuscitation when compared animals treated at higher dose of DAF (50 μg/kg) plus Hextend® (Figure 2).

3.4 DAF prolongs animal survival. To determine whether DAF increases survival of hemorrhaged animals, we monitored animal mortality and cumulative survival was plotted (Figure 3). Administration of Hextend® with or without DAF increased animal survival ($p<0.01$). Superior survival rates were observed in animals treated with a high dose of DAF (50μg/kg b.w.) when compared with those treated with only Hextend® ($p<0.05$) alone or in combination with DAF.

3.5 DAF limits lung injury associated with hemorrhage and resuscitation. H&E

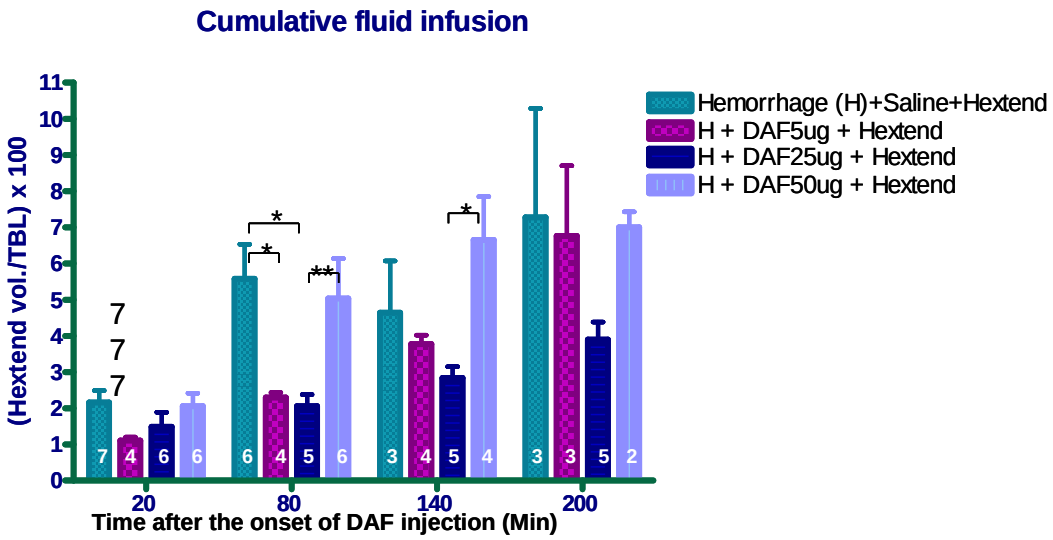

Figure 2. DAF treatment reduces fluid requirement in a swine hemorrhagic model managed with hypotensive resuscitation. The ratio of cumulative fluid infusion over total blood loss (TBL) was calculated for each group at 20, 80, 140 and 200 minutes after Hextend resuscitation. The number of animals are given on the bars. * $P < 0.05$; ** $P < 0.01$ (two-way ANOVA; Bonferroni post tests).

stained histological sections (Figure 4A) and injury scores (Figure 4B) showed marked lung injury after hemorrhage and/or resuscitation. Histopathological analysis of lung tissues revealed destruction of the alveolar architecture with severe alveolar hemorrhage and moderate inflammation compared with Sham operated. Treatment with DAF alone resulted in optimal reduction in tissue damage when compared to all other groups. Significant lung tissue protection was also observed in animals treated with DAF (5µg/kg b.w.) plus Hextend when compared to Hextend alone or Hemorrhage alone.

3.6 DAF limits intestinal injury associated with hemorrhage and resuscitation. Histological changes (Figure 5A) and injury scores (Figure 5B) of small intestines from the same animals as described above were examined. When compared with small intestines obtained from Sham pigs, intestines obtained from pigs subjected to hemorrhage or hemorrhaged and resuscitation exhibited epithelial cell sloughing, villi denuding, necrosis and inflammation, whereas animals treated with DAF (50 µg/kg) alone or DAF (5µg/kg) plus Hextend® showed significantly reduction in intestinal injury (Figure 5B). When higher dose of DAF (50 µg/kg-bw) was used in combination with Hextend® it resulted in augmented hemorrhage-induced intestinal damages compared with that of lower dose DAF (5 µg/kg) followed by resuscitation.

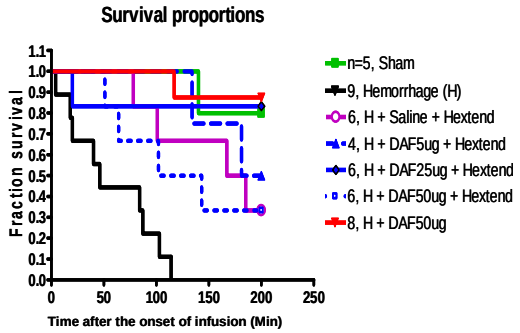

Figure 3. Treatments with DAF and/or Hextend® clearly increased survival of hemorrhaged animals. Pigs were subjected to the protocol and randomized in a blinded fashion. Mortality was monitored and cumulative survival was plotted and logrank test was applied ($p<0.05$). H + DAF50µg vs. H + Hex, $P < 0.05$; H + DAF50µg vs. H + DAF50µg + Hex, $P < 0.05$; H vs H+DAF50µg, $p<0.0001$; H vs H+DAF5µg+Hex, $p<0.01$; H vs H+DAF25µg+Hex, $p<0.01$; H vs H+DAF50µg+Hex, $p<0.05$; H + Hex vs. H + DAF5, 25 or 50µg/kg + Hex, $P > 0.05$; H + DAF50µg vs. H + DAF25µg + Hex, $P > 0.05$; H + DAF50µg vs. H + DAF5µg + Hex, $P > 0.05$.

3.7 Effect of DAF in C5a deposition in animal lungs. To understand whether DAF exerts its major molecular function in complement inhibition, we detected the expression of C5a in lungs from the animals by immunoblot analysis (Figure 6A and 6B). Increased amounts of C5a were found in lungs from animals undergoing hemorrhage and hemorrhage followed by resuscitation compared with Sham animals ($p<0.001$). Treatment though with DAF either at a dose of 50 µg/kg in hemorrhaged animals or a dose of 5µg/kg in the animals with hemorrhage plus resuscitation limited significantly the amounts of C5a in the lungs ($p<0.05$).

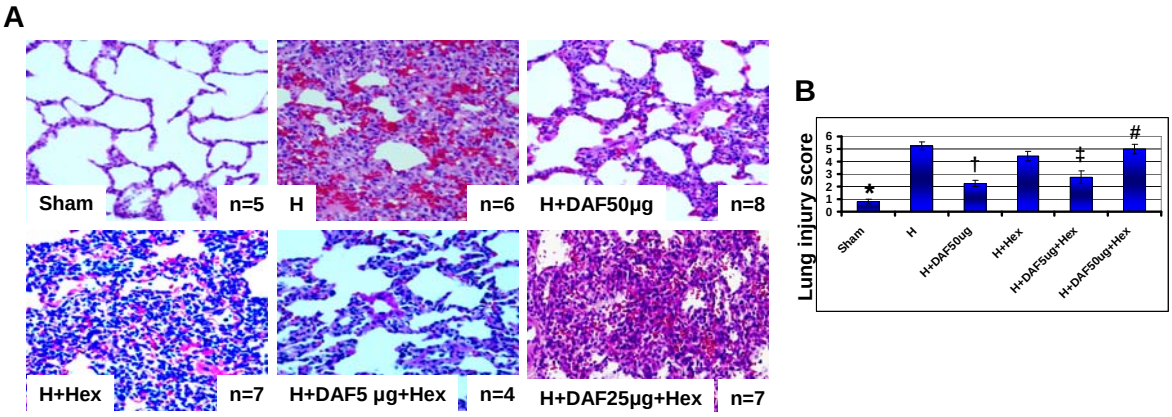

Figure 4. DAF mitigates lung injury in a swine hemorrhagic model. A, Representative H&E stained histological sections of lung from pigs subjected to the protocol.x400. B, lung injury scores were calculated using the scale shown in *Materials and Methods* for each animal. * p<0.001 vs. H, H+Hex and H+DAF50µg+Hex, p<0.05 vs. H+DAF5µg+Hex; † p<0.001 vs. H, H+Hex; ‡ p<0.001 vs. H, p<0.05 vs. H+Hex; # p<0.01 vs. H+DAF5µg+Hex, p<0.001 vs. H+DAF50µg (One-Way ANOVA, Tukey's post test).

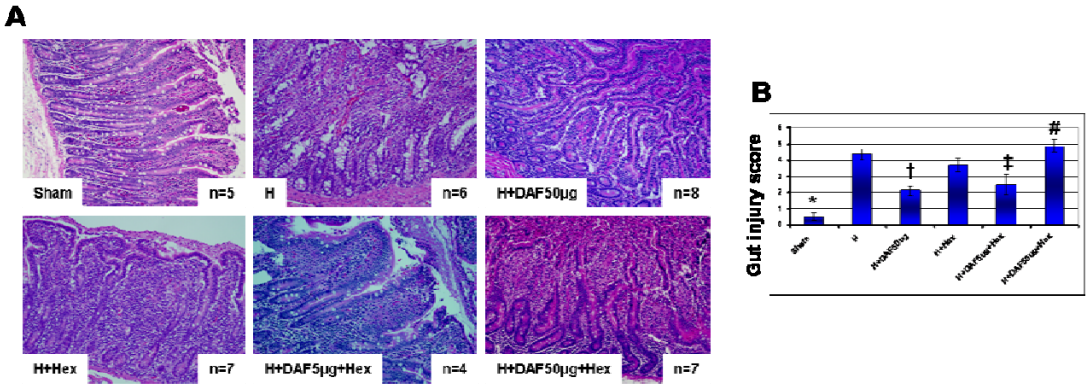

Figure 5. DAF attenuates HS-mediated intestinal injury of pigs. A, Representative histological changes of small intestines in the pigs were shown. B, Intestinal injury scores evaluated by a standard shown in *Materials and Methods*. (x200). Group data were compared using one-way ANOVA followed by Tukey's Multiple Comparison Test with p values of < 0.05 considered significant.Tukey: * p<0.001 vs. H, H+Hex and H+DAF50µg+Hex; p<0.05 vs. H+DAF5µg+Hex ; † p<0.001 vs. H; p<0.05 vs. H+Hex; ‡ p<0.05 vs. H; # p<0.01 vs. H+DAF5µg+Hex, p<0.001 vs.H+DAF50µg.

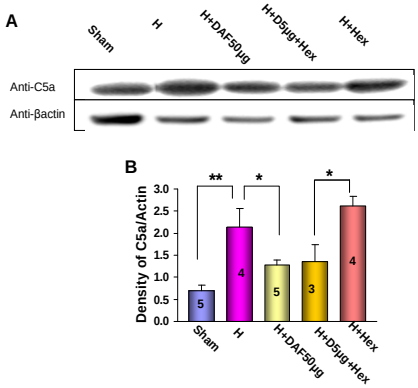

Figure 6. DAF reduces C5a deposition in lungs from animals subjected to hemorrhage or hemorrhage plus resuscitation. (A) Extracted proteins from lung tissues were separated in SDS gel, transferred onto PVDF membrane, then immunoblotted with anti-C5a and anti-β-actin antibodies. (B) C5a was quantified by densitometry and the ratio over β-actin was calculated for each sample. *p<0.05, **p<0.01.

4. DISCUSSION

Hemorrhagic blood loss or resuscitation following hemorrhage leads to complement activation (Yao, et al., 1998; Szebeni, et al, 2003; Peckham, et al., 2007). DAF inhibits the alternative complement pathway by accelerating decay of the convertase enzymes formed by C3b and factor B (Lublin and Atkinson, 1989; Miwa and Song, 2001). Thus, DAF treatment following hemorrhage attenuates complement activation caused by hemorrhage or hemorrhage and resuscitation. Several important findings were observed in the present study. First, treatment with either high dose of DAF (50µg/kg) alone or smaller doses of DAF (5-25µg/kg) followed by Hextend® dramatically increased survival;

second, the presence of DAF in the resuscitation fluids protected animals significantly from tissue damage in multiple organs; third, smaller doses of DAF displayed significant reduction of resuscitation fluid requirements; and, finally, administration of DAF corrected altered hemodynamic and metabolic parameters triggered by hemorrhagic shock.

Hypovolemic resuscitation is the current standard of care and has proved successful in many critical hemorrhagic shock patients. However, growing evidence shows that fluid infusion causes excess fluid extravasation into the interstitial space which may worsen trauma-related complications and can lead to dilutional coagulopathy (Hai, 2004; Moore, et al., 2004; Revell, et al., 2003; Stern, 2001). Starch-containing fluids such as Hextend® may also cause derangements in coagulation and pruritus (Hardy, et al., 2006; Bork, 2005). Therefore strategies aimed at reducing or eliminating the need for resuscitation fluid infusion has been identified as a major requirement for both military and civilian medicine. Another requirement identified by special operations medics as well as tactical combat casualty care experts relates to identification of volume-sparing adjuncts. Specifically, it should reduce the carrying load of medics in forward military environments and with delayed evacuation or mass casualty scenarios where availability of resuscitation fluids may be limited and requires significant logistical footprint. In this study, we report significant reduction of resuscitation fluid requirements with intravenous bolus injection of DAF. The volume sparing effects of DAF are consistent with our previous unpublished data from a rat hemorrhagic model and it suggests that DAF may have the capability to regulate microvascular function.

Although Hextend® alone improves some hemodynamic and metabolic parameters, it also aggravates organ injury. Therefore, the present study provides an attractive candidate to mitigate these side effects of Hextend® by demonstrating the efficacy and safety of bolus injection of DAF as either adjunct therapy or replacement to resuscitation fluid.

The present study also revealed a potential limitation related to the therapeutic range of DAF when combined with Hextend. Animals receiving the highest dose of DAF (50μg/kg b.w.) followed by Hextend® infusion not only failed to show beneficial effects but also masked any positive effects of Hextend® alone. Further studies will be needed to clarify this outcome. Complement inhibition may interfere with the coagulation cascade (Horstick, et al., 2001; Tassani P, et al., 2001). It is also known that colloidal and starch fluids may also cause dilutional coagulopathy (Kentnera, et al., 2005; Vollmar and Menger, 2004). The increased mortality associated with the administration of high dose of DAF and Hextend may be caused by the induction of severe coagulopathy. We are currently in the process of determining the status of the coagulation cascade in blood samples which were collected from all groups throughout the experiment.

DAF administered at the beginning of resuscitation increases survival, reduces resuscitation requirements, prevents tissue damage and also provides a basis for replacement therapy to hypovolemic resuscitation. All of which has the potential to dramatically reduce medic load and logistic footprint during far-forward medical support to military operations. Furthermore, the effects of DAF in preventing onset of decompensation during early stages of shock poses an attractive therapeutic approach in civilian emergency medical response to trauma such as those observed followed automobile crashes.

In conclusion, the present study reveals for the first time in a military relevant model of hemorrhagic shock that bolus injection of DAF given within the time frame observed for the arrival of the first responder in current military operations may be utilized as an important life saving tool leading to decreased mortality and possibly morbidity of severely injured casualties.

ACKNOWLEDGMENT(S)

The skillful technical assistance of Ms Irene Gist, Mr. Michael J. Falabella, and Mr. Parag Apte, as well as the staff from the Department of Pathology and Department of Veterinary Surgery at Walter Reed Army Institute of Research is gratefully appreciated.

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