May 15, 2007

Michael B. Given, Ph.D.
Program Officer, Casualty Care Management
Office of Naval Research
875 North Randolph Street, Suite 14 (Code 341)

Ref: Final Report Submission for ONR Awards no: N00014-05-MP-2-0006 (Uniformed Services Univ./HJF) and N00014-06-1-0192 (Massachusetts General Hospital).
Principal Investigator: Hasan Alam, MD

Dear Dr. Given,

Attached is the final technical report for the above referenced project. This project was completed at two separate institutions, because I moved from the Uniformed Services University (USUHS) to the Massachusetts General Hospital (MGH) in 2005. The unused funds on this project were returned by the USUHS to the Office of Naval Research, and reissued as a new grant to the MGH. This is reflected on the face sheet, which contains two sets of grant/project numbers and a longer overall period of reporting.

Six copies of the completed package have been distributed as required. I am also sending you copies of the newer manuscripts that have been published since the last report. I hope that you find the report satisfactory. Please do not hesitate to contact me in case you have any questions or need any more information.

Sincerely,

Hasan B. Alam, MD, FACS
Massachusetts General Hospital/ Harvard Medical School, Boston, MA.
Director of Research. Division of Trauma, Emergency Surgery, and Surgical Critical Care
Hemorrhagic shock is the leading cause of death in civilian and combat trauma. Even when the injured survive long enough to be transported to a medical facility, hemorrhage still remains the leading cause of preventable late death and complications. Effective hemorrhage control and better resuscitation strategies have the potential of saving lives. However, resuscitation can exacerbate cellular injury caused by hemorrhagic shock. Utilizing the funding provided by the ONR, we have clearly established that resuscitation fluids play a critical role in this injury pattern. Furthermore, we have demonstrated that these adverse effects can be avoided through simple modifications. We have also designed novel strategies for cellular protection. In parallel, advanced hemostatic battlefield dressings have been developed and validated. The goal of this research has been to improve the care of the critically injured, and a number of our findings have already been incorporated into new military doctrine (e.g. use of new hemostatic dressings, limited volume resuscitation), saving numerous lives.
FINAL TECHNICAL REPORT

GRANT #: N00014-05-MP-2-006 (Uniformed Services Univ./HJF) and N00014-06-1-0192 (Massachusetts General Hospital)

PRINCIPAL INVESTIGATOR: Hasan B. Alam, MD

INSTITUTION(S): Uniformed Services University of the Health Sciences, Bethesda and Massachusetts General Hospital, Boston (PI moved to Boston in 2005).

GRANT TITLE: Far Forward Treatment of Hemorrhagic Shock


REPORTING PERIOD: The period covered by this report is October 1, 2002-February 28, 2007.

OBJECTIVE:
The overall goal of our research was to improve the outcome of combat casualties. During this period of funding, three complimentary areas of research were pursued. First, we studied different methods of resuscitation following hemorrhagic shock. Second, we developed and tested surgical techniques and equipments to improve the care of injured soldier in the battlefield. Third, we tested various hemostatic agents to identify the best agent for use in the battlefield.

APPROACH:

Resuscitation (Area 1): Our team developed a number of small and large animal model of hemorrhagic shock. Using these clinically relevant animal models, the impact of various resuscitation strategies on cellular injury, organ function, hemodynamic profile, and immune/inflammatory response was studied. We also developed novel resuscitation fluids that did not exhibit the adverse properties of conventional fluids. These new fluids were then extensively tested in a variety of pre-clinical models.

Development and testing of new equipment (area 2): We wanted to demonstrate that development of new equipment and refinement of techniques would make life saving interventions suitable for battlefield application. Two such devices were developed and tested in appropriate animal models, including a small portable pump for induction of hypothermic metabolic arrest (following lethal uncontrolled hemorrhage) and a simple, portable device for evacuation of air and blood from the body cavities (e.g. blood from pleural space).

Hemorrhage control (area 3): We developed a lethal but potentially salvageable swine model of severe groin injury. This model was used to perform a series of pre-clinical
trials, which resulted in FDA approval of a new zeolite hemostat. This zeolite hemostat (QuikClot) and another dressing that was tested in these experiments (HemCon) have been deployed to the battlefield. We also tested various new formulations of zeolites that were developed to mitigate the exothermic reaction (seen with the original QuikClot) and make the product easier to use.

ACCOMPLISHMENTS (Throughout award period):

1. **Resuscitation**: It is now well recognized that administration of resuscitation fluids is not completely innocuous and may actually augment post-shock cellular damage. In an effort to design better resuscitation techniques, we have studied in detail the mechanisms by which resuscitation strategies affect cellular functions. Our experiments have demonstrated that resuscitation with the conventional lactated Ringer’s solution (mixture of D- and L-isomers of lactate) results in an up-regulation of various markers of cellular injury, including programmed cell death (apoptosis). Apoptosis in key organs can be markedly reduced by eliminating D-lactate, and by substituting lactate with beta-hydroxybutyrate (ketone Ringer’s) or sodium pyruvate (pyruvate Ringer’s). Influenced by this emerging data about the adverse effects of D-lactate, one major manufacturer of resuscitation fluids (Baxter Corp) has switched to using pure L-isof orm of lactate.

We have also shown that these novel Ringer’s solutions exert their protective effects through modifications of key regulatory proteins (nuclear and cytoplasmic). Modifications of nuclear regulatory proteins (i.e. histones) in turn influences transcription of genes, which controls a number of downstream pathways. These mechanisms are also potential targets for therapeutic interventions. For example, our data show that administration of some pharmacological agents (HDAC inhibitors) after hemorrhage can produce histone acetylation patterns that are almost identical to the ones seen after ketone Ringer’s resuscitation. This approach also results in reversal of shock-induced regulatory imbalances, and improves survival.

To test various resuscitation strategies, we first used small animal (rodents) volume controlled hemorrhage models, along with in-vitro studies using human blood exposed to resuscitation fluids. After obtaining convincing comparative data, for the final study we designed a clinically relevant large animal model of shock with a number of salient features. These included: 1) uncontrolled hemorrhage from intra-abdominal vascular injuries, 2) three phases of resuscitation simulating pre-hospital, intra-operative, and the recovery periods, 3) clinically relevant volumes and types of resuscitation fluids, 4) surgical repairs of vascular injuries, 5) clinically meaningful end points of resuscitation, and 6) comprehensive monitoring of hemodynamic parameters and organ function. The findings of this experiment were presented at two premier surgical meetings (American Association for the Surgery of Trauma, and the American College of Surgeons), and some of the findings are already in peer reviewed literature (Ayuste et al. J Trauma 2006). This study confirmed the earlier findings that resuscitation with conventional lactated Ringer’s causes a significant increase in apoptotic cell death in lung and liver. Furthermore, this can be avoided if
D-lactate is eliminated from the resuscitation fluid. We also noted that, similar to rodents, resuscitation strategies influence cellular regulation in swine at the level of gene transcription through differential acetylation of histone proteins.

2. **New Equipment:** Two devices were developed and tested in appropriate animal models during this period. The first was a small portable pump developed by the Cleveland Clinic Foundation for the induction of hypothermic metabolic arrest. This was tested in a swine model of lethal uncontrolled hemorrhage and found to be very effective (Alam *J Trauma* 2006 and Casas *Artificial Organs* 2005). As this pump was very small, disposable, battery operated and very cost effective, we found it to be logistically superior to the conventional heart-lung bypass machine. A simple, portable device for evacuation of air and blood from the body cavities was developed and a patent filed (Alam 2003). This device has been licensed by Bard Inc. and will be marketed for evacuation of pleural effusions in the summer of 2007. We are currently negotiating possibilities for licensing and development of a similar device for the evacuation of pleural blood in trauma victims.

3. **Hemorrhage control:** Uncontrolled hemorrhage remains the major cause of preventable battlefield deaths. Identification of an effective battlefield dressing has been the focus of active research in our lab over the last few years resulting in the deployment of zeolite hemostat (QuikClot) by the Marine Corps. Although very effective, zeolites generate heat when exposed to blood and there has been some concern about potential for tissue injury with their use. Zeolite granules are also difficult to remove from the wounds and a better delivery system is needed to facilitate application and removal of the hemostat. In 2005, we worked in close collaboration with another group that has been funded by the ONR (Galen Stucky, PhD, Univ. Calif. Santa Barbara) to develop a new generation of zeolites that are less exothermic. Using an ion substitution method, Dr. Stucky's team replaced calcium ion in the standard QuikClot zeolite with a number of less exothermic ions to manufacture a new generation of zeolites. After initial in-vitro screening, promising versions were selected for further in-vivo testing by our team at USUHS. Using a swine model of lethal groin injury (femoral artery, vein and soft tissues) we screened; five different formulations of the new zeolites, and a beaded version of the conventional zeolite packaged in a fabric bag. We also screened the new formulation of Chitosan dressing (HemCon, Hemorrhage Control Technologies, Lake Oswego, OR), and two new products: 1) Hemostatic Polymer Bandage (ARES Lab, West Sacramento, CA) and, 2) Chitosan-starch polymer fleece dressing (developed by Loma Linda University, and the Medafor Corporation, Minneapolis, MN). Based on the results of the screening, three new zeolites (Ag, Ba, and Na exchanged formulations), bagged zeolite, and HemCon were selected for a randomized pre-clinical trial where use of the bagged zeolite was associated with 90% survival (compared to 0% in control group). The maximum wound temperatures after the application of zeolites was not very different in this study (48-55°C) compared to our previously published data (51-57°C). However, in contrast to our last study (Alam et al. *J Trauma* 2004), almost no histological evidence of tissue injury was noted in the current study. Possible reasons for the lack of tissue injury may include differences in...
the rate of heat generation, duration of exothermic reaction, and physical barrier between the zeolite and tissues (in the bagged zeolite group). These possibilities deserve further investigation. These data were presented at the ATACC meeting (August 2005, St. Petersburg, FL), and the Western Trauma Association annual meeting (February 2007), and the full manuscript has recently been published in the Journal of Trauma (Ahuja 2007). In all, we have published 3 original studies and 2 review papers on this topic as listed in the manuscript section.

CONCLUSIONS:

1. Exuberant activation of circulating white blood cells through excessive fluid resuscitation can cause wide spread organ injury. This is especially true for conventional lactated Ringer’s solution and artificial colloids.
2. Cellular injury in various organs can be reduced through modifications of the conventional Ringer’s solutions.
3. Elimination of D-isomer of lactate from the lactated Ringer’s solution is clearly beneficial. Additional cellular protection can be achieved by substituting lactate with equal amounts of a ketone body (beta-hydroxybutyrate) or sodium pyruvate.
4. Resuscitation strategies can influence key cellular regulatory mechanisms in a fluid specific fashion. These include; 1) transcriptional regulation of genes through modifications of nuclear histone proteins, and 2) post-translational modifications of proteins involved in cell survival.
5. Development of new equipment can provide logistical advantage in austere environments, and potentially make life saving interventions suitable for battlefield application.
6. Induction of profound total body hypothermia can preserve viability of key organs during repair of lethal injuries and improve survival following lethal injuries. Protective hypothermia can be induced using portable, battery operated, small pumps.
7. Rapid and effective hemorrhage control improves survival following lethal injuries.
8. Advanced hemostatic dressings are superior to standard dressings for control of lethal external hemorrhage.
9. Advanced hemostatic dressings can be refined to improve their safety profile and while maintaining the efficacy.

SIGNIFICANCE: The goal of our research is to improve the treatment of combat casualties, and a large number of our findings have already been incorporated into new military doctrine (e.g. use of advanced hemostatic dressings, limited volume resuscitation). Historic data demonstrates that the single major cause of treatable death in combat casualty is hemorrhage. Historically, 20% of the combat casualties were killed in action (KIA), with about 90% dying before reaching the field hospital. The cause of death is hemorrhage in 50% and neurologic trauma in 36% of these soldiers. The rest are from devastating multiple injuries. Even when the injured survive long enough to be transported to a medical facility, hemorrhage still remains the leading cause of
preventable late death and complications. In a review of Vietnam War data, almost 40% of soldiers that were KIA due to exsanguination had a source of hemorrhage that could have been controlled by simple hemostatic measures.

Due to the changing strategies of engagement in future military conflicts, medical support must also change in order to accommodate future needs. Today, battlefronts are more rapid and non-linear. The logistical footprint is much smaller and often there are no full fledged hospitals in close proximity to the frontlines. Conflicts in urban settings are often associated with delays in evacuation. Therefore, the treatment strategies for the injured must adjust to accommodate the changing scenarios of conflicts. Due to the recent advances combat casualty care (and protective armor), the KIA rate in the ongoing conflict in Iraq and Afghanistan is the lowest in the history of modern warfare.

**PATENT INFORMATION:**

**AWARD INFORMATION:**
During the period of this funding the principal investigator has received a number of awards and academic promotions:

**REFERENCED PUBLICATIONS:**

**PEER REVIEWED JOURNAL ARTICLES**


35. Lin T, Chen H, Kouostova E, Sailhamer E, Li, Yongqing, Shults C, Liu B, Rhee P, Kirpatrick J, and Alam HB. Histone deacetylase as therapeutic target in a rodent


BOOK CHAPTERS:

REFERENCED ABSTRACTS


NATIONAL/INTERNATIONAL MEETINGS


5. September 2005- 65rd Annual meeting, American Association for the Surgery of Trauma, Atlanta, GA. Oral presentation. Ayuste E, Ahuja N, Koustova E, Rhee P, Alam HB. Hepatic and pulmonary apoptosis following hemorrhagic shock in swine can be reduced through modifications of conventional Ringer's solution.


Poster Presentation. Energy substrate supplemented resuscitation increases brain monocarboxylate transporter levels in rat model of hemorrhagic shock.


OTHER ACADEMIC ACTIVITIES (INVITED LECTURES-NATIONAL/INTERNATIONAL MEETINGS)
7. November 2006- Harvard Critical Care and Trauma Symposium, Boston, MA. Topic: Resuscitation is bad for you (the way we do it).
27. August 24, 2005- Division of Trauma, Emergency Surgery, and Surgical Critical Care Core Lecture Series. Massachusetts General Hospital, Boston, MA. Topic: Therapeutic hypothermia.
32. November 2004- Visiting Professor Grand Rounds at the Georgetown University Hospital, Washington, DC. Topic: Advances in Resuscitation strategies.


36. October 2004. Visiting Professor Grand Rounds at the Massachusetts General Hospital, Boston, MA. Topic: Novel Resuscitation Strategies


