INDUCED HYPOTHERMIA DURING RESUSCITATION FROM HEMORRHAGIC SHOCK ATTENUATES MICROVASCULAR INFLAMMATION IN THE RAT MESENTERIC MICROCIRCULATION

BY

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BS, UNIVERSITY OF MICHIGAN, 2009

Submitted to the graduate degree program in Clinical Research and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

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Chairperson Edward F. Ellerbeck, MD, MPH

Date approved: April 16th, 2013

Abstract

Introduction: Hemorrhagic shock is a major cause of morbidity and mortality in trauma patients. Microvascular inflammation occurs during resuscitation following hemorrhagic shock, and is a major cause of multiple organ dysfunction and late mortality. Hypothermia has traditionally been associated with poor outcomes in trauma patients, but pre-clinical evidence suggests that hypothermia may have some benefit in selected patients. Our objective was to evaluate the effect of induced hypothermia on microvascular inflammation during resuscitation from hemorrhagic shock.

Methods: Intravital microscopy was used to visualize mesenteric venules of anesthetized rats in real time to evaluate leukocyte adherence per 100 μm venule length and mast cell degranulation. An optical Doppler velocimeter was used to measure centerline red blood cell velocity in order to calculate shear rate. Measurements were obtained at a baseline control period in all animals. Animals then were divided into normotensive or hypotensive groups. Animals in the shock group underwent mean arterial blood pressure reduction to 40-45 mmHg for 1 hour via blood withdrawal. During the first two hours of resuscitation, body temperature of the hypothermic group was maintained at 32-34°C, while the normothermic group was maintained between 36-38°C. The hypothermic group was then rewarmed for the final two hours of resuscitation.

Results: Leukocyte adherence was significantly lower after 2 hours in hypothermic resuscitation (n=5) compared with normothermic resuscitation (n=6): (3.4±0.8 vs 8.3±1.3, p=0.011). Upon rewarming, leukocyte adherence was not significantly different between hypothermic and normothermic shock groups: (5.4±1.1 vs 9.5±1.6, p=0.081). No significant

elevation was observed in normotensive normothermic (n=3) or hypothermic animals (n=4). Shear rate decreased significantly from the control period after normothermic resuscitation ($p<0.05$), with no other groups having significant changes. Mast cell degranulation was significantly decreased in the hypothermic (1.02±0.04) vs normothermic (1.22±0.07) shock groups ($p=0.038$) after the experiment compared to the control measures; no significant degranulation occurred in normotensive animals.

Conclusions: Hypothermia during resuscitation attenuates microvascular inflammation in rat mesentery following hemorrhagic shock. Further study is needed to determine the underlying mechanisms of hypothermia in reducing microvascular inflammation during resuscitation, defining optimal degree of hypothermia, and the timing of this innovative therapy.

Acknowledgements

First and foremost I would like to thank my mentor Dr. John Wood, who has been an excellent source of knowledge and support throughout the year. From the time I came in with the hypothermia idea, you have allowed me to run with it, even when the progress was amazingly slow for a period of time. You have no doubt had a major impact on my scientific development, and for that I am thankful. I am honored to count you as a mentor.

I also would like to thank Dr. Michael Moncure, who I have had the privilege of working with since early in my first year of medical school. I am appreciative to you for introducing me to clinical research, and allowing me the autonomy to develop several projects while working with you. You have demonstrated the virtues of being a fine academic surgeon and I hope to emulate your enthusiasm in that regard in the near future.

I am also thankful for the support of Dr. Ed Ellerbeck, who convinced me to pursue this program in the first place. It has been a great experience and has helped develop my academic career immensely. I am also appreciative of Dr. Ellerbeck's guidance throughout the process, and enthusiasm in supporting my work.

I would additionally like to thank the three gentlemen listed above for agreeing to serve on my thesis committee.

This research wouldn't have been possible without the wonderful support of our research technician Naomi Holloway. She is a gifted, hardworking technician who was invaluable in completing the research and teaching me the nuts and bolts of microcirculatory laboratory

technique. I would also like to extend my thanks to the Department of Surgery for supporting my research in the laboratory, especially Dr. James Thomas, chairman of the department. The Department of Molecular and Integrative Physiology also played a key role in supporting this project, and I would like to thank them as well.

I would like to thank Anita Macan, Theresa Shireman, and Diane Durham for keeping the program running and organized. I would also like to thank the rest of the faculty and staff that I worked with throughout the year. This was an excellent program, and I am grateful for you all.

Last but not least, I would like to thank my family and friends who have all supported me throughout this process. It was tough for me to not go straight into my fourth year of medical studies, but with all the support from this group, I believe I made the right decision.

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Introduction

Background

Trauma continues to be a major cause of morbidity and mortality in the general population.[1] Along with traumatic brain injury, hemorrhagic shock shares a large responsibility for these poor outcomes.[2] Even with optimal resuscitation, delayed morbidity and mortality occurs in this population secondary to microvascular inflammation. Although major efforts have been made in both the laboratory and clinical research environment to overcome this inflammatory response to injury and resuscitation, little improvement in standardized care has been realized.[3]

Currently, victims of severe hemorrhagic shock from traumatic injury are treated with volume resuscitation. Blood products are the preferred resuscitation fluids for this patient population, but prehospital and continuous in hospital use of blood is costly and potentially dangerous. Isotonic resuscitation fluids, such as normal saline and lactated ringers, are also used frequently in this population. If shock is refractory to these measures after surgical control of bleeding, vasoactive medications may be given as a last resort to maintain blood pressure. There has been little change in this treatment paradigm over the last several decades, meaning continued poor outcomes for those suffering from severe hemorrhage.

If patients are transported quickly to a trauma center and receive optimal resuscitation as discussed above, patients may very well survive the injury for the first few hours. Unfortunately, there is still major danger lurking for these patients. Hemorrhagic shock has been shown to induce microvascular inflammatory responses that are not attenuated with volume resuscitation, and are even made worse in several cases.[4] Various mechanisms of this injury have been

hypothesized, including ischemia-reperfusion injury and systemic hypoxia.[3,5] It is likely a mixture of both of these, with the addition of signaling through cytokines and sympathetic activation that leads to this exaggerated inflammatory response. This causes severe microvascular inflammation, leading to multiple organ failure and delayed mortality after the initial injury and resuscitation.

Numerous studies have looked at the cellular and molecular mechanisms contributing to microvascular inflammation. It is now thought that multiple pathways can lead to induction of this response, and the pathways are inherently interrelated (Figure 1). One proposed mechanism involves generation of reactive oxygen species at the cellular level secondary to inhibited respiration due to an insult (hemorrhage, hypoxia, or ischemia). These reactive oxygen species initiate cytokine formation through mediators such as TNF-α and IL-1β, and endothelial activation occurs.[6] This increases leukocyte adherence and emigration. Additionally, mast cells located in the perivascular tissue are induced to release granules by activation with Ig-E.[7] These granules contain histamine, additional inflammatory cytokines, and even some anti-inflammatory markers that modulate immune function. This complex pathway leads to localized tissue death due to edema and apoptosis, ending with multiple organ dysfunction and death of the patient.

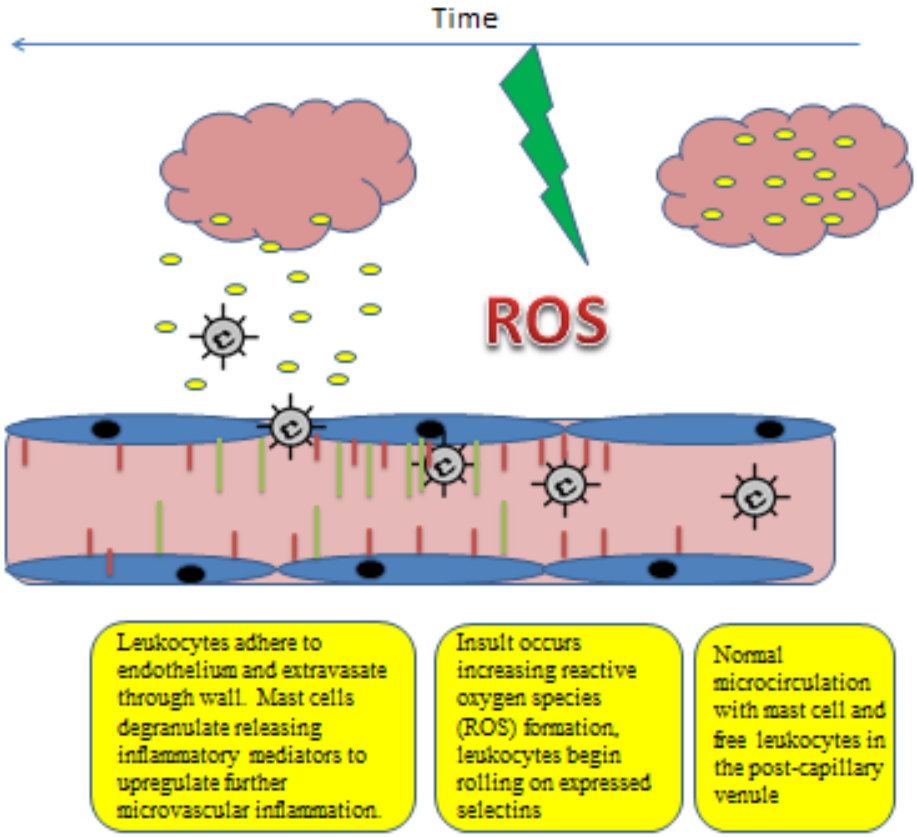

Figure 1: Mechanism of Microvascular Inflammation

A long held axiom of trauma care has been temperature regulation of the injured patient, essentially the maintenance of normothermia (36-38°C) during resuscitation.[8] This practice has been based off several observational studies in the past that have shown a relationship between hypothermia (temperature less than 35°C) and increased mortality. Hypothermia has even become accepted as a member of the "deadly triad" of trauma resuscitation (Figure 2). However, there have been no definitive studies pointing to hypothermia as a causative factor of death in the trauma patient. Additionally, some have suggested that hypothermia on presentation to the

trauma bay may be an index for injury severity, and may also be a compensatory mechanism to preserve tissue viability in the patient.[9]

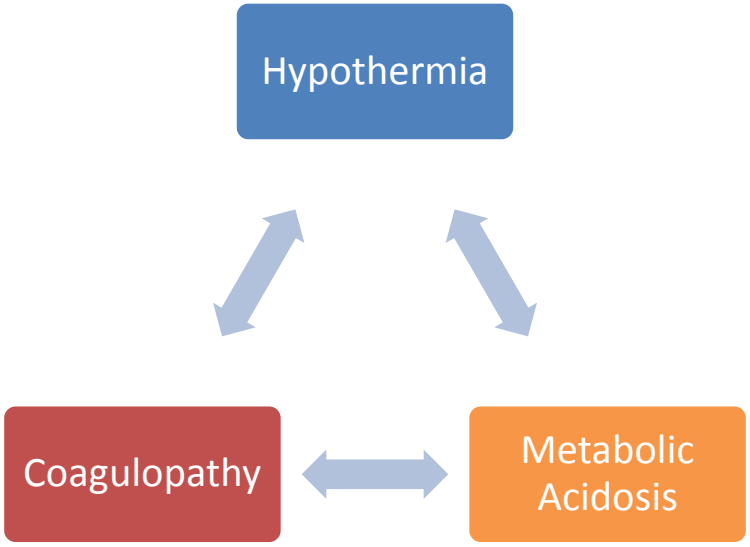

Figure 2: "Triad of Death" in Traumatology

Hypothermia has seen a recent increase in usage as a therapeutic modality.[10] It has been used in the conduction of cardiac surgery procedures for over 50 years. Hypothermia has also been shown to have a neuroprotective effect on patients with stroke and traumatic brain injury. Additionally, induced hypothermia after cardiac arrest has been shown to decrease mortality and increase neurological function.[11,12] A recent case series has even shown that induced hypothermia protocols can be used after traumatic cardiac arrest with positive outcomes.[13]

Study Objective

Our objective in this study was to determine the effect of induced hypothermia during resuscitation from hemorrhagic shock on microvascular inflammation. We have developed a successful model of microvascular inflammation during hemorrhagic shock in our laboratory by

examining rat mesenteric venules via intravital microscopy.[4] This study was designed to evaluate mild hypothermia (32-34°C) during resuscitation as this poses the least risk of coagulopathy, and is easy to achieve and maintain without aggressive cooling techniques in the trauma patient.[14] Additionally, we also wanted to study the effects of rewarming the hypothermic patient on microvascular inflammation, as this is the common practice in traumatology at this point in time. We chose to evaluate leukocyte adherence and mast cell degranulation, two important indices of microvascular inflammation and dysfunction, during the course of our experiments.[5,15,16]

Methods

Regulatory Considerations

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center. The University of Kansas Medical Center is accredited by the American Association for Accreditation of Laboratory Animal Care; all procedures were carried out in accordance with National Institute of Health guidelines.

Surgical Procedures

Sprague-Dawley rats weighing between 250 to 350 grams were obtained from Harlan Laboratories (Indianapolis, IN). Animals were allowed full access to food and water prior to the experiment. Rats were anesthetized with sodium pentobarbital (40-60 mg/kg IM injection, additional doses as needed). Jugular venous cannulation was performed for administration of fluids and blood with a Polyethylene (PE) cannula (PE-50, 0.58mm ID). Carotid artery cannulation was likewise carried out with a PE-50 cannula for withdrawal of blood and monitoring of mean arterial pressure (MAP) with a continuous blood pressure analyzer

(Micromed, Louisville, KY). A tracheostomy was performed with PE-240 tubing for definitive airway management throughout the experiment. Midline laparotomy incision was conducted with radiocautery (Harvard Apparatus, Holliston, MA).

Intravital Microscopy

The animal was placed in the left lateral decubitus position on a Plexiglas stage mounted to a Zeiss Axiovert inverted microscope. A loop of mesentery was draped over a glass cover slip in the center of the stage to view a mesenteric venule. Saline warmed to 37°C was superfused over the mesentery to prevent drying, and a square of Saran wrap was also placed over the exposed mesentery to assist in this regard. Mesenteric venules were selected for study based on the following 3 criteria: 1) straight, unbranched vessels at least 100 μm in length; 2) no adjacent vessels within 100 μm of the venule; 3) vessel diameter of 20-40 μm. Images of the mesentery (40x objective, 10x eyepiece) were recorded using Mini-DV videocassette recorder (Sony, Tokyo, Japan) using a video camera and time-date generator (Panasonic, Kadoma City, Japan).

Venular diameter was obtained using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). An optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University, College Station, TX) was used to measure red blood cell (RBC) centerline velocity in mesenteric venules. Average RBC velocity was calculated by dividing centerline velocity by 1.6. Shear rate was then calculated as 8 multiplied by RBC velocity divided by venular diameter.

Leukocyte Adherence and Mast Cell Degranulation

Leukocyte adherence and mast cell degranulation were measured during offline playback of recorded videotapes. Leukocyte adherence was measured over each minute by counting the

number of leukocytes that remained stationary for longer than 30 seconds in a 100 μm length of venule. Tapes were analyzed two times by and experienced observer to ensure accurate counting of leukocytes. Mast cell degranulation was identified by superfusing the mesentery with ruthenium red, a chemical that stains degranulated mast cells. Images of mast cells were collected during the control period, then once again at the conclusion of the experiment. These images were converted to digital grayscales and phase inverted. Relative light intensity of each mast cell was measured using the ImageJ program (NIH, Bethesda, MD), and the degree of degranulation was quantified as a mast cell degranulation index (MDI) equal to the ratio of the end of experiment to control period intensities.

Experimental Protocol

The experimental groups (Figure 3) consisted of normothermic normotensive animals (n=3), normotensive with hypothermia (n=4), normothermic hemorrhagic shock (n=6), and hemorrhagic shock with hypothermia (n=5). Temperature was recorded with a rectal thermometer, and maintained at 36-38°C with a homeothermic blanket and a heat lamp. Animals were allowed to stabilize after surgery for a 30 minute control period prior to beginning the experiment. Hemorrhagic shock was induced by withdrawal of blood from the carotid artery into a syringe containing 100 unit of heparin. A MAP of 40-45 mmHg was maintained for a period of one hour. At the end of this period, the shed blood was returned to the animal via the jugular cannula, as well as two times the volume of blood in normal saline. For those undergoing hypothermia, animals were allowed to naturally begin cooling during hemorrhagic shock, with a target of 32-34°C at the end of the shock period. Animals needing additional cooling at this point were subjected to air cooling via a fan. The hypothermia was maintained for a two hour time period, after which the animals underwent re-warming to 36-38°C for 2 hours.

Leukocyte adherence and physiologic variables were recorded every 15 minutes throughout the experiment. Mast cell degranulation was recorded at the end of the control period and at the end of the experiment.

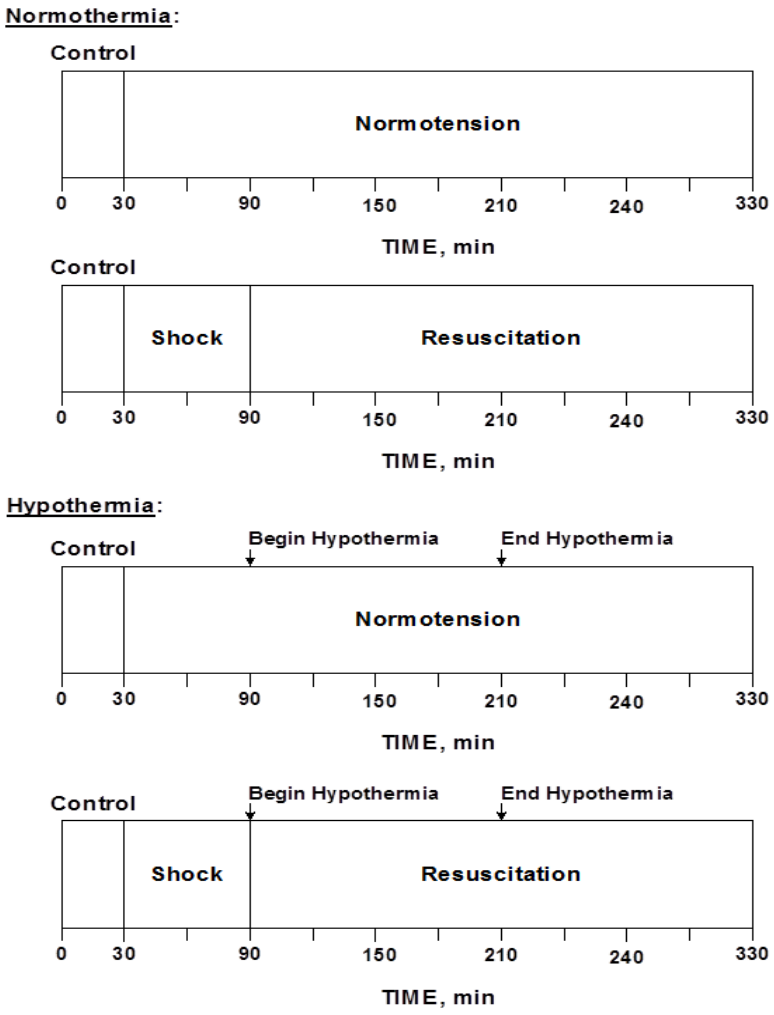

Figure 3: Experimental Design and Group Configuration

Chemicals

Ruthenium red was obtained from Sigma (St. Louis, MO).

Statistical Analysis

Data was expressed as mean ± SE for continuous variables. MAP and rectal temperature were analyzed with one-way ANOVA with bonferoni's post-hoc correction to characterize all between group differences at each time point. Student's *t* test was used to make direct comparisons in differences of leukocyte adherence between hypothermic shock and normothermic shock groups, between normothermic normotensive and hypothermic normotensive groups, and between each shock group individually with the normothermic normotensive group (acting as a longitudinal baseline comparison) at hourly time points during resuscitation. Shear rate was evaluated in the same manner. Paired *t* test was used to compare shear rates from the control period to the final resuscitation time point within each group to determine change from the control period. Differences in mast cell degranulation index were compared using Student's *t* test. Differences were considered significant when $p \leq 0.05$. Statistical analysis was carried out using SAS 9.3 (SAS Institute, Cary, NC).

Results

Physiological Measures

Figure 4 displays rectal temperatures recorded throughout the experiment. Animals in the hypothermic shock group gradually cooled during shock, and were maintained at 32-34°C for 2 hours. The rewarming took place rapidly over approximately one hour in this group, and was maintained at 36-38°C for the remainder of the experiment. There were no significant differences between the like temperature groups. The hypothermic groups had significantly lower temperatures than the normothermic groups from start of resuscitation to 30 minutes into the rewarming period ($p<0.05$). MAP of the animals is displayed in Figure 5 below. Shock was

maintained for 60 minutes, and resuscitation provided initial pressures near baseline. This pressure gradually decreased as the experiment went on during the 4 hour resuscitation period in all 4 groups. The shock groups had significantly lower MAPs then the normotensive groups during the shock period ($p<0.05$). Additionally, the hypothermic normotensive group had a significantly higher MAP then the other groups from 30 minutes of shock to 30 minutes into the rewarming ($p<0.05$) period; this was expected due to known increases in MAP with hypothermia.

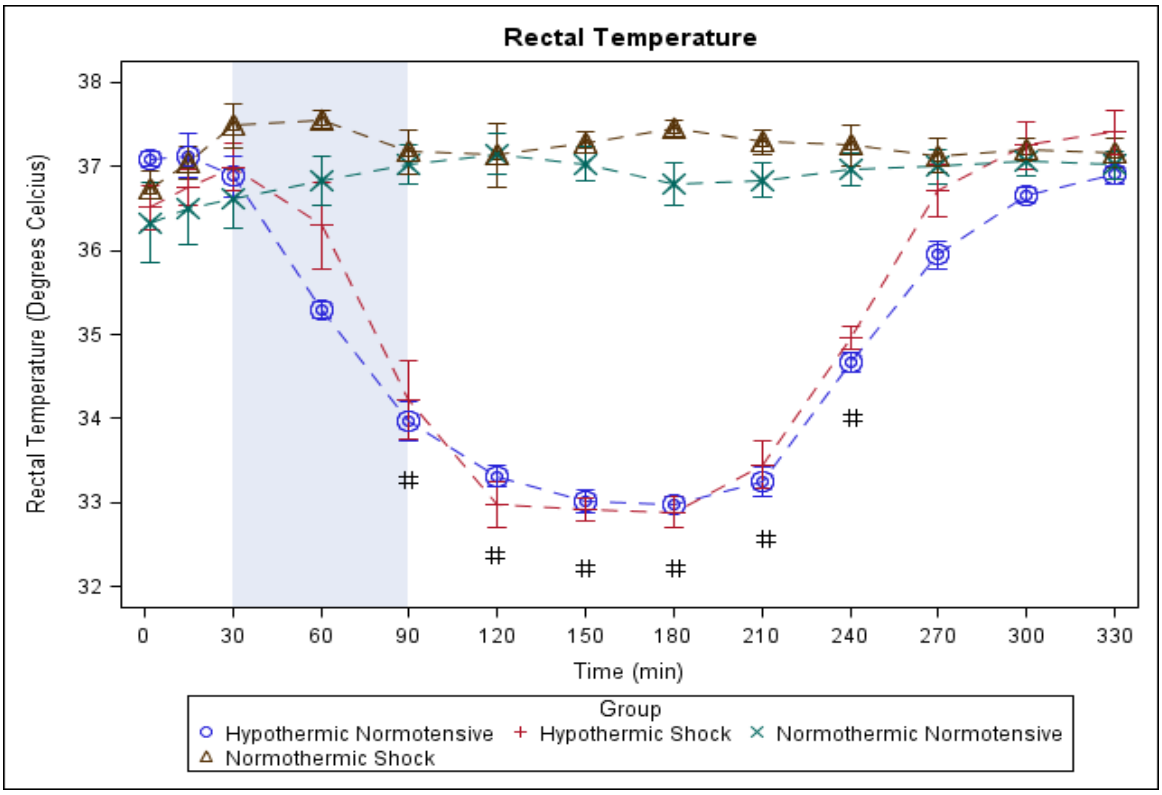

Figure 4: Rectal Temperatures; #: significant difference between hypothermic groups and normothermic groups ($p<0.05$).

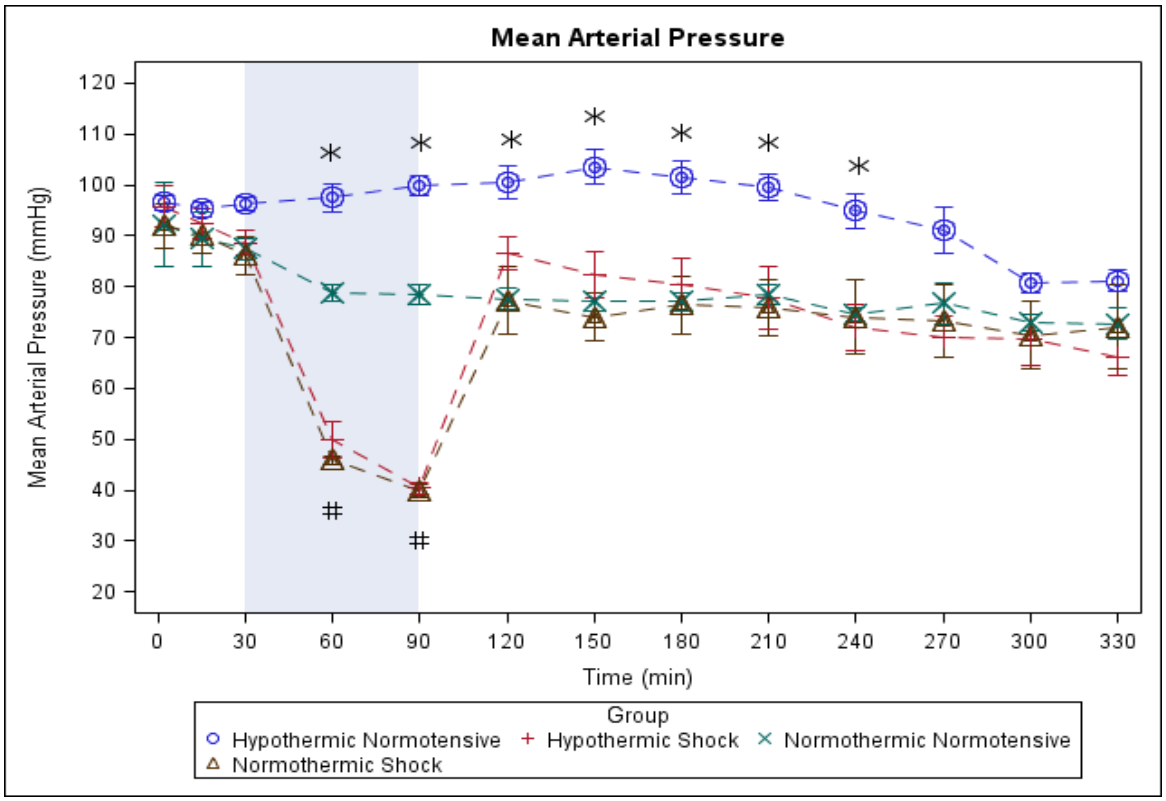

Figure 5: Mean Arterial Blood Pressure; #: Significant difference between shock and normotensive groups ($p<0.05$). *: Significant difference between hypothermic normotensive and all other groups ($p<0.05$).

Calculated shear rates are shown in Figure 6. Shear rates in the hypothermic shock group did not significantly change over the course of the experiment when compared to control time period measurements ($p>0.05$), while there was a significant decrease within the normothermic shock group over the course of the experiment from the control period measure to the end of 4 hours of resuscitation ($p<0.05$). There were no significant changes in shear rates within the two normotensive groups.

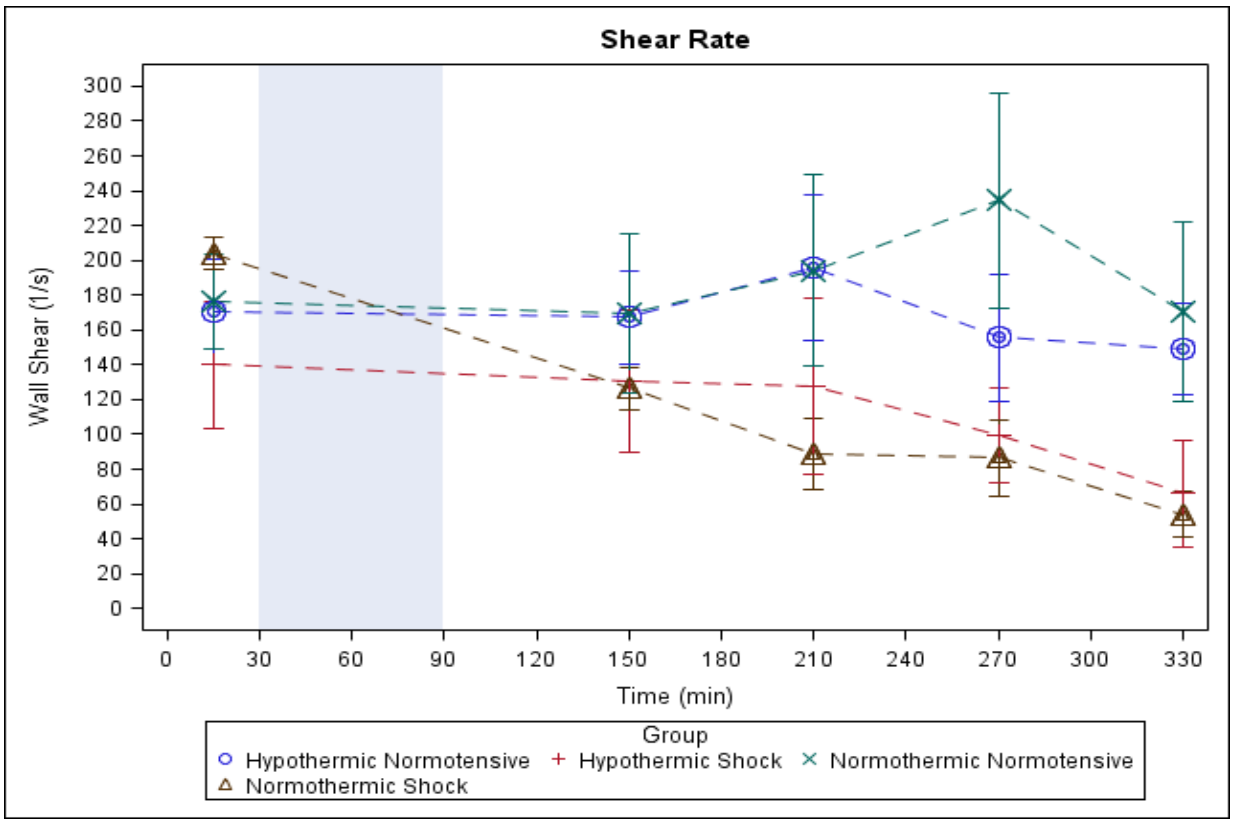

Figure 6: Wall Shear Rate; There were no significant differences between groups. However, there was a significant decrease within the normothermic shock group between the control period and the end of resuscitation at 4 hours.

Leukocyte Adherence

Figures 7-10 shows photographs of rat mesenteric venules in the normothermic sham, hypothermic sham, normothermic shock, and hypothermic shock groups at control, 2 hours into resuscitation, and 4 hours into resuscitation.

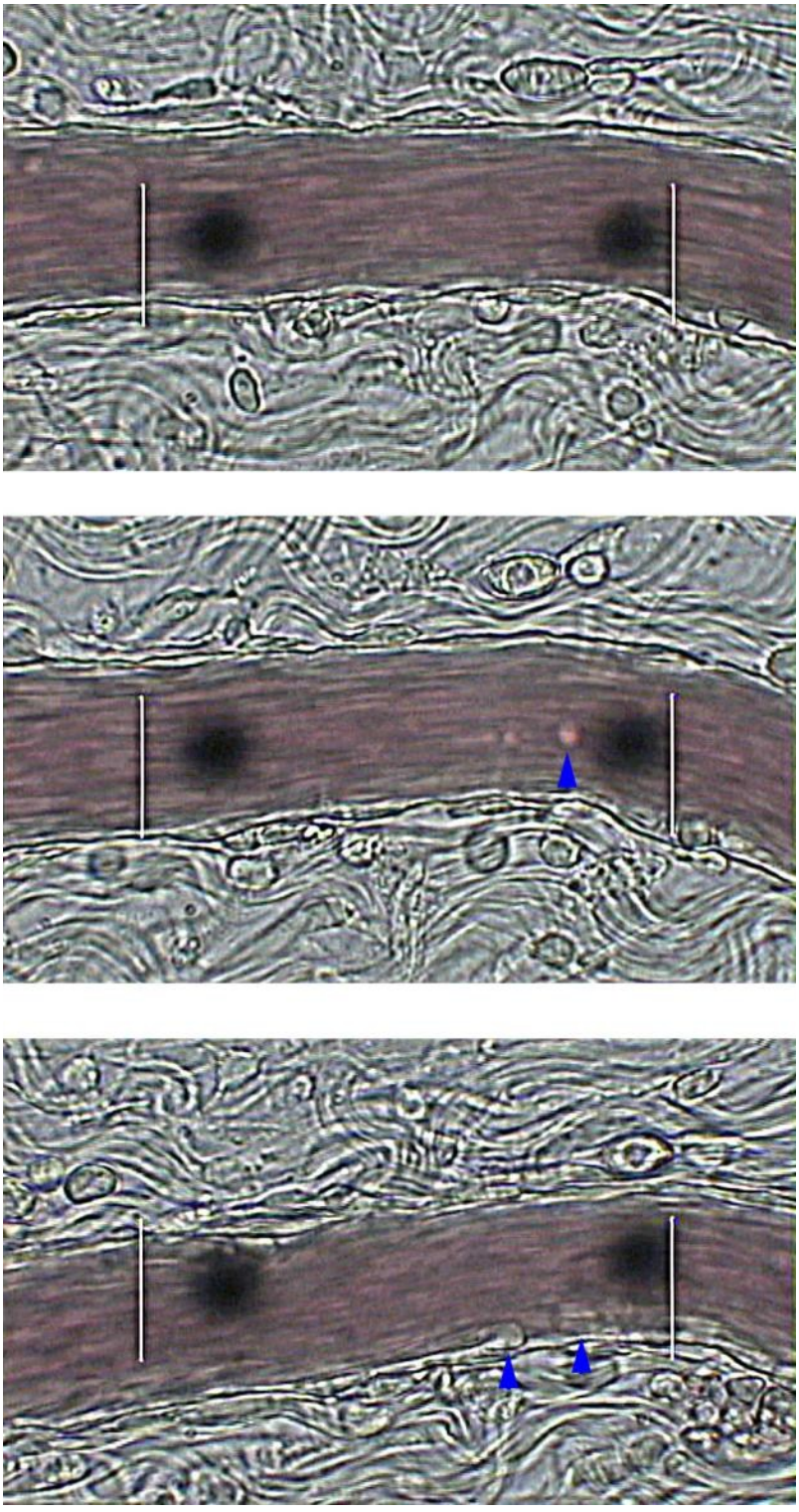

Figure 7: Normothermic Normotensive Mesenteric Venule; From top to bottom: Control, 2 hours post resuscitation, and 4 hours post resuscitation. Blue arrows indicate adherent leukocytes. Black dots are part of the optical Doppler velocimeter and white bars outline 100 μm length segment.

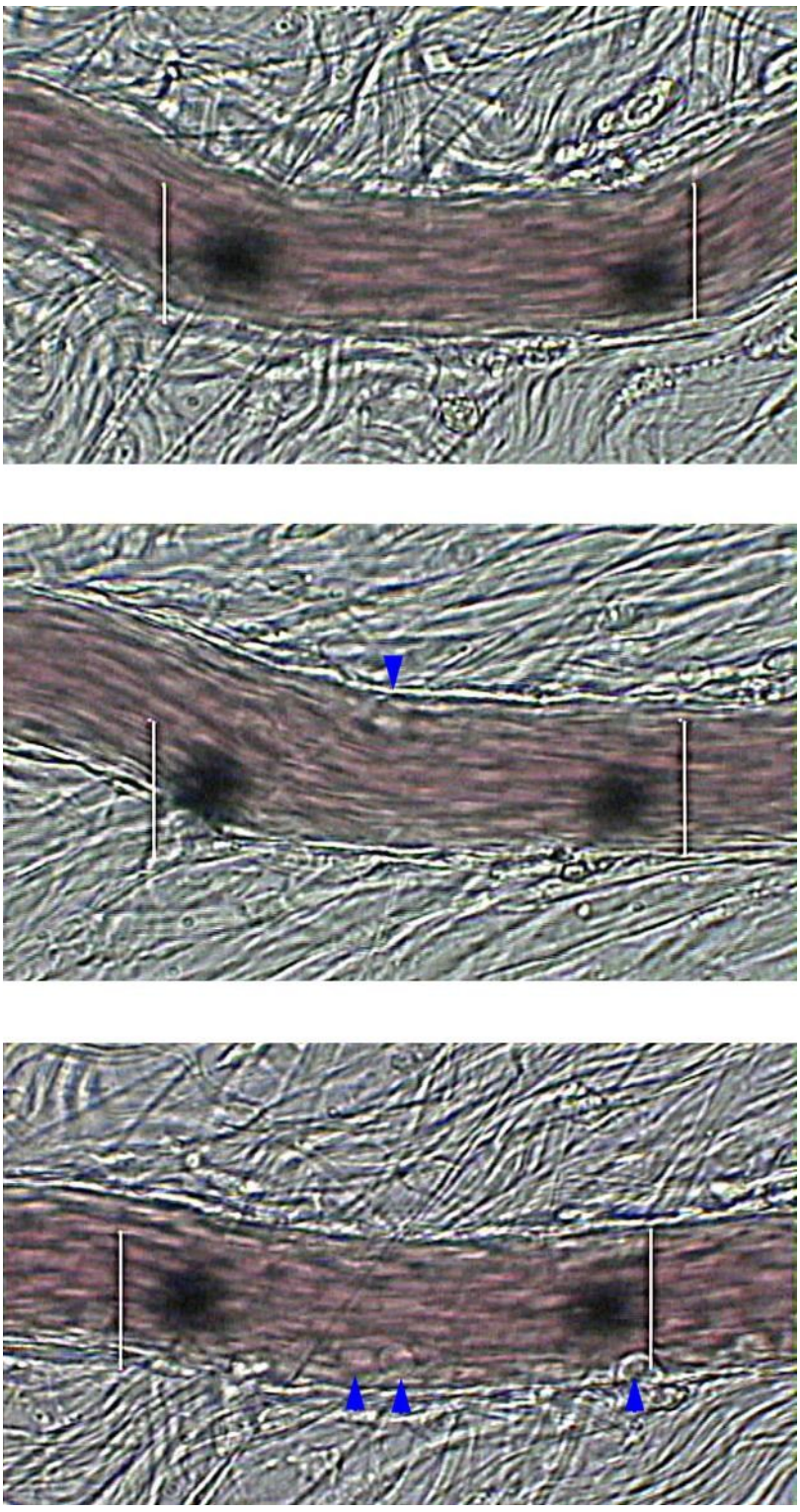

Figure 8: Hypothermic Normotensive Mesenteric Venule; From top to bottom: Control, 2 hours post resuscitation, and 4 hours post resuscitation. Blue arrows indicate adherent leukocytes. Black dots are part of the optical Doppler velocimeter and white bars outline 100 μm length segment.

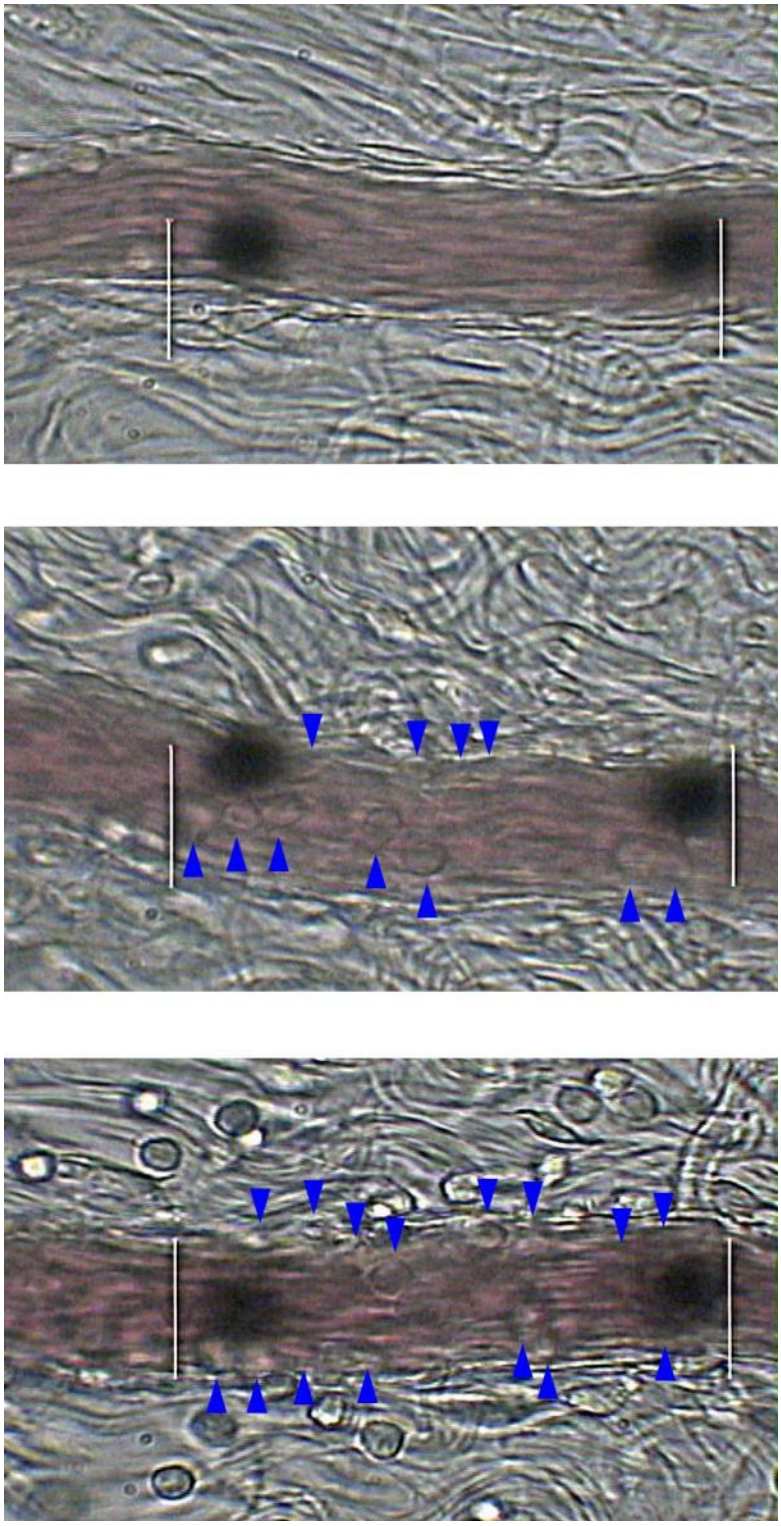

Figure 9: Normothermic Hemorrhagic Shock Mesenteric Venule; From top to bottom: Control, 2 hours post resuscitation, and 4 hours post resuscitation. Blue arrows indicate adherent leukocytes. Black dots are part of the optical Doppler velocimeter and white bars outline 100 μm length segment.

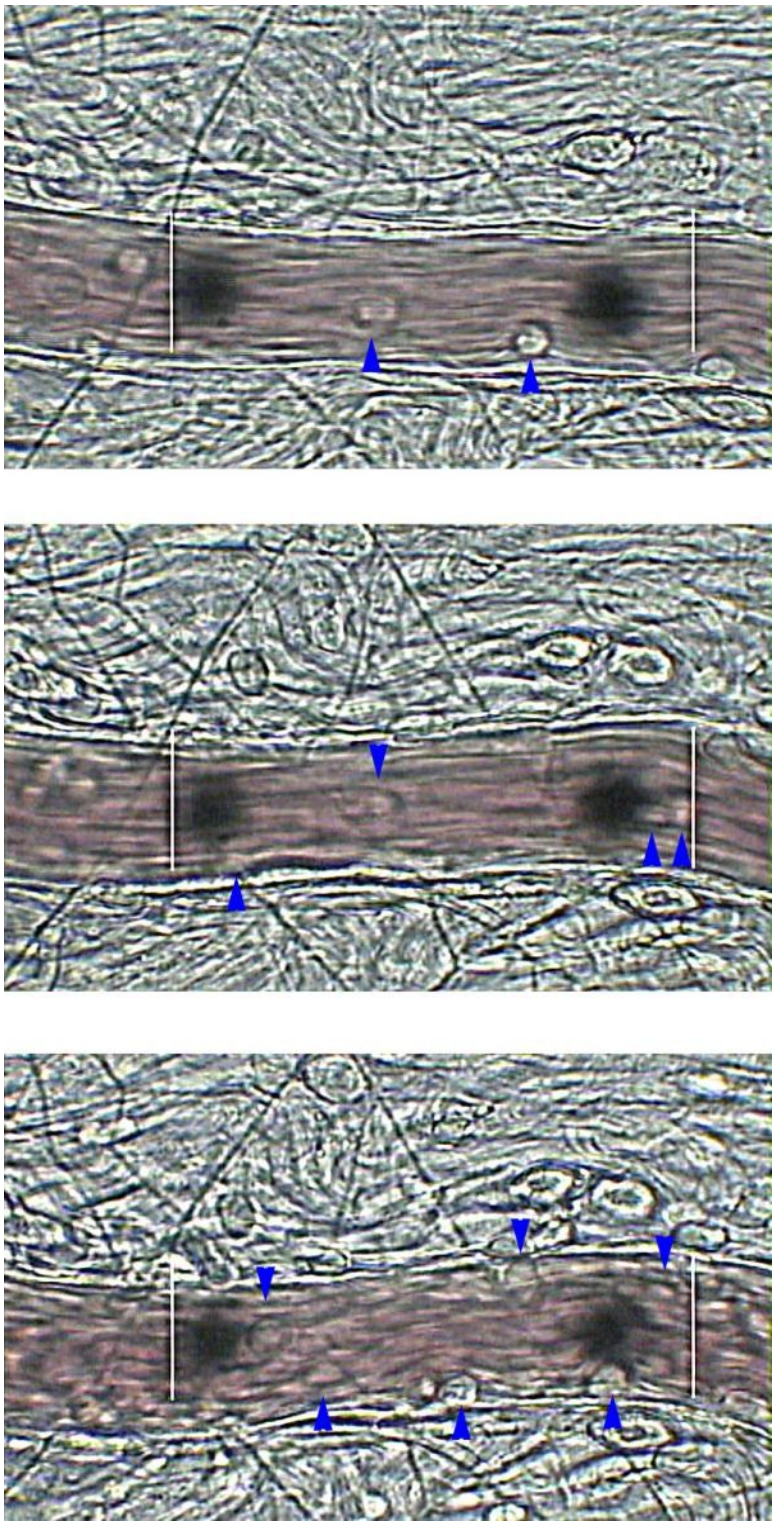

Figure 10: Hypothermic Hemorrhagic Shock Mesenteric Venule; From top to bottom: Control, 2 hours post resuscitation, and 4 hours post resuscitation. Blue arrows indicate adherent leukocytes. Black dots are part of the optical Doppler velocimeter and white bars outline 100 μm length segment.

The number of adherent leukocytes in a 100 μm length of venule is shown for all 4 experimental groups in Figure 11. Leukocyte adherence was significantly lower in the hypothermic shock group than in the normothermic shock group at the 2 hour time point (3.4±0.8 vs 8.3±1.3, $p=0.011$), but this difference became non-significant at the end of the rewarming period (5.4±1.1 vs 9.5±1.6, $p=0.081$). The increase in leukocyte adherence in the hemorrhagic shock groups as compared to the normothermic normotensive animals became significant at 1 hour after beginning resuscitation in the normothermic shock group, while the increase became significant at 3 hours (during rewarming) in the hypothermic shock group (both $p<0.05$). Normotensive animals did not have significant changes in leukocyte adherence between the two groups.

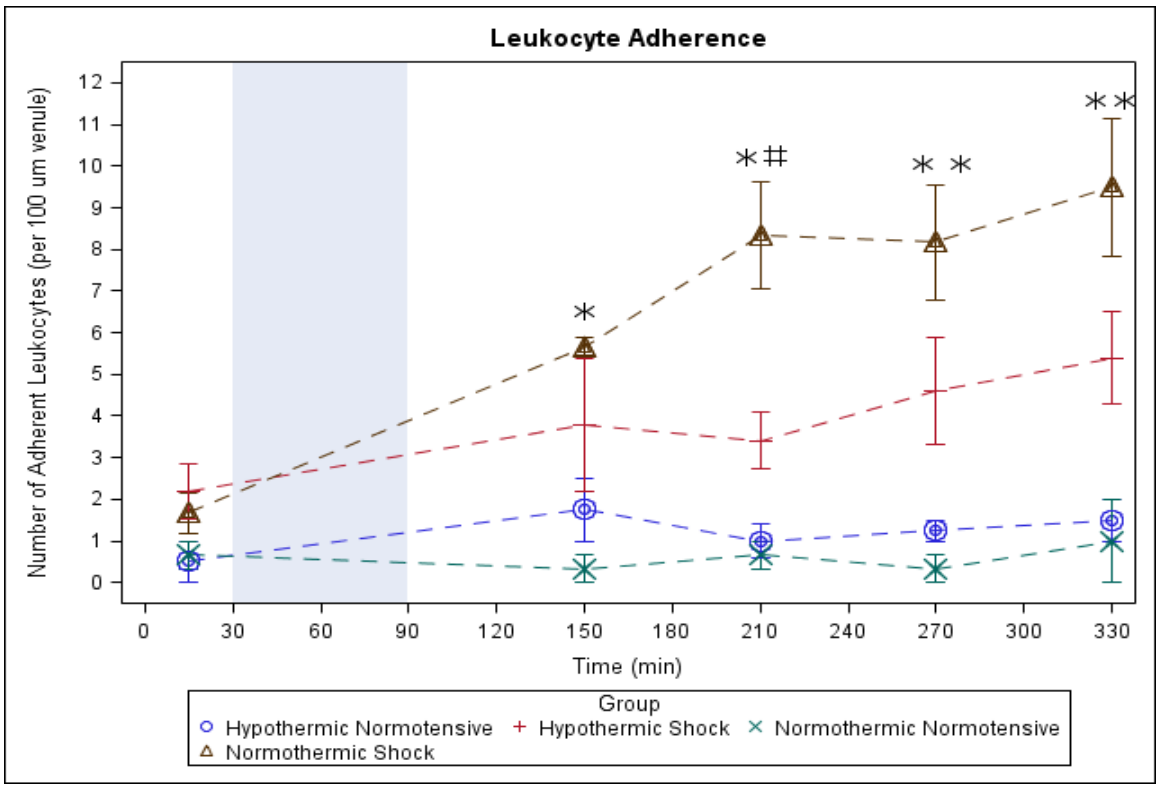

Figure 11: Leukocyte Adherence in Mesenteric Venules; #: Significant difference between normothermic shock and hypothermic shock group (p=0.011). *: Significant difference between normothermic shock and normothermic normotensive groups (p<0.05). **: Significant difference between both shock groups and the normothermic normotensive group (p<0.05).

Mast Cell Degranulation

Figures 12 and 13 show photographs of mast cells for the normothermic shock group and the hypothermic shock group (respectively) during the control period and after 4 hours of resuscitation. Mast cell degranulation index was significantly decreased after the 4 hours resuscitation period compared to control period measures in the hypothermic shock group (1.02±0.04) as compared to the normothermic shock group (1.22±0.07, p=0.038). No significant degranulation occurred in the normothermic or the hypothermic normotensive groups.

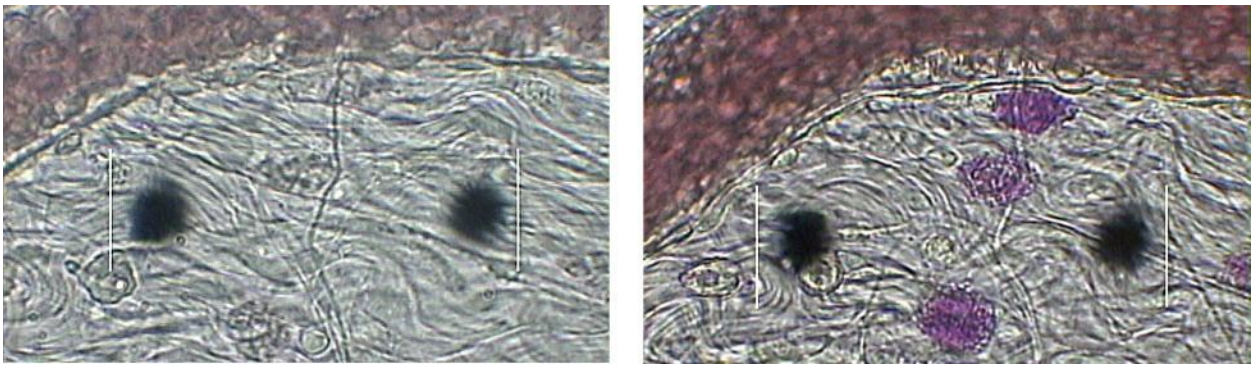

Figure 12: Mast Cell Degranulation in Normothermic Shock; Left: Control period. Right: After 4 hours of resuscitation. Pink color represents pickup of ruthenium red dye due to mast cell degranulation. Black dots are part of the optical Doppler velocimeter, and the white lines represent a 100 μm length.

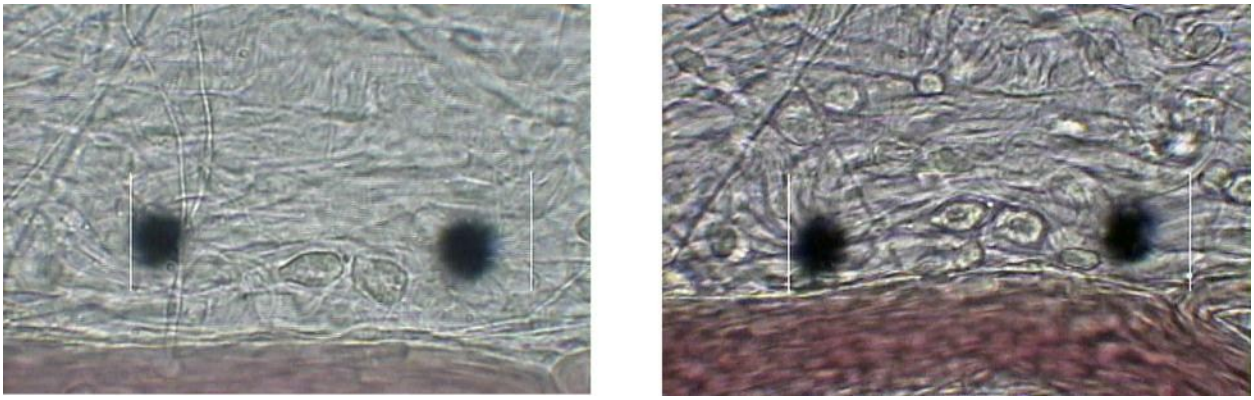

Figure 13: Mast Cell Degranulation in Hypothermic Shock; Left: Control period. Right: After 4 hours of resuscitation. Pink color represents pickup of ruthenium red dye due to mast cell degranulation. Black dots are part of the optical Doppler velocimeter, and the white lines represent a 100 μm length.

Discussion

We have shown that induced hypothermia during resuscitation from hemorrhagic shock significantly attenuates leukocyte adherence and mast cell degranulation in the rat mesenteric microcirculation. The decreased leukocyte adherence between hypothermic and normothermic resuscitation was statistically significant after the hypothermic period, but was not statistically significant after rewarming occurred. We also observed a significant decrease in shear rates

during the resuscitation period in the normothermic cohort, while there was no significant decrease during resuscitation of hypothermic animals.

Leukocyte adherence has been shown previously to be a mediator of microvascular dysfunction during hemorrhagic shock.[4] The increase in leukocyte adherence has been linked to several other indices of inflammation, such as mast cell degranulation, reactive oxygen species (ROS) formation, and increased vascular permeability. It is thought that ROS formation secondary to the ischemia-reperfusion injury that occurs during hemorrhagic shock and resuscitation is a cause of endothelial dysfunction that leads to increased leukocyte adherence and vascular permeability. Mast cell degranulation occurs in response to oxidative stress as well as when inflammatory mediators extravasate through the damaged endothelium. The degranulation of mast cells further increases ROS formation and leukocyte adherence. This cycling cascade continues for several hours post-injury, and is responsible for much of the delayed morbidity and mortality in patients suffering from hemorrhagic shock injury.

There have been several previous laboratory studies examining the effect of hypothermia on hemorrhagic shock.[17,18] Although the results have generally been favorable, there have been others that have not shown benefits. Childs and colleagues showed that pretreating animals with mild and moderate hypothermia prior to hemorrhagic shock attenuated ROS formation and vascular permeability during the resuscitation period.[19] These findings support the results we have discussed above, and generally fit with the physiology of microvascular inflammation discussed above. It is important to note that their study pretreated with hypothermia and maintained constant temperature throughout the experiment, while we used a model that involved allowing animals to cool and maintaining the hypothermic range as would be done in actual resuscitation settings. Additionally, it has been shown that moderate hypothermia

decreases systemic oxidative stress during intestinal ischemia-reperfusion injury in the rat.[20] It seems clear that reduced ROS has some role in the protective effect of hypothermia during shock and resuscitation, but the exact mechanism of this process will require further study.

Other laboratory studies have directly examined mortality during hemorrhagic shock with hypothermia. Several studies indicate decreased early mortality with mild to moderate hypothermia during resuscitation from hemorrhagic shock in rat models.[21-24] The few studies that did look at long term outcomes had mixed results.[22,25,26] Additionally, large laboratory animal models have shown that environmental (natural cooling) hypothermia has similar mortality to normothermia.[27] These studies are routinely conducted with a small number of animals, and this could account for some of the variations in outcomes. It is also important to point out that the method of hemorrhagic shock induction is different between studies, and this makes aggregating the results difficult. We chose to use a pressure-controlled hemorrhagic shock model in our study due to earlier studies suggesting animals with hypothermia had increased MAPs during volume-controlled shock, which is a confounder for looking at the effect of hypothermia alone on the microcirculation.[22,24,28]

The mechanism of protection of the microvasculature with induced hypothermia during resuscitation from hemorrhagic shock remains unclear. A study in rats showed that IL-1β and IL-6 were decreased in hypothermic resuscitation, but the study did not have enough power to call it a significant difference.[29] The same study did find that IL-10, an anti-inflammatory cytokine, was elevated in hypothermia, but once again the study was underpowered to detect the difference. An interesting finding was that TNF-α was significantly increased after hypothermic resuscitation. This has also been suggested by other studies looking at traumatic brain injury with hypothermia for neuroprotection. Although this response seems counter-intuitive, delayed

increased levels of TNF-α may help decrease certain ROS products, and may decrease metabolic demand on myocardium and peripheral tissues. These cytokines also play a role in modulating selectins, intracellular adhesion molecules, and mast cells. This supports our findings of decreased leukocyte adherence and mast cell degranulation in the hypothermic resuscitation.

Findings in the human population regarding hypothermia in the trauma population have been largely negative.[8] Many observational studies have linked hypothermia on arrival to the hospital as a negative prognostic indicator. Additionally, one prospective study showed that rapid rewarming of hypothermia using intravascular warming was superior to passive, slower rewarming in outcomes. These findings have led to the creation of a dogma in the trauma field that hypothermia is to be avoided at all costs during resuscitation. However, a recent case series reported successful outcomes following induced hypothermia after traumatic cardiac arrest.[13] Additionally, numerous studies have shown a neuroprotective effect of hypothermia in patients suffering from traumatic brain injury.

There is also some debate about what hypothermia in a trauma patient actually means: is hypothermia a pathological factor leading to increased morbidity and mortality, or is it a physiological compensatory mechanism? While many would argue that it is a pathological factor based on clinical data, one needs to consider that most of this data is observational in nature, and cannot determine causality. In laboratory studies, it has been shown that hemorrhagic shock lowers the thermoregulatory set point.[30] It is also known that hypothermia decreases metabolic demand and tissue oxygenation requirements, and has a positive effect on MAP due to peripheral vasoconstriction. It would seem from this data that hypothermia is a physiological compensatory mechanism. Further prospective and randomized studies in humans may help to determine this once and for all, but there are several barriers to this research:

namely, mixed laboratory results and lack of large animal models proving the concept. These should be goals of further research.

Our study has several strengths. We used a pressure-controlled hemorrhagic shock model in our study in order to limit the increased MAP effect of hypothermia, as to eliminate this as a confounder. We allowed the animal to cool during shock, and simply maintained the hypothermia during the first two hours of resuscitation, simulating clinical conditions that patients experience. We also examined 2 hours of rewarming, to see if the effect carried over after acute resuscitation.

There also exist several limitations to our model. Since our model involves non-survival surgical methods, we were not able to assess mortality differences between normothermic and hypothermic resuscitation. It remains unclear what the proper duration of hypothermia during the resuscitation period should be; there are various lengths used in the current literature, and we selected 2 hours due to our current hemorrhagic shock models looking at 2 hour resuscitation period outcomes. Although we carried out rewarming, it is difficult to tell whether the rise in leukocyte adherence during that time period was due to the warming or simply the length of the experiment. We postulate that this increase was due to warming as evidenced by the plateau in leukocyte adherence reached near the end of hypothermia, and no increase was observed during rewarming of the hypothermic normotensive animals.

One important question that developed as a result of our methodology was whether hypothermia actually prevents microvascular inflammation or delays the onset. Since we only kept the subjects hypothermic for the first 2 hours of resuscitation, we are unable to predict what effects longer periods of hypothermia may have in terms of continued microvascular

inflammation. Additionally, we observed increased leukocyte adherence in the rewarming period, but we have no way of currently knowing if this was caused by the rewarming, or if this was simply a delayed inflammatory response secondary to the hypothermic period. One important factor to note is that the rewarming in our experiments took place rapidly, at approximately 2-3 °C per hour. This is much faster than recommended rewarming times with therapeutic induced hypothermia, and may have led to the increase in leukocyte adherence. Further studies to investigate this novel finding will be needed to clarify what is happening in this instance.

Conclusions

We conclude that induced hypothermia (32-34°C) during resuscitation from hemorrhagic shock decreases leukocyte adherence and mast cell degranulation in the rat mesenteric circulation. Furthermore, hypothermia did not cause microvascular inflammation in normotensive animals. Probable mechanisms for this observed benefit include decreased ROS, altered cytokine signaling, and maintenance of the microvascular endothelial integrity. Further research is warranted to examine the optimal time of hypothermia, the optimal degree of cooling, and the effect of rewarming on microvascular inflammation.

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