Resistance to multi organ damage after hemorrhagic shock induced ischemia/ reperfusion in arctic ground squirrels

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During hemorrhagic shock (HS), the body undergoes global ischemia as blood pressure drops below the threshold at which tissues can be adequately perfused. Resistance to ischemia/reperfusion (I/R) injury is a characteristic of hibernating mammals. The present study sought to determine if arctic ground squirrels (AGS) are protected from HS induced I/R and if any protection is dependent upon their hibernation season. Rats, euthermic AGS, and interbout arousal AGS were subject to HS by withdrawing blood to a mean arterial blood pressure (MAP) of 35 mmHg. Low MAP was maintained for 20 min and then the animals were reperfused with Ringers. The animals' temperature was maintained at 36.5-37.5 °C. After reperfusion, animals were monitored for 3 hrs then tissues and blood were collected for histopathology, clinical chemistries, and cytokine level analysis. In addition, a group rats and AGS were monitored for three days after HS to access survival and physiological impairment. AGS were able to survive three days beyond HS with no impairment noticeable after 12 hrs whereas rats did not survive to 12 hrs. Regardless of season, blood chemistries indicate no organ damage in the ground squirrel while the rat showed signs of liver damage. In addition, both winter and summer AGS had a lower cytokine response after HS than did rats. Funded by USAMRMC W81XWH009-2-0134.

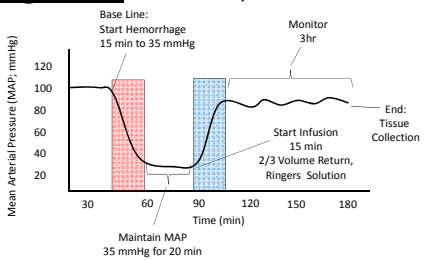
INTRODUCTION

- Worldwide hemorrhagic shock is the number one cause of death in trauma patients, the majority of those die from multi organ dysfunction syndrome [1, 2].
- During hemorrhagic shock (HS), the body undergoes global ischemia as blood pressure drops below the threshold at which tissues can be adequately perfused with blood.
- Resistance to ischemic injury is a characteristic of hibernating mammals, including ground squirrels.
- There is debate on if this resistance is dependant on hibernation season or if it is an intrinsic plasticity of the organism.

QUESTION: Are AGS protected from HS-induced ischemia reperfusion (I/R) injury on the whole organism and tissue–specific levels and if any protection is dependent upon their hibernation season.

METHODS

Figure 1: HS isobaric procedure



Prior to hemorrhage (baseline), immediately after resuscitation and 3 hours after resuscitation, blood was sampled and analyzed physiological parameters (Table 1). Blood sampled during hemorrhage and 3hr after resuscitation was also analyzed for markers of organ damage and levels of inflammatory cytokines as indicators of systemic inflammation. After 3 hours, organs were collected and fixed for histological analysis (Table 1). The primary endpoints were: 1) plasma markers for organ damage, 2) histopathological damage to tissues, 3) circulating and tissue levels of inflammatory cytokines. In addition, a group of animals were monitored for 72 hours after reperfusion to assess survival rates and any physiological impairments. During hemorrhage, infusion, and monitoring period, animals' body and head temperatures were maintained between 36.5-37.5 °C. Sham animals were instrumented and monitored in the same way as HS animals, except blood was not withdrawn for hemorrhage. AGS were tested during their summer (euthermic) and winter (interbout arousal) season. All AGS were housed at 2°C, 4L:20D conditions, year round. Interbout arousal AGS were aroused 20 hours prior to experiment. All animals were fasted 20 hours prior to experiment.

Table 1: Components of analysis

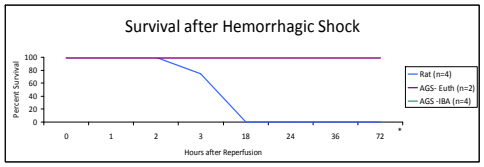
Analysis	Parameters
Physiological	HR, MABP, temperature (head, core, limb), pO_2, O_2 Sat, pCO_2, pH, bicarb, base excess, blood glucose, blood lactate, complete blood count (CBC)
Blood chemistries	blood urea nitrogen (BUN), creatinine, bilirubin, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)
Histopathology	heart, lungs, intestine, kidney, spleen, and liver
Cytokine Immunochemistry	IL-1 alpha, IL-1 beta, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, INF-gamma, GM-CSF (Luminex-EDTA plasma)

Table 2:
AGS seasonal group parameters

Summer/Euthermic	Winter/IBA (prior to induced arousal)
Tb > 35°C	Tb < 5°C
RR > 80 rpm	RR < 5 rpm
Alert and responsive	Wood shavings on back undisturbed
No spontaneous torpor ≥ 4 wk	> 3 prior torpor bouts

RESULTS

Figure 2: Rats do not survive 18 hrs after HS while AGS survive a minimum of 72 hrs afterward



*AGS were euthanized at the 72hr timepoint

Figure 3: AGS recover to normal behavior by 18 hrs after HS

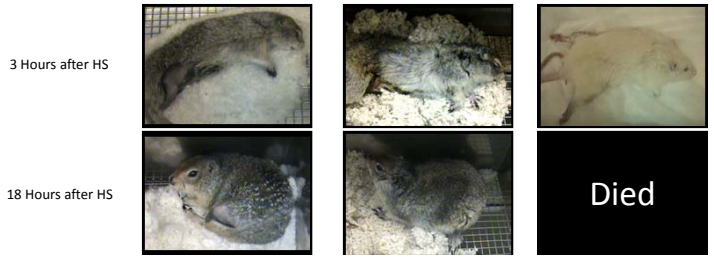
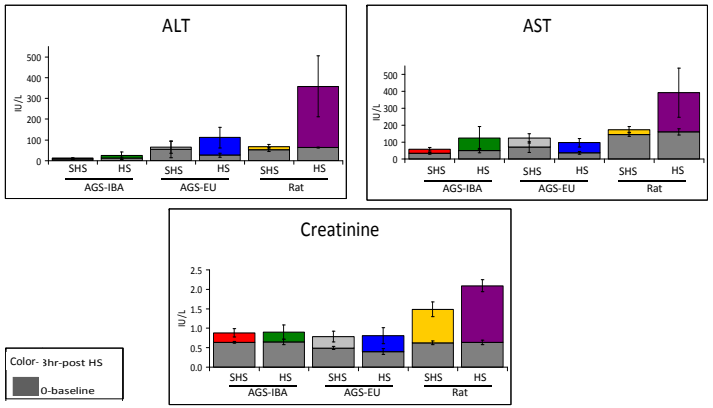
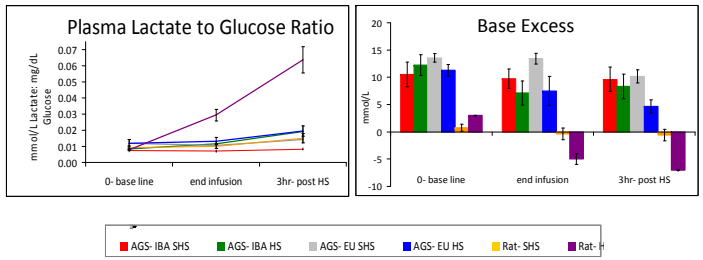


Figure 4: AGS do not show early indicators of organ damage in the kidney (creatinine) or liver (ALT, AST) regardless of hibernation season



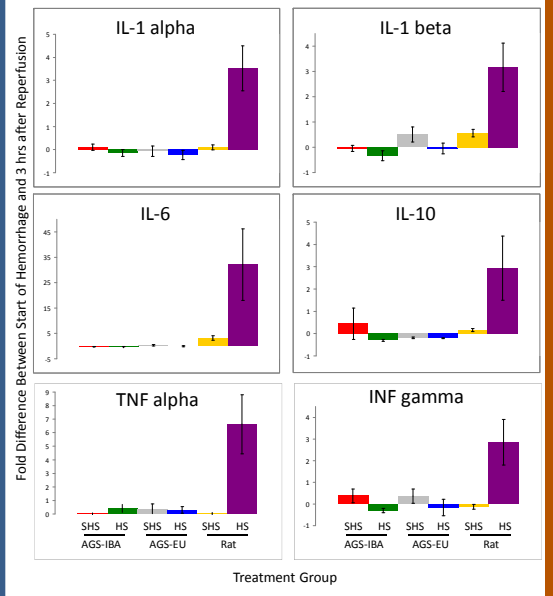
Data are expressed as Mean ± SEM. N ≥ 6 for all groups. SHS- Sham Hemorrhagic Shock, HS- Hemorrhagic Shock, IBA- Interbout Arousal, EU- Euthermic.

Figure 5: Metabolic shift indicated by an increase in lactate production and negative base excess occurs in rats during and after HS but not in AGS



Data are expressed as Mean ± SEM. N ≥ 6 for all groups. SHS- Sham Hemorrhagic Shock, HS- Hemorrhagic Shock, IBA- Interbout Arousal, EU- Euthermic.

Figure 6: Circulating plasma cytokine levels do not increase in AGS after HS



Data are expressed as Mean ± SEM. N ≥ 6 for all groups .
SHS- Sham Hemorrhagic Shock, HS- Hemorrhagic Shock, IBA- Interbout Arousal, EU- Euthermic.

CONCLUSIONS

Independent of hibernation season, AGS were resistant to HS induced I/R injury on the whole organism and tissue specific level.

Both euthermic and interbout arousal AGS

- can survive without apparent physiological deficit for 3 days after HS at euthermic body temperatures when blood loss corresponds to an MAP of 35 mmHG (~30% total blood volume)
- Maintain a high base excess during and after HS
- Do not show blood serum markers for organ damage
- Do not have a systemic inflammatory cytokine response after HS I/R injury

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