

EFFECTS OF ADENOSINE ON THE ORGAN INJURY AND DYSFUNCTION CAUSED BY HEMORRHAGIC SHOCK

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ABSTRACT

Objectives: Adenosine has been shown in animal and human studies to decrease the post-ischemic myocardial injury by lowering the levels of tumor necrosis factor- α . The objectives of the study was to examine the protective effects of adenosine on the organ injury (liver, kidney, pancreas) associated with hemorrhagic shock in rats.

Methodology: The study was conducted at Cardiovascular Physiology laboratory, King Saud University, Riyadh in 2007-2008. Anesthetized male Sprague- Dawley rats were assigned to hemorrhage and resuscitation treated with 20mM adenosine, untreated, or similar time matched control groups (n=6 per group). Rats were hemorrhaged for one hour using a reservoir model. Arterial blood pressure was monitored for one hour, and maintained at a mean arterial blood pressure of 40 mmHg. Adenosine 20mM was injected intra-arterially, before resuscitation in the adenosine treated group. Resuscitation was performed by reinfusion of the shed blood for 30 minutes. Arterial blood samples were analyzed for biochemical indicators of multiple organ injury: 1) liver function: aspartate aminotransferase (AST), alanine aminotransferase (ALT), 2) renal function: urea and creatinine, 3) pancreatic function: amylase.

Results: In the control group there was no significant rise in the serum levels of (i) urea and creatinine, (ii) aspartate aminotransferase (AST) and alanine aminotransferase (ALT), (iii) amylase. While in the adenosine treated group, resuscitation from one hour of hemorrhagic shock resulted in significant rises in the serum levels of (i) urea and creatinine, (ii) aspartate aminotransferase (AST) and alanine aminotransferase (ALT), (iii) amylase. Treatment of rats with 20mM adenosine before resuscitation following one hour of hemorrhagic shock decreased the multiple organ injury and dysfunction caused by hemorrhagic shock.

Conclusion: Adenosine attenuated the renal, liver and pancreatic injury caused by hemorrhagic shock and resuscitation in rats. Thus, the inflammatory response to shock may contribute to the multiple organ failure developed after hemorrhagic shock and resuscitation.

KEY WORDS: Adenosine, hemorrhagic shock, multiple organ failure, TNF- α , inflammation.

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INTRODUCTION

Multiple organ failure (MOF) is recognized as the leading cause of death following traumatic injury.¹⁻³ Most trauma deaths result from either insufficient tissue perfusion due to excessive blood loss, or the development of inflammation, infection and organ injury following resuscitation.⁴ In the past decade, there have been no effective interventions for post-injury

MOF.⁵ Despite the improvement in intensive care medicine, the mortality of hemorrhagic shock remains very high.⁶ Trauma victims who survive their initial injuries face a risk of death of multiple organ failure.⁷ Thus, there is still a great need for new approaches to improve therapy and outcome for patients with hemorrhagic shock.

The exact mechanism of MOF following hemorrhagic shock is unclear. Recent evidence suggest that the overproduction of pro-inflammatory cytokines may mediate the progression of shock to MOF and death.⁸⁻¹⁰ Many factors may be involved, as the marked increased production of oxygen free radicals which in turn triggers cytokines and TNF- α production and development of organ injury.^{10,11} However, little is known regarding the role of these radicals in hemorrhagic shock. Research is directed toward the protection and maintenance of liver, cardiac, intestinal and renal function following hemorrhagic shock.

There are multiple therapeutic interventions that lower the inflammatory response to shock. Adenosine, an endogenous nucleoside, possesses several properties that could be valuable in protection following hemorrhagic shock insult. Adenosine may redistribute blood flow and increase vital organ perfusion. Adenosine can decrease whole body and myocardial oxygen consumption¹² and improve recovery of energy metabolism after intestinal ischemia-reperfusion.¹³ Adenosine has been shown in animal and human studies to lower the post-ischemic myocardial injury.¹⁴⁻¹⁶ The exact mechanism is not known. However, it may be mediated via attenuating the inflammatory response to shock. Adenosine may produce anti-inflammatory effects by inhibiting neutrophil adhesion to endothelial cells and inhibiting neutrophil superoxide anion generation and degranulation.¹⁷ Moreover, adenosine reduces the release of pro-inflammatory cytokine.¹⁸ Despite the intensive research that has been done on the role of adenosine in protecting the heart following ischemic insults, little is known regarding the protective effects of adenosine following hemorrhagic shock and resuscitation.

The present study evaluated the effects of adenosine on the organ injury (liver dysfunction, kidney dysfunction and pancreatic dysfunction) caused by hemorrhagic shock and resuscitation in rats.

METHODOLOGY

The study was conducted at the Cardiovascular Physiology laboratory at King Khalid University Hospital, College of Medicine, King Saud University, Riyadh, Saudi Arabia in 2007-2008. Sixteen male Sprague Dawley rats weighing 400-500 gm were used. Rats were anesthetized with urethane (125mg/kg i.p.) and cannula was placed in the carotid artery for measurement of arterial blood pressure, for withdrawal of blood and resuscitation. Heparin sodium (2000 I.U.) was then injected intra-arterially. Rats were randomly assigned to 4 groups: (1) hemorrhagic shock untreated, (2) hemorrhagic shock treated with adenosine, (3) sham hemorrhage untreated and (4) sham hemorrhage treated with adenosine. After 15 minutes stabilization, rats were hemorrhaged using a reservoir (a 10 ml syringe). Blood was withdrawn from the carotid artery until MAP reached 35-40 mmHg, over a period of 60 minutes. At 90 minutes, rats were resuscitated by reinfusion of shed blood, together with saline when required to achieve normotension (80-110mmHg). If the blood pressure drop below 30 mmHg or failed to be restored to normal, experiments were excluded. The same surgical procedures were performed for the sham hemorrhage groups except that rats were not hemorrhaged.

In the treated groups, rats were injected with adenosine (20 μ M) intra-arterially via the carotid artery cannula before resuscitation, followed by 30 minutes resuscitation as described above.

After one hour of hemorrhagic shock and 30 minutes resuscitation, 1.5 ml of blood was collected into a serum gel S/1.3 tube (Sarstedt, Germany) from the catheter placed into the carotid artery. The blood sample was centrifuged (1610 \times g for 3 minutes at room temperature) to separate plasma. All plasma samples were analyzed within 24 hours at the central

Table-I: Serum levels of urea, creatinine, AST, ALT and amylase in rats resuscitated following 1 hour of hemorrhagic shock, treated with adenosine or sham controls.

Group	Sham Hemorrhage	Hemorrhage	Hemorrhage+Adenosine
<i>Serum levels expressed as means \pm SD</i>			
Urea	795.5 \pm 112.4	1221 \pm 50.9	930 \pm 134.4
Creatinine	70 \pm 5.7	121.5 \pm 7.8	74 \pm 15.6
AST	137.5 \pm 10.6	174 \pm 4.2	147.5 \pm 3.5
ALT	75.5 \pm 0.7	92 \pm 4.2	78.5 \pm 0.7
Amylase	795.5 \pm 112.4	940.5 \pm 64.3	930 \pm 134.4

Data expressed as means \pm SD.

Biochemistry laboratory at King Khalid University Hospital. The following marker enzymes were measured in the plasma as biochemical indicators of multiple organ injury/dysfunction: (1) Renal dysfunction was assessed by measuring the rises in plasma levels of creatinine (as an indicator of impaired renal function) and urea (as indicator of impaired excretory function of the kidney)¹⁹; (2) Liver function was assessed by measuring the rise in plasma levels of aspartate aminotransferase (AST, a non-specific marker for hepatic injury), alanine aminotransferase (ALT, as indicator of hepatic parenchymal injury)⁵; (3) Pancreatic injury was assessed by the rises in serum levels of lipase, a specific indicator for the development of pancreatic injury and (4) Neuromuscular injury was assessed by measuring the levels of creatinine kinase.

Statistical analysis: Data are presented as means and standard deviations. Data was analyzed with analysis of variance (ANOVA). Data were

considered statistically significant when yielding a *P*-value less than 0.05.

RESULTS

As previously reported, hemorrhagic shock induced acute renal injury as evidenced by the significant rise in the serum levels of urea and creatinine (Fig-1 and Table 1), compared to the sham controls. The protective effect of treatment with adenosine before resuscitation was evidenced by markedly lowering the serum levels of urea and creatinine in the hemorrhage treated group as compared to the hemorrhage untreated one (Figure-1).

As compared to sham hemorrhage group, resuscitation from one hour of hemorrhagic shock resulted in significant rise in the serum levels of AST and ALT compared to sham group, demonstrating the development of hepatocellular injury triggered by hemorrhage and resuscitation. Treatment with adenosine before resuscitation lowered the levels of AST

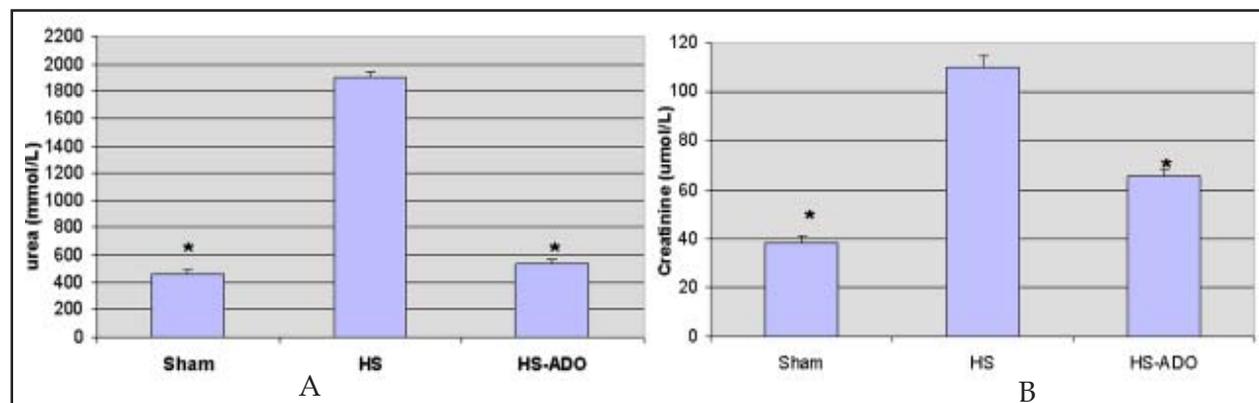


Figure-1: Serum levels of (A) urea and (B) creatinine in rats resuscitated following 1 hour hemorrhagic shock (HS), treated with adenosine (HS+ADO) or sham controls. **P*<0.05 when compared with HS.

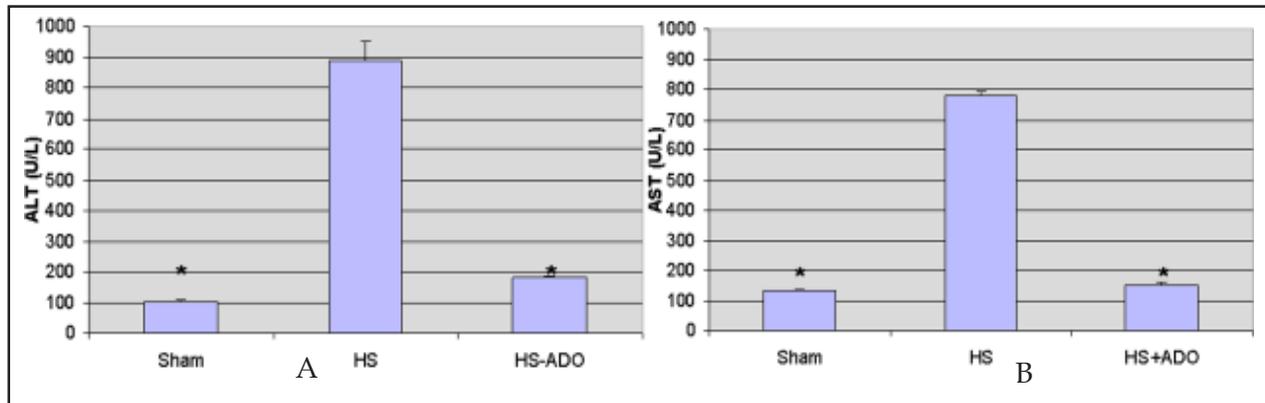


Figure 2: Serum levels of (A) AST and (B) ALT in rats resuscitated following 1 hour of hemorrhagic shock (HS), treated with adenosine (HA+ADO) or sham controls. * $P < 0.05$ when compared with HS

and ALT in the hemorrhage treated group (Figure-2).

Resuscitation following one hour of hemorrhagic shock did result in non-significant rises in the serum levels of amylase, demonstrating the possibility of pancreatic injury (Figure 3). Pretreatment of rats with adenosine non-significantly decreased the levels of amylase (Figure 3).

DISCUSSION

In this study, we have evaluated the effects of treatment with adenosine on multiple organ failure associated with hemorrhagic shock in rats. Hemorrhage for 60 minutes followed by resuscitation with shed blood for 30 minutes resulted in (i) liver dysfunction, (ii) renal dysfunction and (iii) pancreatic injury. We have previously confirmed that²⁰ the model of hemorrhagic shock used here results in myocardial contractile dysfunction and myocardial injury. We reported that treatment with $20\mu\text{M}$ adenosine (intra-arterially) before resuscitation of hemorrhagic shock protected the myocardium against post-resuscitation injury and dysfunction by improving the myocardial contractile function and preserving the myocardial structure.²¹ The present study showed that the treatment with $20\mu\text{M}$ adenosine (intra-arterially) before resuscitation of hemorrhagic shock abolishes (i) the liver injury, (ii) the renal dysfunction and (iii) the pancreatic injury caused by hemorrhage and resuscitation.

Hemorrhagic shock and resuscitation leads to injury to target organs including the heart, liver, brain and kidney.²² The progression of hemorrhagic shock to multiple organ failure is associated with an increase in mortality⁵ Hemorrhagic shock has been shown to result in an inflammatory response with the activation of neutrophils and the release of a number of inflammatory mediators.¹⁰

Adenosine has been shown to protect the organs following ischemic insults by an anti-inflammatory effects via inhibition of neutrophil infiltration to the myocardium and lowering the levels of the inflammatory mediators.²³ Another possibility could be due to the vasodilator effect of adenosine on the coronary bed as it will increase blood flow and enhance oxygen delivery to the organs.²⁴ Despite the research that has been done on the role of adenosine in protec-

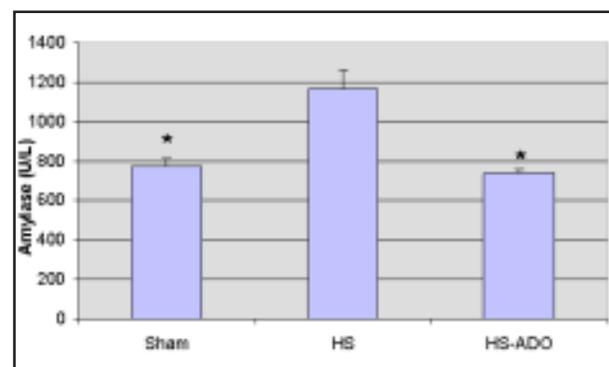


Figure-3: Serum levels of pancreatic amylase in rats resuscitated following 1 hour of hemorrhagic shock (HS), treated with adenosine (HA+ADO) or sham controls.

tion against post-ischemic insults, little is known about the role of adenosine in protection following hemorrhagic shock and resuscitation. In our study we have shown that adenosine protected the liver, kidney and pancreas against post-resuscitation dysfunction. Our results are consistent with the previous reports³ that hemorrhage and resuscitation leads to organ ischemia.^{2,22}

In conclusion, this study demonstrated that adenosine is protective of the renal, liver and pancreatic function if given before resuscitation of hemorrhagic shock. Our result is consistent with the previous hypothesis that hemorrhages and resuscitation leads to organ injury and dysfunction.

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