Original Article

MYOCARDIAL PROTECTION BY ETHYL-ISOPROPYL AMILORIDE, A SPECIFIC NA^+-H^+ EXCHANGE INHIBITOR, FOLLOWING HEMORRHAGIC SHOCK

Mona Soliman[1], Abdul-Majid Al-Drees[2]

ABSTRACT

Background: Hemorrhagic shock and resuscitation is well known to result in myocardial dysfunction and injury. Stimulation of the Na^+-H^+ exchanger plays an important role in the pathway of myocardial injury. The purpose of the present study was to examine the protective effects of blocking the cardiac Na^+-H^+ exchange, using 100mM ethyl-isopropyl amiloride (EIPA), a specific Na^+-H^+ exchanger blocker, on myocardial contractile function on *ex vivo* resuscitation of isolated rat heart following one hour of hemorrhagic shock.

Methodology: Sprague- Dawley rats were assigned to hemorrhage, hemorrhage + EIPA, sham hemorrhage and sham hemorrhage + EIPA groups. Rats were hemorrhaged for one hour. Hearts were harvested and *ex vivo* treated and resuscitated by perfused in the Langendorff System. Myocardial function was determined.

Results: The results showed that inhibition of the Na^+-H^+ exchanger using EIPA improved the post-resuscitation myocardial contractile function.

Conclusion: Blocking the Na^+-H^+ exchanger using 100mM EIPA following 60 minutes of hemorrhagic shock improved myocardial function.

KEY WORDS: Hemorrhage, Rat, Isolated heart, Contractility, Ethyl-Isopropyl Amiloride, Langendorff.

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1. Dr. Mona Soliman, MBBS, MSc, PhD
Assistant Professor
2. Dr. Abdul-Majid Al-Drees, PhD
Assistant Professor
1-2: Department of Physiology
College of Medicine
King Khalid University Hospital
Riyadh - Saudi Arabia.

Correspondence

Dr. Mona Soliman, MBBS, MSc, PhD
Assistant Professor, Dept. of Physiology,
College of Medicine,
King Khalid University Hospital,
P.O. Box 2925 (29),
Riyadh – 11461,
Saudi Arabia.
E-Mail: monaslmn@yahoo.com

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INTRODUCTION

Despite the intensive improvement in resuscitation strategies, trauma remains the most common killer in modern countries.[1] The exact mechanism of post-resuscitation myocardial dysfunction and failure is unclear. It is well known that hemorrhagic shock results in intracellular acidosis due to anaerobic metabolism. This will stimulate the Na^+-H^+ exchanger, which plays an important role in pH regulation,[2] causing an increase in intracellular Na^+ and a subsequent increase in intracellular Ca^{2+} via stimulation of the Na^+-Ca^{2+} exchanger. Calcium overload represents a major component of post-resuscitation contractile dysfunction and cell injury.[3] Inhibition of the Na^+-H^+ exchanger is associated with decrease tissue

contents of Na^+ and Ca^{2+}.[4,5] Moreover, inhibition of the Na^+-H^+ exchanger has been demonstrated to protect against ischemia-reperfusion injury and enhance contractile recovery.[6-9]

Despite the extensive research that has been done on the role of inhibition of the Na^+-H^+ exchanger in case of ischemia-reperfusion injury, its myocardial protective role following resuscitation of hemorrhagic shock has not been investigated. Our laboratory has previously shown the myocardial protective effects of treatment with amiloride, a non-specific Na^+-H^+ exchange blocker, before *ex vivo* as well as *in vivo* resuscitation of hemorrhagic shock in rats.[10] These results may be of great clinical importance as it may open a new field for treatment of trauma patients.

The aim of the present study was to assess the myocardial protective effects of using a more specific Na^+-H^+ exchange blocker, 100mM ethyl-isopropyl amiloride (EIPA),[11] on myocardial contractile function on *ex vivo* resuscitation of isolated rat hearts following one hour of hemorrhagic shock.

METHODOLOGY

Animal Preparation: Male Sprague- Dawley rats were injected intra-peritonealy (i.p.) with heparin sodium 2000 I.U 15 minutes prior to anesthesia. The rats were then anaesthetized using urethane 125mg/kg intra-peritoneal. The left carotid artery was cannulated using polyethylene tubing size 60, and was connected to an in-line pressure transducer for continuous blood pressure monitoring. Animals were allowed to stabilize for a period of 30 minutes. The animals were assigned randomly to hemorrhage, hemorrhage + EIPA, sham hemorrhage and sham hemorrhage + EIPA groups.

Experimental Protocol: Rats were hemorrhaged using a reservoir (a 10 ml syringe) that was connected to the carotid artery via a three way stopcock.[10] Blood was aspirated at a rate of 1 ml/min. Blood was continuously withdrawn or re-infused to the animal to maintain a mean arterial pressure of approximately 40 mmHg. The same surgical procedure was performed as for the sham hemorrhage groups except that rats were not hemorrhaged.

Ex Vivo Resuscitation of Isolated Hearts: After one hour of hemorrhagic shock, hearts were harvested and perfused *ex vivo* for 60 min using the Langendorff apparatus,[2] with Krebs-Henseleit- Bicarbonate (KHB) buffer consisting of the following (in mM): sodium chloride, 118; calcium chloride, 1.25; potassium chloride, 4.7; sodium bicarbonate, 21; magnesium sulphate, 1.2; glucose, 11; potassium biphosphate, 1.2; and EDTA, 0.5. In the treatment group, hearts were perfusion with 100 μM EIPA for 5 minutes, then shifted to perfusion with KHB for 55 minutes.

A saline- filled cellophane balloon-tipped catheter was placed into the left ventricle LV via the mitral valve and was used to measure LV pressure and balloon volume. The balloon was inflated by injecting 0.4-0.5 ml saline to adjust the LVEDP to 5 mmHg, then no further adjustments were made and LVEDP was recorded. Hearts were stimulated electrically at 5Hz using an electrical stimulator (6020 Stimulator from Harvard Apparatus). Perfusion rate was maintained at 10ml/min. Perfusate temperature was maintained at 37°C by using a thermocirculator. The perfusate was gassed with a mixture of 95% O_2 + 5% CO_2. The pH of the perfusate was adjusted at 7.4.

Statistical Analysis: All data were initially analyzed with Bartlett's test for homogeneity. Data were analyzed with multivariate analysis of variance (ANOVA). Means were analyzed using Duncan's test and were considered significant when yielding a "p" value less than 0.05. Data are expressed as means ± SEM.

RESULTS

Ex vivo resuscitation of isolated hearts following 60 min of hemorrhagic shock resulted in a significant impairment in the indices of cardiac performance and hemodynamic function. Treatment with EIPA before resuscitation significantly improved the post-resuscitation myocardial function as compared to the saline resuscitated group. Exposure to 60 minutes hemorrhage and *ex vivo* resuscitation led to decrease in the LV generated pressure (Fig-3) (n=6). Treatment with EIPA before *ex vivo*

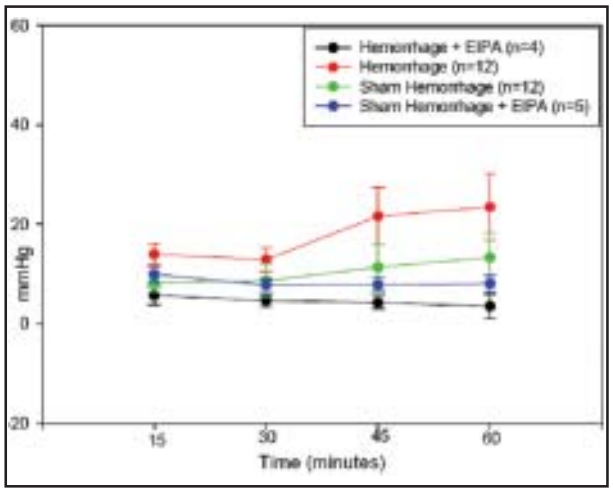

Fig-1: Left ventricular end-diastolic pressure of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour of ex vivo resuscitation.

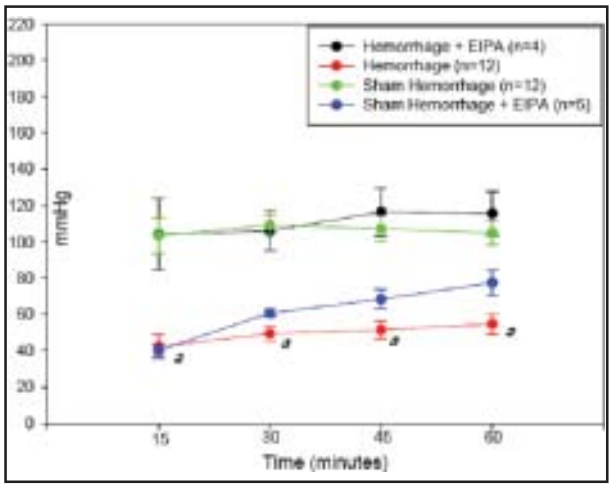

Fig-2: Left ventricular peak systolic pressure of the sham-hemorrhage treated and untreated, hemorrhage treated & untreated group over one hour ex vivo resuscitation. a=p<0.05 compared to hemorrhage + EIPA

resuscitation improved the post-resuscitation recovery of LV generated pressure. As shown in (Fig-1), EIPA attenuated the increase in LVEDP that occurred in the *ex vivo* untreated resuscitated hearts. EIPA markedly improved the hearts contractile function, and protected the heart against the post-resuscitation decrease in LV peak systolic pressure seen in the untreated group (Fig-3,4). Left ventricular maximum + dP/dt (Fig-4) was significantly lower in the hemorrhage group as compared to the sham hemorrhage group and the hemorrhage treated group. Maximum - dP/dt was significantly higher in the hemorrhage group (Fig-5), compared to the hemorrhage treated group (P<0.05).

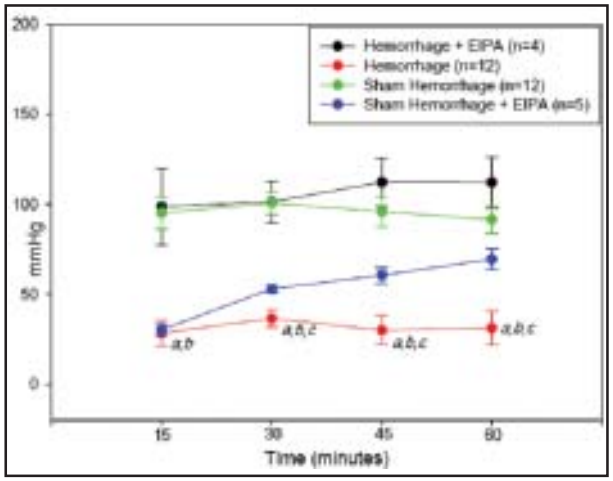

Fig-3: Left ventricular generated pressure of the sham-hemorrhage treated, untreated, hemorrhage treated & untreated group over one hour ex vivo resuscitation
a = p < 0.05 compared to hemorrhage + EIPA
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to sham hemorrhage + EIPA

DISCUSSION

In this study, we have investigated the myocardial protective effects of EIPA, a specific Na^+-H^+ exchange blocker, on the post-resuscitation myocardial injury in *the ex vivo* resusci-

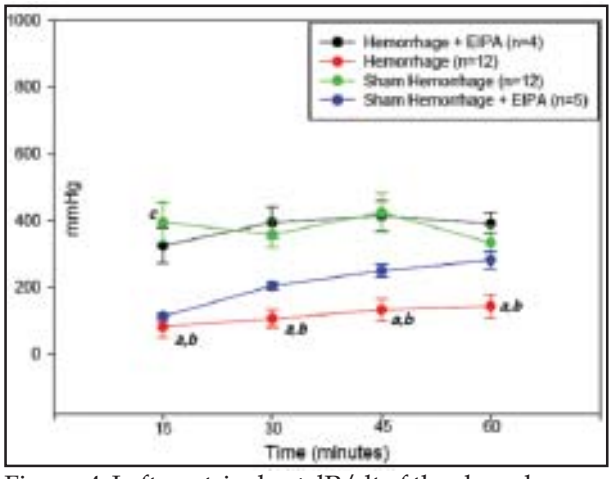

Figure-4: Left ventricular +dP/dt of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour ex vivo resuscitation.
a = p < 0.05 compared to hemorrhage + EIPA
b = p < 0.05 compared to sham hemorrhage
c = p < 0.05 compared to sham hemorrhage + EIPA

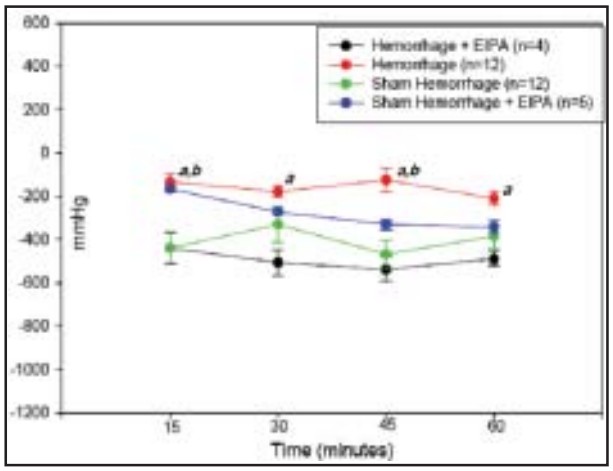

Figure-5: *Left ventricular –dP/dt* of the sham-hemorrhage treated and untreated, hemorrhage treated and untreated group over one hour *ex vivo* resuscitation
a = $p < 0.05$ compared to hemorrhage + EIPA
b = $p < 0.05$ compared to sham hemorrhage

tated rat hearts following 60 minutes of hemorrhagic shock. In isolated *ex vivo* resuscitated hearts, EIPA protected against the post- resuscitation myocardial injury, in terms of improvement of post-resuscitation recovery of LV function. Our results support the hypothesis that inhibiting the Na^+-H^+ exchanger, may exert cardioprotection by preventing the Na^+ and Ca^{2+} overload.[11] Recent evidence has suggested that the Na^+-Ca^{2+} exchange represents an important pathway to cause intracellular Ca^{2+} overload during myocardial and reperfusion.[12] Our laboratory has previously shown the cardioprotective effects of blocking the Na^+-H^+ exchanger, using a non-specific blocker, amiloride.[10]

Thus a pharmacologic intervention using Na^+-H^+ exchange blocker, may be beneficial in the protection against myocardial dysfunction in case of resuscitation of hemorrhagic shock. This finding may open a new strategy for treatment of trauma patients. Despite the extensive research that has been done in the resuscitation strategies,[1,13,14] trauma is still the leading cause of death in the developed countries due to multiple organ failure and myocardial dysfunction.[15]

In conclusion, our results suggest that EIPA is a potent and specific Na^+-H^+ exchange inhibitor, showing myocardial protective effect in the *ex vivo* resuscitated hearts following 60 minutes of hemorrhagic shock. Our finding support the hypothesis that the Na^+-H^+ exchange system is likely to play a major role in the pathophysiology of development of post-resuscitation myocardial injury.[16,17]

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