Insulin treatment before resuscitation following hemorrhagic shock improves cardiac contractility and protects the myocardium in the isolated rat heart

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ABSTRACT

Background: Insulin has been shown to exert positive inotropic effects in several in vivo ex vivo models and in human hearts. Resuscitation following hemorrhagic shock results in myocardial contractile dysfunction. However, the optimal timing for treatment with insulin for the cardioprotection effects is unclear. Objectives: The objective of this study was to test the hypothesis that treatment with insulin before resuscitation provides better cardioprotection than treatment with insulin after resuscitation. Materials and Methods: Rats were assigned to 3 experimental groups (n = 6 per group): (1) Hemorrhagic shock and resuscitation, (2) hemorrhagic shock resuscitated then treated with insulin and (3) hemorrhagic shock treated with insulin before resuscitation. Rats were hemorrhaged for 60 min to reach mean arterial blood pressure of 40 mmHg. Rats were resuscitated in vivo by reinfusion of the shedded blood to restore normotension and monitored for 60 min. Rats were treated or not with insulin 200 µU/g body weight intramuscularly either before or after resuscitation. The maximum of the left ventricular developed pressure (+dP/dt) was measured for 60 min in the isolated perfused hearts using the Langendorff method. Blood samples were obtained for measurements of tumor necrosis factor-alpha (TNF-α). Results: Treatment with insulin before resuscitation following hemorrhagic shock significantly elevated max dP/dt compared with insulin treatment after resuscitation and the untreated group. TNF-α levels were lower in the insulin treatment before resuscitation compared to the treatment after resuscitation and the untreated group. Conclusion: Insulin treatment before resuscitation following hemorrhagic shock provides better cardiac protection than treatment with insulin after resuscitation, as evidenced by the improved myocardial contractility, preservation of myocardial structure. The mechanism of cardiac protection involves decrease in the inflammatory response to shock by lowering the levels of TNF.

Key Words: Contractility, hemorrhage, insulin, resuscitation, shock

INTRODUCTION

The effects of insulin on the heart has been of great interest for researches with the recognition of the side-effects of hyperglycemia on heart resulting in decrease myocardial contractility, organ dysfunction, and mortality. Studies have shown the beneficial effects of insulin therapy in cardiac surgery patients. Several studies have shown that insulin therapy reduced the mortality in patients with acute myocardial infarction. Previous studies have demonstrated that insulin may play an important role in attenuating both myocardial ischemia and reperfusion injury.
The exact mechanism of cardioprotection by insulin is not fully understood. Studies have shown that the cardioprotective effects of insulin are mediated in part metabolically by altering the hyperglycemic effects on the heart. In addition, insulin exerts its cardioprotective effects by promoting cardiomyocyte survival pathway by activation of the phosphatidylinositol 3 kinase and its antiapoptotic effect. Another important mechanism of insulin cardioprotection is via reducing the oxidative stress by reducing the nitric oxide synthase (NOS) production. Data showed that insulin has anti-inflammatory effects and that glucose proinflammatory and pro-oxidant actions suppress the anti-inflammatory effects of insulin.

Hemorrhagic shock and resuscitation (HS-R) has been shown to result in myocardial contractile dysfunction, failure, and injury. The exact mechanism is not known. Activation of the inflammatory pathways by hemorrhage and resuscitation and the production of inflammatory mediators have been shown to result in myocardial injury. Another mechanism of cardiac injury following hemorrhagic shock is by the activation of NOS and the production of NO.

Timing of insulin therapy in relation to ischemia was found to be a crucial factor for its cardioprotective effects. It has been shown that insulin administration prior to ischemia provides better cardioprotection than insulin administered only at reperfusion in the isolated ischemic rat heart.

The aim of the present study was to test the hypothesis that treatment with insulin before resuscitation following hemorrhagic shock provides better cardioprotection than treatment with insulin after resuscitation in hemorrhagic shock rat models. The study measured the myocardial contractility and examined myocardial structure as well as the levels of tumor necrosis factor-alpha (TNF-α) for the possible involvement of inflammatory pathways.

MATERIALS AND METHODS

Animal preparation
The National Plan for Sciences and Technologies, King Saud University approved this study. Male Sprague-Dawley weighing 300-350 g were anesthetized using intraperitoneal injection of urethane (125 mg/kg). The rats were injected with heparin sodium intraperitoneally (2000 IU) 15 min prior to anesthesia to prevent coagulation of the blood in the isolated hearts and vasculature.

Experimental protocol
The animals were assigned randomly to the three experimental groups (n = 6 per group):
1. HS-R,
2. Hemorrhagic shock treated with insulin before resuscitation (HS-I-R) and
3. Hemorrhagic shock treated with insulin after resuscitation (HS-R-I) [Figure 1].

Hemorrhagic shock and resuscitation rat model
The left carotid artery was cannulated, and the mean arterial blood pressure (MABP) was monitored using a blood pressure transducer. The animals were allowed to stabilize for a period of 30 min. After stabilization, hemorrhagic shock was induced by aspiration of blood at a rate of 1 ml/min to reach MABP of approximately 35-40 mmHg using a reservoir (a 10 ml syringe). The rats were resuscitated in vivo by reinfusion of the shed blood to restore normotension, and the MABP was monitored for 60 min.

Measurement of myocardial contractile function in the isolated hearts
After 60 min hemorrhagic shock and resuscitated, with or without treatment, hearts were excised quickly and mounted on a cannula of the Langendorff apparatus. Retrograde perfusion was performed at a flow rate of 10 ml/min with Krebs Hanselte bicarbonate buffer (KHB, in mM: NaCl 118, CaCl$_2$ 1.25, KCl 4.7, NaHCO$_3$ 21, MgSO$_4$ 1.2, glucose 11, KH$_2$PO$_4$ 1.2, and ethylenediaminetetraacetic acid 0.5). Perfusate temperature was maintained at 37°C and was gassed with a mixture of 95% O$_2$ and 5% CO$_2$ at a pH of 7.4 as described previously.

Hearts were stimulated electrically at 5 Hz using an electrical stimulator (6020 stimulator from Harvard apparatus). Left ventricle pressure was measured by the use of a saline-filled cellophane balloon-tipped catheter which was placed into the left ventricle via the mitral valve and inflated to maintain an end diastolic pressure at 5 mmHg. Left ventricular maximum developed pressure (+dP/dt) was calculated by the acknowledge software as the first derivative of pressure over time. Data were collected and analyzed using the BIOPAC system and the acknowledge software.

Insulin treatment
In the preresuscitation insulin treated group, insulin 200 µU/g body weight in 1 ml saline was injected intramuscularly before resuscitation. The postresuscitation insulin group (HS-R-I), insulin was injected after resuscitation period of 60 min [Figure 1].

Measurement of tumor necrosis factor-alpha
Blood (0.5 ml) was collected from the left carotid artery cannula at the end of the experimental period harvesting the heart, and

![Figure 1: Experimental protocol](image)
centrifuged at 2500 g for 10 min and plasma was stored at −80°C until analysis for TNF-α measurement. Serum samples were analyzed by ELISA (R&D systems).

Statistical analysis
Data were presented as means ± standard deviation. Data were analyzed with one-way ANOVA. The values of \( P < 0.05 \) were considered significant. The Student’s \( t \)-test was used to compare mean values between the two experimental groups.

RESULTS
Baseline measurements are shown in Table 1. There was no significant difference in baseline measurements. Coronary perfusion pressure was comparable among all experimental groups [Table 1].

Left ventricular max dP/dt was significantly increased in the experimental group treated with insulin before resuscitation compared to the group treated after resuscitation. After resuscitation, left ventricular dP/dt was significantly lower compared to the resuscitated groups treated with insulin [Figure 2].

The TNF-α levels were significantly elevated in the HS-R untreated group compared to the HS-I-R and HS-R-I groups [Figure 3]. Treatment with insulin before resuscitation significantly lowered the levels of TNF-α compared to the group treated after resuscitation.

DISCUSSION
The present study showed that treatment with insulin before resuscitation following hemorrhagic shock improved cardiac contractility compared to treatment after resuscitation in HS-R rat model. Insulin has been shown to induce cardioprotection effects.\(^{28}\) The exact mechanism of the cardioprotection is not known. Insulin has been shown to have positive inotropic effects in animals and in human hearts.\(^{25,26}\) Several cardioprotection effects of insulin have been identified. Insulin has been shown to have antioxidant effects by preventing the formation of free radicals after myocardial ischemia-reperfusion in rats.\(^{27}\) Another extensively studied mechanism of insulin cardioprotection is by inhibiting apoptosis in the context of ischemia-reperfusion.\(^{28}\) The present study showed that the positive inotropic effect of insulin was mediated, in part, via the anti-inflammatory effect.

The present study demonstrated that the levels of TNF-α was elevated in the HS-R without treatment. Hemorrhagic shock has been shown to result in myocardial contractile dysfunction and injury.\(^{15,29}\) One of the possible mechanisms of cardiac injury following HS-R is by activation of the inflammatory pathways,\(^{30}\) and increasing the levels of inflammatory mediators, including TNF-α.\(^{31,32}\) Treatment with insulin before resuscitation significantly lowered the levels of TNF-α compared to the untreated group and even compared to treatment before resuscitation. Insulin is known to exert anti-inflammatory effects.\(^{33}\) Hyperglycemia is known to activate inflammatory pathways.\(^{33}\) In addition, insulin has been shown to have direct anti-inflammatory effects by inhibiting interleukin-6.\(^{34}\) Insulin has been also shown to inhibit the pro-inflammatory effects of TNF-α.\(^{35}\) Studies have shown that insulin lowered the levels of TNF-α and reduced apoptosis in cardiomyocyte preparation in...
ischemia-reperfusion. In human studies, insulin has been shown to inhibit TNF-α in human aortic endothelium.[36]

The optimal timing for insulin administration for heart cardioprotection remains unclear. Timing for insulin therapy is important in ischemia for its cardioprotective effects. Studies have shown that insulin treatment after ischemia was effective in reducing the infarct size in isolated rat heart of acute myocardial infarction.[15] In 2014, Sato et al. demonstrated that treatment with insulin prior to ischemia provides better cardioprotection than insulin administration only at reperfusion.[39] However, studies have demonstrated that insulin treatment before cardiopulmonary bypass (coronary bypass graft) is cardioprotective in human.[39] Several studies emphasize the importance of timing of insulin treatment in relation to myocardial ischemia.[10] However, the optimal timing for treatment with insulin in resuscitation of hemorrhagic shock is not known. The present study showed that treatment with insulin before resuscitation was significantly effective in cardioprotection than after resuscitation as demonstrated by the significant increase in myocardial contractility.

LIMITATIONS

One limitation of the present study is that blood glucose levels were not measured.

Future studies are needed to examine the effects of treatment with insulin on cardiac function and monitor the blood glucose changes.

CONCLUSION

Treatment with insulin before resuscitation following hemorrhagic shock provided better cardioprotection than insulin treatment after resuscitation. The present study suggested that one of the possible mechanisms of cardioprotection by insulin is by inhibiting the inflammatory pathways.

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