

Protective Effects of Estradiol on Myocardial Contractile Function Following Hemorrhagic Shock and Resuscitation in Rats

Mona Soliman

Department of Physiology, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia

Abstract

Background: Hemorrhagic shock (HS) results in myocardial contractile dysfunction. Studies showed that 17 β -estradiol protects the myocardium against contractile dysfunction. The study investigated the cardioprotective effects of treatment with 17 β -estradiol before resuscitation following 1 h of HS and resuscitation.

Methods: Male Sprague-Dawley rats were assigned to 2 sets of experimental protocols: *Ex vivo* and *in vivo* treatment and resuscitation. Each set had three experimental groups ($n = 6$ per group): Normotensive (N), HS and resuscitation (HS-R) and HS rats treated with 17 β -estradiol (E) and resuscitated (HS-E-R). Rats were hemorrhaged over 60-min to reach a mean arterial blood pressure of 40 mmHg. In the *ex vivo* group, hearts were resuscitated by perfusion in the Langendorff system. In the 17 β -estradiol treated group, 17 β -estradiol 280 μ g/kg was added for the first 5 min. Cardiac function was measured. Left ventricular generated pressure (LVGP) and +dP/dt were calculated. In the *in vivo* group, rats were treated with 17 β -estradiol 280 μ g/kg s.c. after 60-min HS. Resuscitation was performed *in vivo* by the reinfusion of the shed blood for 30-min to restore normotension.

Results: Treatment with 17 β -estradiol before resuscitation in *ex vivo* treated and resuscitated isolated hearts and in the *in vivo* treated and resuscitated rats following HS improved myocardial contractile function. In the *in vivo* treated group, LVGP and +dP/dt max were significantly higher in 17 β -estradiol treated rats compared to the untreated group (LVGP 136.40 \pm 6.61 compared to 47.58 \pm 17.55, and +dP/dt 661.85 \pm 49.88 compared to 88.18 \pm 0.85). Treatment with 17 β -estradiol improved LVGP following HS.

Conclusions: The results indicate that treatment with 17 β -estradiol before resuscitation following HS protects the myocardium against dysfunction.

Key words: Contractility; Heart; Hemorrhage; Rats; Resuscitation

INTRODUCTION

Hemorrhagic shock (HS) and trauma are one of the major causes of death.^[1] Despite the research that has been done to improve the resuscitation strategies, myocardial dysfunction and multiple organ injury remain one of the major causes of mortality and morbidity following HS and resuscitation.^[1-3] Resuscitation strategies are concerned to reestablish tissue perfusion, but they do not prevent multiple organ injury. The precise mechanism of multiple organ injury and failure is not well-established. Previous studies suggested the involvement of inflammatory pathways and the production of nitric oxide.^[4]

It is well-known that sex is a major determinant of the outcome of patients after trauma and sepsis.^[5,6] Estrogens, progesterone, and testosterone mediate a number of the differences in the response to trauma based on different sex.^[6] Studies have shown

that estradiol decreases lung injury after HS down-regulating the inflammatory mediator production.^[7] Estradiol exerts protective effects after ischemia reperfusion.^[8,9] Experimental studies have shown that female rats are resistant to organ injury after HS.^[10] In addition, intestinal blood flow is protected in female than in male rats after HS.^[11]

The present study examined the protective effects of treatment with estradiol before resuscitation on myocardial contractile function following HS in a rat model.

Address for correspondence: Dr. Mona Soliman,

Department of Physiology, College of Medicine, King Saud University,
P.O. Box: 2925 (29), Riyadh 11461, Saudi Arabia
E-Mail: monaslman@yahoo.com

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Received: 30-12-2014 **Edited by:** Xiu-Yuan Hao

How to cite this article: Soliman M. Protective Effects of Estradiol on Myocardial Contractile Function Following Hemorrhagic Shock and Resuscitation in Rats. Chin Med J 2015;128:2360-4.

Access this article online

Quick Response Code:



Website:
www.cmj.org

DOI:
10.4103/0366-6999.163390

METHODS

The Ethical Committee at the National Plan for Sciences and Technologies, King Saud University approved the study.

Preparation of animals

Male Sprague-Dawley rats were anesthetized by injecting urethane 125 mg/kg intraperitoneally (i.p.). Rats were injected i.p. with heparin sodium 2000 I.U. The left carotid artery was cauterized using polyethylene tubing size 60. For measurement of blood pressure, the left carotid artery was connected to the pressure transducer. Animals were left to stabilize for 30 min. The animals were assigned randomly to two experimental protocols: (a) HS followed by *ex vivo* treatment and resuscitation and (b) HS followed by *in vivo* treatment and resuscitation.

Hemorrhagic shock model

Hemorrhage was done by utilizing a 10-ml syringe that is attached to the three-way stopcock connected to the carotid artery. Hemorrhage was achieved by opening the stopcock and aspirating gradually with the syringe. Hemorrhage was done at a rate of 1 ml/min. Mean arterial blood pressure (MABP) was maintained at approximately 40 mmHg. Blood was withdrawn or re-infused to the animal to maintain the blood pressure. For the normotensive group, the same methods were done but the rats were be hemorrhaged.

Resuscitation was done *in vivo* by reinfusion of the shed blood after 60 min hemorrhage to restore blood pressure. Blood pressure was recorded for 30 min.

Perfusion of hearts outside the body

Hearts were excised and removed. Hearts were perfused outside the animal body (*ex vivo*). The Langendorff system was used for hemodynamic measurements. Hearts were perfused with normal physiological buffer Krebs-Henseleit-Bicarbonate (KHB) solution for 60 min.

Hearts were hanged by the aorta to the cannula in the Langendorff system. The hearts were perfused with KHB buffer consisting of the following (in mmol/L): Sodium chloride, 118; calcium chloride, 1.25; potassium chloride, 4.7; sodium bicarbonate, 21; magnesium sulfate, 1.2; glucose, 11; potassium biphosphate, 1.2; and ethylenediaminetetraacetic acid (EDTA), 0.5. An incision was made in the apex of the left ventricle using a No. 15 scalpel blade. A cellophane balloon filled with saline and attached to a catheter was inserted into the left ventricle. The catheter was placed through the mitral valve. The catheter was used to measure the left ventricular pressures by attaching the catheter to a transducer that is placed at the levels of the heart and aorta. Stimulation of the hearts was performed electrically at a rate of 300 beats/min using the electrical stimulator (6020 stimulator from Harvard Apparatus). The perfusion pressure (PP) was adjusted to be constant at 50 mmHg. The left end-diastolic pressure was adjusted and maintained at a pressure of 5 mmHg by injecting saline in the left ventricular balloon. The temperature of the perfusate fluid was adjusted and stays constant at 37°C. The perfusion fluid was oxygenated using a mixture of 95%

O₂ + 5% CO₂. The pH of the perfusion fluid was maintained at 7.4.

Study design

Ex vivo treatment and resuscitation

After 60-min HS, hearts were harvested and resuscitated *ex vivo* by perfusion using the Langendorff apparatus for 60-min [Figure 1]. In the treated group, hearts were perfused with 17β-estradiol for 5 min followed by 55 min of *ex vivo* resuscitation in the Langendorff system.

Hearts were excised quickly and rapidly placed onto a Langendorff apparatus, and perfused at a flow rate of 10 ml/min with KHB buffer (KHB, in mmol/L: NaCl 118, CaCl₂ 1.25, KCl 4.7, NaHCO₃ 21, MgSO₄ 1.2, glucose, 11, KH₂PO₄ 1.2, and EDTA 0.5). Perfusate temperature was maintained at 37°C and was gassed with a mixture of 95% O₂ and 5% CO₂ at a pH of 7.4 as described previously.^[12]

Ex vivo experimental groups

Three experimental groups (*n* = 6) were assigned:

1. Normotensive rats (*ex vivo*-N): The rats underwent the same surgical preparation, and continuous blood pressure measurements were obtained for the 60-min experimental period. Hearts were harvested and perfused *ex vivo* in the Langendorff system for 60 min with the physiological buffer.
2. Hemorrhagic shock rats (HS-*ex vivo* R): After a 30-min stabilization period, the rats were hemorrhaged to 40 mmHg for 60 min. Hearts were then harvested and resuscitated *ex vivo* in the Langendorff system for 60 min.
3. *Ex vivo* treatment with 17β-estradiol and resuscitation (HS-*ex vivo* E-R): After a 30-min stabilization period, the rats were hemorrhaged to 40 mmHg for 60 min. Hearts were then harvested and treated with 17β-estradiol *ex vivo* by perfusion for 5 min in the Langendorff system followed by *ex vivo* resuscitation for 55 min with KHB.

In vivo treatment and resuscitation

In the *in vivo* resuscitated groups, rats underwent the same surgical procedure and protocol for hemorrhage as described earlier. The rats were then resuscitated *in vivo* by reinfusion of the shed blood to restore normotension, and the MABP was monitored for 30 min. In the treated group, 17β-estradiol, 280 μg/kg s.c. injected after 60 min of HS. The rats were then resuscitated and monitored for 30 min. Hearts were then excised and perfused in the Langendorff system for measurement of myocardial contractile function, described later.

In vivo experimental groups

Experimental protocols

Three experimental groups (*n* = 6) were assigned:

1. Normotensive rats (*in vivo*-N): The rats underwent the same surgical preparation, and continuous blood pressure measurements were obtained for 60-min experimental HS period and 30 min of resuscitation, total of 90 min. Hearts were then excised and perfused in the Langendorff system for 60 min for measurement of myocardial function.

- Hemorrhagic shock and *in vivo* resuscitation (HS-*in vivo* R): After a 30-min stabilization period, the rats were hemorrhaged to 40 mmHg for 60 min. The rats were then resuscitated by reinfusion of the shed blood to restore normotension and monitored for 30 min. Hearts were then excised and perfused in the Langendorff system for 60 min for measurement of myocardial function.
- Treatment with 17 β -estradiol before *in vivo* resuscitation following HS (HS-*in vivo* E-R): After a 30-min stabilization period, the rats were hemorrhaged to 40 mmHg for 60 min. 17 β -estradiol 280 μ g/kg s.c. was injected after 60 min of HS. The rats were then resuscitated and monitored for 30 min. Same procedure was followed for measurement of myocardial function.

Mean arterial blood pressure

Mean arterial blood pressure was monitored for the 60-min duration of hemorrhagic shock and for 30 min after resuscitation in the *in vivo* treated group.

Hemodynamic measurements

Using the Langendorff apparatus, hemodynamic parameters were measured in the isolated hearts for 60 min. Left ventricular end-diastolic pressure, left ventricular peak systolic pressure, coronary PP was recorded. The left ventricular \pm dP/dt, which is the left ventricular index of contractility, was calculated.

Statistical analysis

Data were presented as mean \pm standard deviation (SD). Data were analyzed with one-way analysis of variance

(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

Mean arterial blood pressure

In the first experimental group, rats were hemorrhaged to 40 mmHg and MABP was monitored for 60 min. As shown in Figure 1a, there was no difference between the hemorrhage and the hemorrhage *ex vivo* treated groups. In the *in vivo* resuscitated group, rats were hemorrhaged to 40 mmHg and MABP was monitored for 60 min. The rats were either treated or not *in vivo* and resuscitated by reinfusion of the shed blood to restore normotension. MABP was monitored for additional 30 min. As shown in Figure 1b, there was no difference between the two hemorrhage groups treated or not. In Figure 1c, there was no difference in MABP between the treated and untreated resuscitated HS groups.

Myocardial contractile function

In this group, rats underwent the same surgical procedure but the rats were not hemorrhaged or resuscitated. There was no contractile dysfunction.

Ex vivo treatment with 17 β -estradiol and *ex vivo* resuscitation

Figure 2a shows the changes in the left ventricular generated pressure (LVGP) the end of the 1 h *ex vivo* resuscitation following HS. LVGP was significantly lower in the HS group compared to the normotensive group, while the hemorrhage treated group showed a significant increase in the LVGP

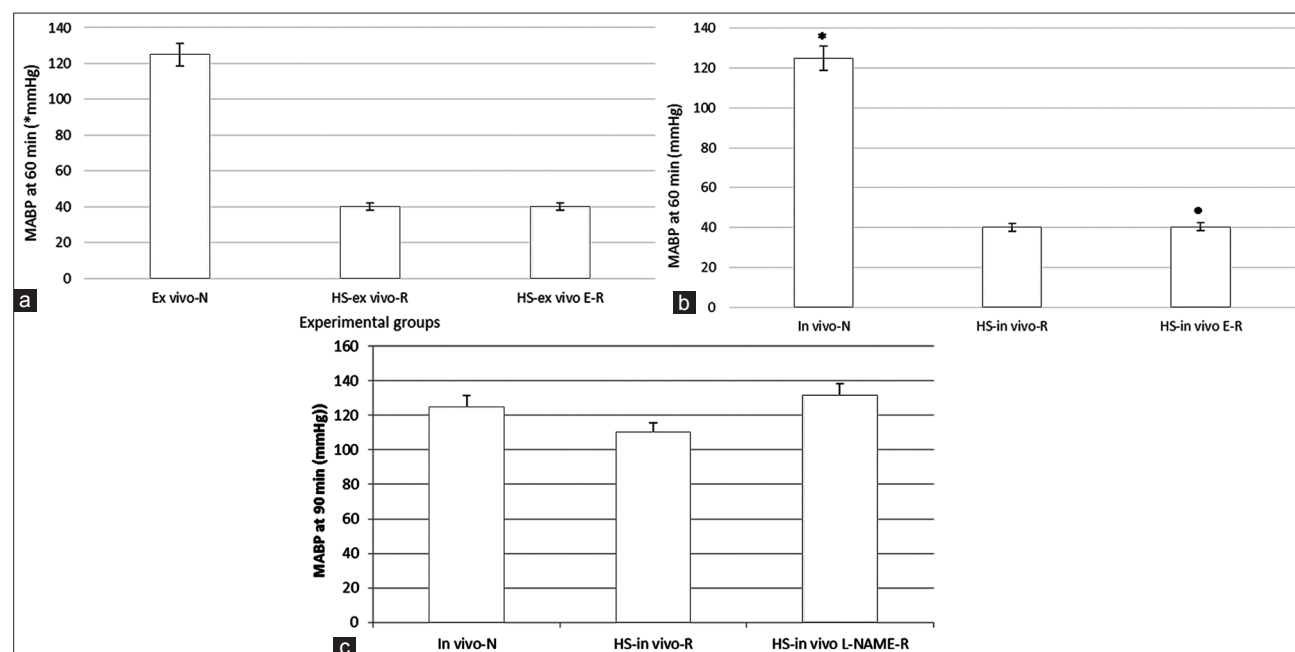


Figure 1: Recording of mean arterial blood pressure (MABP). (a) The *ex vivo* resuscitated group (MABP) after 60-min. (1) Normotensive rats (*ex vivo*-N), (2) Hemorrhagic shock (HS) rats (HS-*ex vivo* R), and (3) *ex vivo* treatment with 17 β -estradiol and resuscitation (HS-*ex vivo* E-R). (b) *In vivo* resuscitated group (MABP). (a) Recording of MABP for 60-min. (c) Recording of MABP for 60-min hemorrhage and 30-min resuscitation. (1) Normotensive rats (*in vivo*-N), (2) HS rats (HS-*in vivo* R), and (3) *in vivo* treatment with 17 β -estradiol and resuscitation (HS-*in vivo* E-R). All values are means \pm standard deviation. * $P < 0.05$ versus hemorrhagic shock group, * $P < 0.05$ versus HS group ($n = 6$ per group).

compared to the hemorrhage group ($P < 0.05$). Left ventricular maximum $+dP/dt$ [Figure 2b] was significantly lower in the hemorrhage group as compared to the normotensive group and significantly higher in the hemorrhage treated compared to the hemorrhage group ($P < 0.05$).

***In vivo* treatment with 17 β -estradiol and *in vivo* resuscitation**

Figure 3 shows the changes in the LVGP and $+dP/dt$ after 1 h of HS followed by *in vivo* resuscitation. LVGP was significantly lower in the HS group compared to the normotensive group ($P < 0.05$). The hemorrhage treated *in vivo* with 17 β -estradiol showed a significant increase in the LVGP compared to the hemorrhage group ($P < 0.05$). Left ventricular maximum $+dP/dt$ [Figure 3b] was significantly lower in the hemorrhage group as compared to the normotensive group and significantly higher in the hemorrhage treated compared to the hemorrhage group ($P < 0.05$).

DISCUSSION

Although previous studies have demonstrated that 17 β -estradiol has cardioprotective properties, the present data are the first data demonstrating that treatment with 17 β -estradiol before resuscitation following HS protect against myocardial contractile dysfunction. Treatment with 17 β -estradiol before resuscitation following HS improved myocardial contractile function and protect the myocardium against failure. The present results are in agreement with the study of Hsu *et al.*^[13] who demonstrated the cardioprotective effects of estradiol following hemorrhage. However, the present study is the first to demonstrate that treatment with 17 β -estradiol before resuscitation following HS protect the

myocardium against myocardial contractile dysfunction and failure in the *ex vivo* as well as the *in vivo* resuscitated rats.

The *ex vivo* resuscitated group, gives direct information about the cardioprotective effects of the treatment with estradiol outside the body, away from any endocrine, neural and other inflammatory pathways interference. While the *in vivo* treatment and resuscitation is important to prove the cardioprotective effects inside the body to confirm the protective effects with the interaction with other regulatory mechanisms that acts in response to hemorrhage and resuscitation.

Hemorrhagic shock is one of the major causes of death in trauma.^[14] Despite the improvement in resuscitation strategies, myocardial and multiple organ dysfunction and failure remain the major causes of mortality and morbidity after resuscitation following HS.^[15] Resuscitation fluids are concerned with the reestablishment of tissue perfusion; however, they do not protect against multiple organ injury and failure. The precise mechanism of multiple organ injury after resuscitation is not well-established. There are multiple mechanisms that contribute in the pathogenesis of cardiac dysfunction, including energy depletion, intracellular acidosis, and activation of inflammatory pathways and excessive production of nitric oxide. HS and resuscitation can produce inflammatory responses with the production of inflammatory mediators, which might be more harmful than the original hemorrhage^[16-18]

It is well-known that sex is a major determinant in the outcome of trauma patients.^[5,19] Clinical and experimental studies have shown that females are more tolerant to injuries than males.^[5,19,20] Evidence indicates that men and women have a different response to injury. Recent studies have

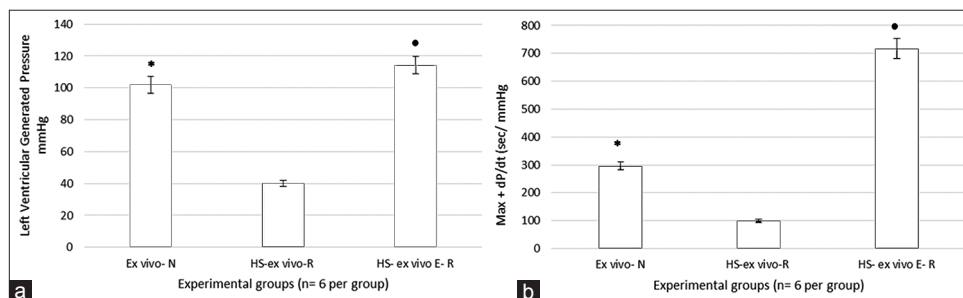


Figure 2: Myocardial contractile function in the *ex vivo* resuscitated group. (a) Left ventricular generated pressure. (b) Left ventricular $+dP/dt$. All values are means \pm standard deviation. * $P < 0.05$ versus hemorrhagic shock (HS) group, * $P < 0.05$ versus HS group ($n = 6$ per group).

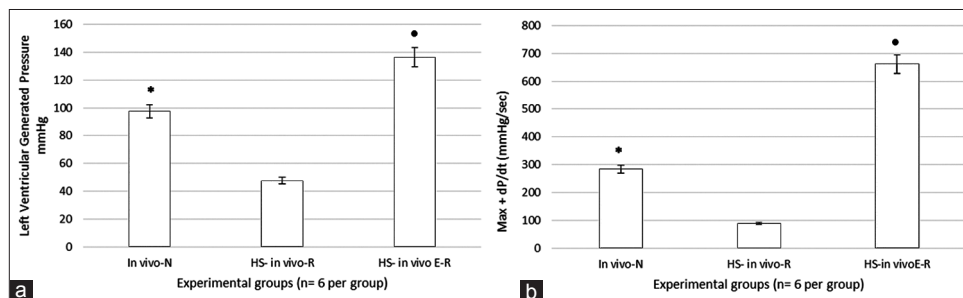


Figure 3: Myocardial contractile function in the *in vivo* resuscitated group. (a) Left ventricular generated pressure (LVGP). (b) Left ventricular $+dP/dt$. All values are means \pm standard deviation (SD). In (a and b), LVGP and $+dP/dt_{max}$ was significantly improved in estradiol-treated rats compared to untreated. All values are means \pm SD. * $P < 0.05$ versus hemorrhagic shock (HS) group, * $P < 0.05$ versus HS group ($n = 6$ per group).

shown that there are differences in immune responses to diseases depending on the gender difference.^[6] Hormones are one of the causes of these different responses in male and female. Experimental studies have shown that sex hormones modulate acute lung injury caused by ischemia-reperfusion.^[6] Estradiol decreases acute lung injury caused by HS in male rats and downregulates the inflammatory mediators.^[7] Furthermore, intestinal blood flow is protected in female than male rats following HS and that the gut of female is more resistant than that of the male to damaging effects of the ischemic injury.^[10] Evidence showed that estradiol may play a protective role against multiple organ injury and dysfunction following HS. The present study demonstrated the cardioprotective effects of administration of estradiol before resuscitation in HS model.

Sex-hormones actions are mediated by their receptors. The mechanism by which estradiol protects against organ dysfunction following ischemia reperfusion is not known. One suggested mechanism is by decreasing the inflammatory response. Multiple organ dysfunction and failure as a complication to systemic inflammatory responses are the major cause of mortality and morbidity following trauma.^[21] The mechanism underlying these actions on the myocardial injury after HS and resuscitation are not totally understood. Future studies are needed to investigate the involvement of inflammatory pathways in the mechanism of cardioprotection effects of estradiol. The results of the present study might be a new therapeutic tool for the management of trauma patients that will help to improve the outcomes after HS and resuscitation.

ACKNOWLEDGMENT

Technical help from Mr. Sabirine Sahiba is acknowledged.

Financial support and sponsorship

This work was supported by a grant from the Research Groups at Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia.

Conflicts of interest

There are no conflicts of interest.

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