

Protective Role of Selective Nitric Oxide Synthase Inhibitor for Treatment of Decompensated Hemorrhagic Shock in Normotensive and Hypertensive Rats

Majid Khazaei, Babak Barmaki, Ali Nasimi

Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:

Associate Prof. Majid Khazaei,
Associate Professor of Physiology,
Department of Physiology, Isfahan
University of Medical Sciences,
Hezarjarib Ave, Isfahan, Iran.
E-mail: khazaei@med.mui.ac.ir

Date of Submission: Aug 14, 2011

Date of Acceptance: Sep 18, 2011

How to cite this article: Khazaei M, Barmaki B, Nasimi A. Protective Role of Selective Nitric Oxide Synthase Inhibitor for Treatment of Decompensated Hemorrhagic Shock in Normotensive and Hypertensive Rats. *Int J Prev Med* 2012;3:47-53.

ABSTRACT

Introduction: Different vasoactive factors can modulate cardiovascular adaptation to hemorrhagic shock including Nitric Oxide (NO). In this study we investigated the effect of the NO synthase inhibitor for treatment of decompensated hemorrhagic shock in normotensive and hypertensive rats.

Methods: Twenty-four male Wistar rats were divided into two groups: The normotensive and hypertensive groups. Hypertension was induced by the DOCA-Salt method for eight weeks. Then, the animals were given hemorrhagic shock by continuously withdrawing blood until the mean arterial pressure (MAP) reached to 40 mmHg. The animals were maintained in the shock state for 120 minutes. Subsequently, they were randomly assigned to L-NAME-treated and non-treated groups and monitored for 60 minutes. The survival time was recorded. Blood samples were taken before and after the shock and 60 minutes after L-NAME administration.

Results: Infusion of L-NAME caused a significant increase in MAP in normotensive animals, however, slightly increased MAP in hypertensive animals. The heart rate did not significantly alter. Hemorrhage caused a marked increase in serum nitrite levels in both groups ($P < 0.05$). L-NAME treatment significantly reduced the serum nitrite concentration in the normotensive group ($P < 0.05$), without any change in the hypertensive group. All animals who received L-NAME treatment survived at the end of experiment. Fifty percent of the hypertensive animals died four hours after the experiment. The 72-hour survival rate was similar in the L-NAME treated groups.

Conclusion: L-NAME infusion during decompensated hemorrhagic shock plays a protective role in the improvement of hemodynamic responses and short-term survival rate in normotensive animals.

Keywords: Hypertension, hemorrhagic shock, nitric oxide

INTRODUCTION

Shock is a condition in which tissue perfusion is inadequate to

maintain the normal oxygen demand.^[1] Cardiovascular adaptation during blood loss is under the control of the sympathetic and parasympathetic divisions.^[2] It is indicated that blood pressure decreases and heart rate increases during blood loss.^[3] Hypotension during hemorrhage is associated with complications such as multiple organ failure, including brain ischemia and the development of secondary infection such as sepsis.^[4] In the decompensation phase, hemorrhagic shock is characterized by a decreased response to vasopressors and hypoperfusion to the peripheral tissues.^[3] Such alterations can result in dysfunction of the vital organs and account for high mortality rates.

Different vasoactive factors can modulate cardiovascular adaptation to hemorrhagic shock such as Nitric Oxide (NO).^[5-8] NO has a key role in cardiovascular homeostasis, including hemorrhagic shock.^[9] Increased free radical oxygen during tissue ischemia in hemorrhagic shock may lead to reduced serum NO level.^[10] On the other hand, increased serum NO concentration has been documented during hypovolemia and cerebral ischemia.^[11] Excessive generation of NO may reduce vascular reactivity to vasoconstrictive factors, such as angiotensin II, during the decompensation phase of hemorrhagic shock^[5-7,12] and reduce hemorrhage-induced tachycardia.^[13] Vascular hyporeactivity may be responsible for the low response of patients with hemorrhagic shock to vasoconstrictive substances.^[7,12]

Clinical studies showed that the mortality rate during blood loss in hypertensive subjects is higher than in the normotensive group.^[14,15] Hypertension is associated with several cardiovascular abnormalities, including endothelial dysfunction.^[16] NO is one of the most important endothelium-derived relaxing factors. There are three isoforms of NO synthase that produce NO from L-arginine. In this study, we aim to investigate the effect of the non-selective NO synthase inhibitor, for treatment of decompensated hemorrhagic shock in normotensive and hypertensive rats.

METHODS

Animals

Experiments were performed on male Wistar rats (Pasteur Institute of Iran) weighing 300–400 g at the time of hemorrhagic shock. The rats were housed, two or three per cage, in the animal room, with a 12-hour light–dark cycle, 20–25°C temperature,

60–70% humidity, and standard rat chow and water *ad libitum*. All experiments were approved by the University Ethical Committee on Animal Research.

Induction of hypertension

Hypertension was induced by the Deoxycorticosterone Acetate (DOCA)-salt method, as previously described.^[17] In brief, the animals were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (7.5 mg/kg). Following this, all the animals were uninephrectomized and the animals in the hypertensive group received a DOCA injection of 30 mg/kg (Aboureihan Co.) subcutaneously, twice a week, for eight weeks. They also received NaCl 1% solution instead of tap water for drinking. The normotensive group received tap water and injection of the solvent of DOCA throughout the study. Systolic blood pressure was recorded every week by the tail cuff pressure.

Hemorrhagic shock model

The animals were fasted for 12 hours, but had free access to water before the experiment. Following this, they were anesthetized by ketamine and xylazine and maintained under anesthesia during the study. The body temperature was maintained around 37°C using a heating pad and monitored by a rectal thermometer. The right femoral artery and vein were cannulated by PE-50 catheters for blood withdrawal and drug administration, respectively. The left femoral artery was cannulated and connected to a physiograph for monitoring of Mean Arterial Pressure (MAP) and Heart Rate (HR) during the experiment.

After a stabilizing period of 30 minutes, decompensated hemorrhagic shock was induced by continuously withdrawing blood using a 1 ml syringe containing 50 IU heparin at a rate of 1 ml per four to five minutes until the MAP reached 40 mmHg, during the total time of 20 minutes.^[18] The animals were maintained in the shock state for the next 120 minutes by withdrawing or re-infusing the shed blood, as necessary, to maintain the MAP at around 40 mmHg.

After the shock period, the normotensive and hypertensive animals were randomly assigned to L-NAME-treated and non-treated groups. L-NAME (10 mg/kg; Sigma Co.)^[19] was dissolved in normal saline (1 ml/kg) and infused through

the femoral vein catheter for 10 minutes. The non-treated groups received normal saline with an equal volume.

Survival time

The MAP and HR were monitored 60 minutes after L-NAME administration, after which the catheters were removed and incisions sutured. After recovering from the anesthesia, the animals were returned to the cages with free access to water and food. The survival time was recorded at the first four hours and every 12 hours up to 72 hours.

Serum nitrite measurement

Blood samples were taken before and after shock and 60 minutes after L-NAME infusion. The blood was centrifuged at 3000 rpm for 20 minutes and serums were kept in separate Eppendorf tubes at -70°C for further analysis. Serum nitrite level was measured using the Griess reagent method, as previously described,^[20] with a detection limit of 2.5 μmol .

Statistical analysis

The results were expressed as mean \pm SE. One-way ANOVA with post hoc test LSD was used to compare the differences between the groups. Student *t*-test was performed to compare the data between the two groups. Survival rate was evaluated with the Chi-square test. *P* less than 0.05 was considered statistically significant.

RESULTS

Changes in hemodynamic parameters

Baseline systolic blood pressure and MAP in the DOCA-Salt hypertensive rats were significantly higher than in the normotensive group (systolic blood pressure: 151.56 ± 5.60 vs. 109.1 ± 6.56 ; MAP: 119.30 ± 4.34 vs. 82.87 ± 3.33), however, the baseline HR measurement showed no significant differences between the groups. Figure 1 illustrates the time course of MAP [Figure 1a] and HR [Figure 1b] during hemorrhagic shock. MAP was maintained around 40 mmHg during the 120 minute shock period. Decompensated hemorrhage caused significant bradycardia five minutes following the bleeding. Then the HR gradually increased during the shock period ($P < 0.05$ vs. basal value), however, there were no significant differences between the groups.

Infusion of L-NAME caused a significant increase of MAP in the normotensive animals, which was significantly different compared to the non-treated group [Figure 2a]. In hypertensive animals, infusion of L-NAME slightly increased the MAP, without significant differences in the non-treated animals [Figure 2b]. HR did not significantly alter after L-NAME infusion in the normotensive and hypertensive groups [Figures 2c and d].

Serum nitrite concentration

Figure 3 illustrates changes in serum nitrite concentrations during the experiment. In the basal state (before shock induction), serum nitrite concentration in normotensive animals was higher than in the hypertensive group (3.97 ± 0.24 versus $2.30 \pm 0.17 \mu\text{mol/l}$; $P < 0.05$). Hemorrhage caused a marked increase in serum nitrite levels in both groups ($P < 0.05$). The L-NAME treatment significantly reduced serum nitrite concentration in normotensive animals ($P < 0.05$), without any change in the hypertensive group.

Survival assay

All animals who received L-NAME treatment survived at the end of experiment. Three of six hypertensive animals (50%) died four hours after the experiment, while, all normotensive animals were alive during this time. After this time, significant differences in survival rate were not observed between hypertensive and normotensive animals [Figure 4].

DISCUSSION

Studies indicated that excessive NO formation is associated with vascular hyporeactivity during blood loss,^[6,9,21] however, the exact role and mechanisms by which NO regulates hemodynamic response is not clear.

In the present study, hemorrhage reduced MAP for five minutes after bleeding and the HR gradually increased in the normotensive and hypertensive groups. It is known that hemorrhage causes a slight increase in HR, which is followed by bradycardia.^[22] In this study we used the DOCA-Salt hypertensive model, which is a known model of moderate hypertension. Hypertension is associated with several vascular abnormalities including endothelial dysfunction.^[16] In the present study, we found that at the basal state, hypertensive

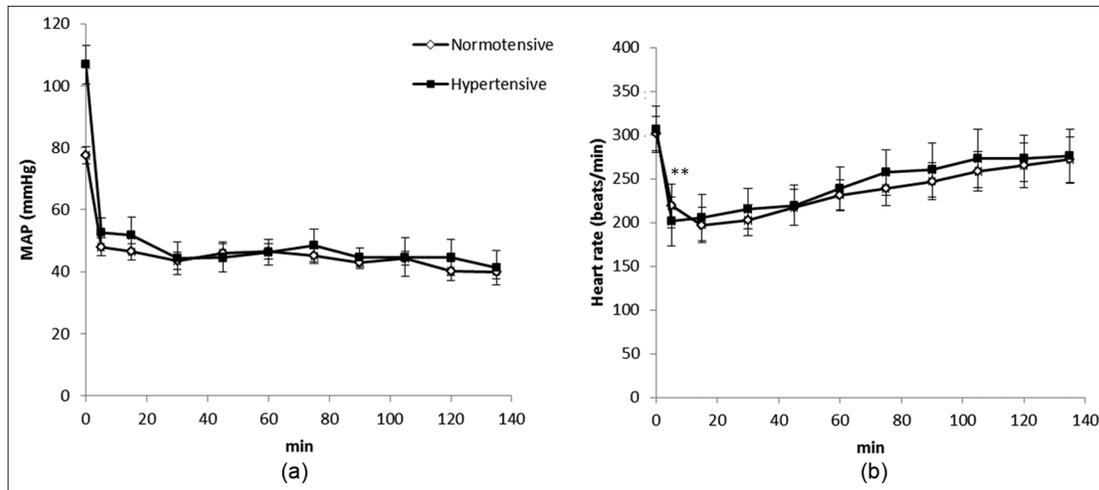


Figure 1: Changes in the mean arterial pressure (MAP) (a) and heart rate (HR) (b) of normotensive and hypertensive rats, during the shock period. * $P < 0.05$ compared to the normotensive group. ** $P < 0.05$ compared to before experiment (0 minute)

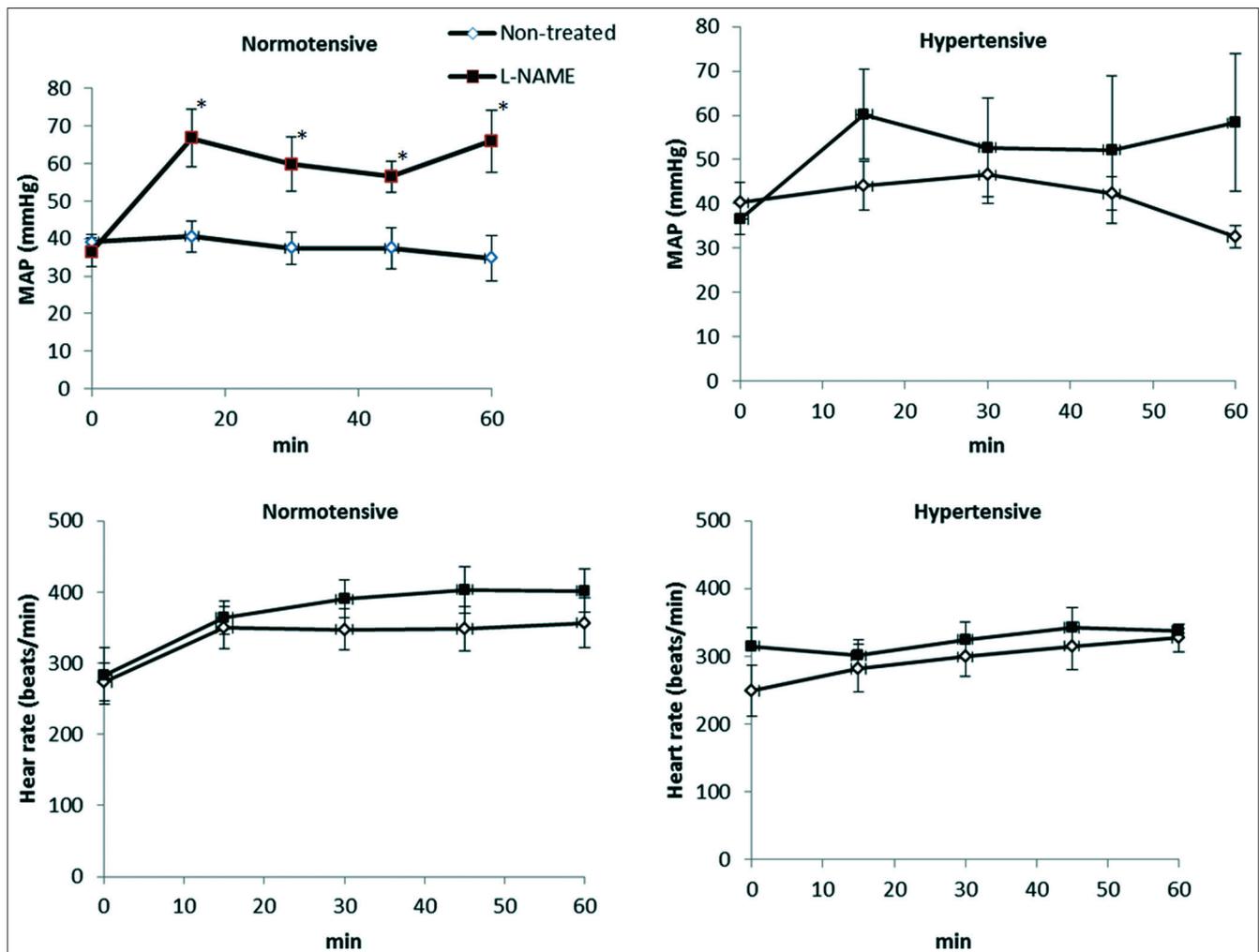


Figure 2: Comparison of mean arterial pressure (MAP) and heart rate (HR) between L-NAME treated and non-treated groups, in normotensive and hypertensive animals. * $P < 0.05$ compared to the non-treated group

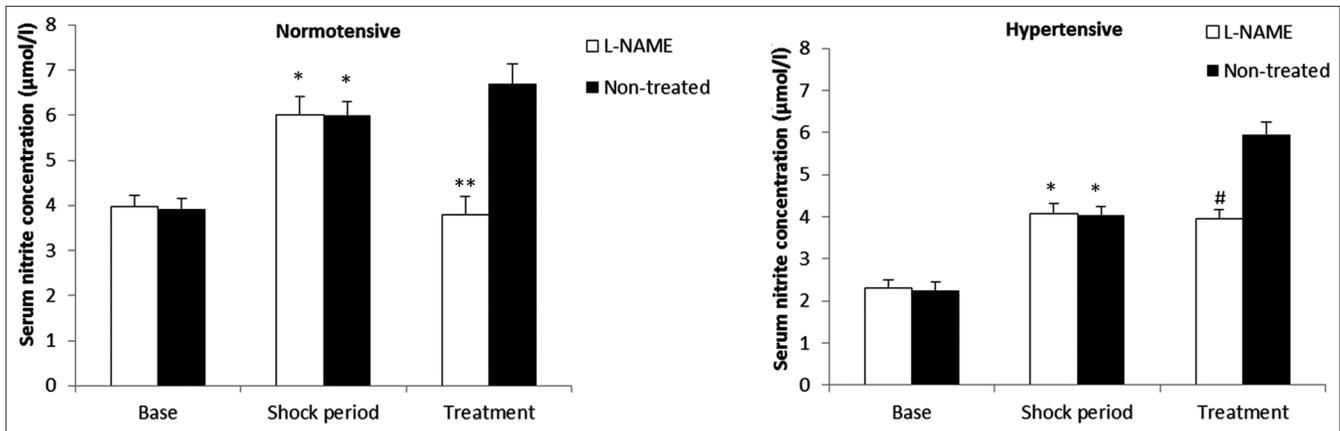


Figure 3: Comparison of serum nitrite levels at different periods in the normotensive and hypertensive groups. *Indicates significant difference compared to base; **indicates significant differences compared to shock period and non-treated group. # $P < 0.05$ compared to the non-treated group

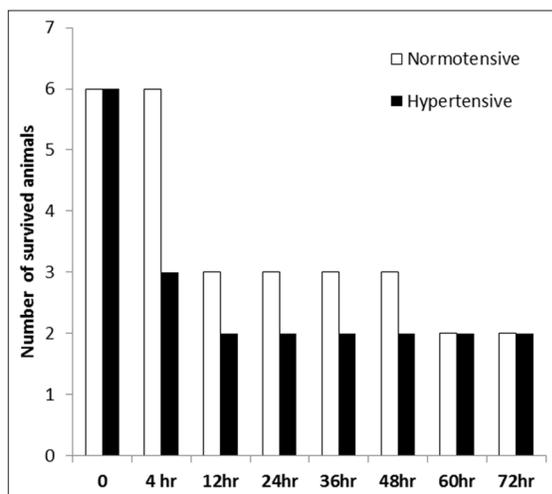


Figure 4: Comparison of survival rate of L-NAME treated normotensive and hypertensive rats

animals had lower serum nitrite concentration than the normotensive group. Nitrite is the stable end-product of NO, which reflects the amount of NO production.^[18] Reduced NO bioavailability in hypertensive subjects has been documented in previous studies,^[10,23,24] and different mechanisms have been suggested for reduced availability of NO during hypertension, including the generation of reactive oxygen species, impaired l-arginine uptake,^[23] or reduced NOS expression.^[24]

During the shock period, we have found elevated serum nitrite concentrations in normotensive and hypertensive animals. NO can produce, by NO synthase, a specifically inducible form (iNOS) during shock and plays a key role in the pathogenesis of various shocks.^[6,9,21,25] Activation

of NO synthase^[26] and upregulation of the inducible form of NO synthase expression^[27] has been documented during shock. Daughter *et al.* also found that NO increases during hemorrhagic shock and contributes to hypotension.^[28]

After L-NAME infusion, we found that MAP increased in normotensive animals, however, did not alter in the hypertensive group. In addition, L-NAME slightly increased the HR in both groups. In contrast to our results, Koch *et al.*, found that L-NAME infusion induced an increase in HR during hemorrhage,^[29] however, Balaszczuk *et al.* demonstrated that L-NAME infusion recovered blood pressure and HR in hemorrhagic shock rats compared to the non-treated group.^[9] Other studies indicated that inhibition of NO attenuates hemorrhage-induced tachycardia.^[30] In the present study, we found a slight increase in HR after L-NAME treatment, which supported the other studies.^[9] Our findings suggest that NO does not play a key role in the regulation of HR during hemorrhage.

We also found that the survival rate in normotensive animals that received L-NAME treatment was higher than in the hypertensive group in the first four hours after shock induction. Low survival rate of hypertensive subjects during hemorrhage has been documented in previous studies.^[31] It is indicated that hypertensive subjects have a defect in the baroreflex response.^[31] Moreover, tissue ischemia and organ failure in the hypertensive animals was more than in the normotensive group at the same blood pressure, after hemorrhagic

shock, which may explain the higher mortality in hypertensive animals.^[31] In contrast to our results, a recent study has found that exogenous NO has a protective role during hemorrhagic shock.^[32] However, other studies have demonstrated that excessive NO production during hemorrhage may lead to organ damage and vascular hyporeactivity to vasoconstrictors,^[5-7,12] which contribute to a low survival rate during severe hemorrhagic shock. In this study, normotensive animals had a higher serum NO level than the hypertensive groups, thus, we can conclude that L-NAME treatment had a greater effect in reducing NO production and improving the survival rate in normotensive animals.

CONCLUSION

L-NAME infusion during decompensated hemorrhagic shock plays a protective role in improving blood pressure and short-term survival rate in normotensive animals.

ACKNOWLEDGMENT

This study was supported by a grant from the Isfahan University of Medical Sciences (grant number # 387413).

REFERENCES

1. Revell M, Greaves I, Porter K. Endpoints for fluid resuscitation in hemorrhagic shock. *J Trauma* 2003; 54: S63-7.
2. Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. *Am J Physiol* 1991; 260: H305-18.
3. Dutton RP. Current concepts in hemorrhagic shock. *Anesthesiol Clin* 2007; 25: 23-34.
4. Durham RM, Moran JJ, Mazuski JE, Shapiro MJ, Baue AE, Flint LM. Multiple organ failure in trauma patients. *J Trauma* 2003; 55: 608-16.
5. Li S, Fan SX, Mckenna TM. Role of nitric oxide in sepsis-induced hyporeactivity in isolated rat lungs. *Shock*. 1996; 5: 122-9.
6. Liu LM, Dubick MA. Hemorrhagic shock-induced vascular hyporeactivity in the rat: relationship to gene expression of nitric oxide synthase, endothelin-1, and select cytokines in corresponding organs. *J Surg Res* 2005; 125: 128-36.
7. Sato S, Suzuki A, Nakajima Y, Iwamoto T, Bito H, Miyabe M. S-Nitroso-N-Acetylpenicillamine (SNAP) during hemorrhagic shock improves mortality as a result of recovery from vascular hyporeactivity. *Anesth Analg* 2000; 90: 362-8.
8. Lange M, Enkhbaatar P, Nakano Y, Traber DL. Role of nitric oxide in shock: The large animal perspective. *Front Biosci* 2009; 14: 1979-89.
9. Balaszczuk AM, Arreche ND, Mc Laughlin M, Arranz C, Fellet AL. Nitric oxide synthases are involved in the modulation of cardiovascular adaptation in hemorrhaged rats. *Vascul Pharmacol* 2006; 44: 417-26.
10. Forstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch* 2010; 459: 923-39.
11. Jiang MH, Kaku T, Hada J, Hayashi Y. Different effects of enos and nnos inhibition on transient forebrain ischemia. *Brain Res* 2002; 946: 139-47.
12. Bucher M, Hobbhahn J, Kurtz A. Nitric oxide-dependent down-regulation of angiotensin ii type 2 receptors during experimental sepsis. *Crit Care Med* 2001; 29: 1750-5.
13. Ajayi AAL, Hercule HC, Pamugo J, Dixon D, Oyekan AO. Interactions of the renin-angiotensin system and alpha-1 adrenoceptors on renal hemodynamics in healthy and acute renal failure rats: The role of nitric oxide. *Blood Press* 2001; 10: 238-46.
14. Sinert R, Guerrero P, Quintana E, Zehtabchi S, Kim CN, Agbemadzo A, *et al.* The effect of hypertension on the response to blood loss in a rodent model. *Acad Emerg Med* 2000; 7: 318-26.
15. Radisavljevic Z. Hypertension-induced dysfunction of circulation in hemorrhagic shock. *Am J Hypertens* 1995; 8: 761-7.
16. Tang EH, Vanhoutte PM. Endothelial dysfunction: A strategic target in the treatment of hypertension? *Pflugers Arch* 2010; 459: 995-1004.
17. Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD, Webb RC. Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. *Hypertension* 2001; 37: 781-6.
18. Md S, Moochhala SM, Siew Yang KL, Lu J, Anuar F, Mok P, *et al.* The role of selective nitric oxide synthase inhibitor on nitric oxide and pge2 levels in refractory hemorrhagic-shocked rats. *J Surg Res* 2005; 123: 206-14.
19. Ng KC, Moochhala SM, Md S, Yap EL, Low SY, Lu J. Preservation of neurological functions by nitric oxide synthase inhibitors following hemorrhagic shock. *Neuropharmacology* 2003; 44: 244-52.
20. Nematbakhsh M, Khazaei M. The effect of estrogen on serum nitric oxide concentrations in normotensive and doca salt hypertensive ovariectomized rats. *Clin Chim Acta* 2004; 344: 53-7.
21. Liu LM, Ward JA, Dubick MA. Hemorrhage-induced vascular hyporeactivity to norepinephrine in select vasculatures of rats and the roles of nitric oxide and endothelin. *Shock* 2003; 19: 208-14.

22. Secher NH, Jacobsen J, Friedman DB, Matzen S. Bradycardia during reversible hypovolaemic shock: associated neural reflex mechanisms and clinical implications. *Clin Exp Pharmacol Physiol* 1992; 19: 733-43.
23. Gkaliagkousi E, Douma S, Zamboulis C, Ferro A. Nitric oxide dysfunction in vascular endothelium and platelets: role in essential hypertension. *J Hypertens* 2009; 27: 2310-20.
24. Levy AS, Chung JC, Kroetsch JT, Rush JW. Nitric oxide and coronary vascular endothelium adaptations in hypertension. *Vasc Health Risk Manag* 2009; 5: 1075-87.
25. Pieber D, Horina G, Sandner-Kiesling A, Pieber TR, Heinemann A. Pressor and mesenteric arterial hyporesponsiveness to angiotensin II is an early event in haemorrhagic hypotension in anaesthetised rats. *Cardiovasc Res* 1999; 44: 166-75.
26. Shirhan M, Moochhala SM, Kerwin SY, Ng KC, Lu J. Influence of selective nitric oxide synthetase inhibitor for treatment of refractory haemorrhagic shock. *Resuscitation* 2004; 61: 221-9.
27. Anaya-Prado R, Toledo-Pereyra LH, Guo RF, Reuben J, Ward PA, Walsh J. The attenuation of hemorrhage-induced liver injury by exogenous nitric oxide, l-arginine, and inhibition of inducible nitric oxide synthase. *J Invest Surg* 2003; 16: 247-61.
28. Daughters K, Waxman K, Nguyen H. Increasing nitric oxide production improves survival in experimental hemorrhagic shock. *Resuscitation*. 1996; 31: 141-4.
29. Koch MA, Hasser EM, Schadt JC. Influence of nitric oxide on the hemodynamic response to hemorrhage in conscious rabbits. *Am J Physiol* 1995; 268: R171-82.
30. Schmetterer L, Dallinger S, Polak K, Eichler HG, Wolzt M. Systemic no synthase inhibition blunts the chronotropic, but not the inotropic response to isoprenaline in man. *Nitric Oxide* 1999; 3: 209-15.
31. Sinert R, Spencer MT, Wilson R, Silverberg M, Patel M, Doty CI, *et al.* The effect of hypertension on uncontrolled hemorrhage in a rodent model. *Acad Emerg Med* 2002; 9: 767-74.
32. Cabrales P, Tsai AG, Intaglietta M. Exogenous nitric oxide induces protection during hemorrhagic shock. *Resuscitation* 2009; 80: 707-12.

Source of Support: Isfahan University of Medical Sciences (grant number # 387413) **Conflict of Interest:** None declared.

Announcement

iPhone App



A free application to browse and search the journal's content is now available for iPhone/iPad. The application provides "Table of Contents" of the latest issues, which are stored on the device for future offline browsing. Internet connection is required to access the back issues and search facility. The application is Compatible with iPhone, iPod touch, and iPad and Requires iOS 3.1 or later. The application can be downloaded from <http://itunes.apple.com/us/app/medknow-journals/id458064375?ls=1&mt=8>. For suggestions and comments do write back to us.