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Original Article

Effects of hypertension on hemodynamic response and serum nitrite concentration during graded hemorrhagic shock in rats

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Abstract

BACKGROUND: Hypertensive patients have higher morbidity and mortality from hemorrhage. In this study, we investigated hemodynamic responses and serum nitrite concentrations during graded hemorrhagic shock and resuscitation in hypertensive (HT) and normotensive (NT) rats.

METHODS: Thirteen male rats were divided into two groups, namely HT (n = 6) and NT (n = 7). Hypertension was induced by deoxycorticosterone acetate (DOCA)-salt method in uninephrectomized rats. After 8 weeks, graded hemorrhagic shock was induced during 34 minutes in four steps separated by 8-minute intervals (totally 16 ml/kg). The animals were kept in this condition for 120 minutes (shock period). Then, they were resuscitated with blood withdrawal. Mean arterial pressure (MAP) and heart rate (HR) were measured throughout the experiment. Blood samples were taken before and after shock induction and at the end of the shock period.

RESULTS: HT rats experienced more MAP and HR reduction during the shock period and less improvement of hemodynamic response after resuscitation compared with the NT group ($p < 0.05$). The survival rate 72 hours post-hemorrhage in the HT group was significantly lower than the NT group (16.7% vs. 71.4%, respectively) ($p < 0.05$). Serum nitrite level in HT animals was lower than the NT group (2.45 ± 0.18 vs. 3.35 ± 0.26 µmol/lit, respectively; $p < 0.05$). In addition, it increased during the shock period in both NT and HT groups ($p > 0.05$).

CONCLUSIONS: More reduction of MAP after hemorrhagic shock, less improvement of MAP and HR after resuscitation and low survival rate in HT animals suggested the impairment of cardiovascular system adaptation of HT animals during blood loss and it should be considered in management of hypertensive subjects.

KEYWORDS: Hypertension, Hemorrhagic Shock, Nitric Oxide, Blood Pressure.

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It has been shown that hypertensive patients have higher morbidity and mortality from hemorrhage[1, 2] which may be a result of deficit in responding to blood loss. Therefore, knowledge regarding the hemodynamic changes and underlying mechanisms is important in early diagnosis and appropriate management of hemorrhagic shock in these patients.

Hemorrhagic shock leads to a sequence of hemodynamic and neuroendocrine responses in the cardiovascular system.[3] This sequence consists of a reduction in blood pressure, an initial increase in heart rate (HR) followed by bradycardia, and then tachycardia in severe or prolonged hypotension.[4] In the decompensation phase, hemorrhagic shock is characterized by decreased response to vasopressors and hypoperfusion of peripheral tissues.[5] It is indicated that endothelial dysfunction during hemorrhagic shock leads to tissue injury.[6]

Several paracrine and endocrine factors are involved to modulate cardiovascular response during shock. Nitric oxide (NO) is an important endothelium-derived relaxing factor which modulates vascular tone, blood pressure and tissue perfusion[7, 8] which may play a major role in pathogenesis of various types of

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shocks.[9-11] It has been suggested that unresponsiveness to angiotensin II in hemorrhagic shock may be resulted from the high generation of NO.[12]

In this study, we investigated hemodynamic changes and serum NO concentration during graded hemorrhagic shock and resuscitation in deoxycorticosterone acetate (DOCA)-salt hypertensive and normotensive rats.

Methods

Animals

Experiments were performed on thirteen male Wistar rats weighting between 300-420 grams. The animals were kept in an animal room under a 12/12h light/dark cycle at 20–25˚C. They received standard rat chow. Animal care was in accordance with the guidelines of the Ethics Committee of Isfahan University of Medical Sciences.

Experimental design

The animals were randomly divided into two groups, namely hypertensive (HT; n = 6) and normotensive (NT; n = 7). Hypertension in uninephrectomized was induced by subcutaneous injection of DOCA (Aboureihan Co., Iran) (30 mg/kg in almond oil, two times a week) rats and also by providing NaCl 1% and KCl 0.2% in their drinking water.[13] The NT group was also nephrectomized and injected with subcutaneous solvent of DOCA. However, tap water was used as their drinking water. Systolic blood pressure of all rats was recorded by tail cuff method every week. Rats with a systolic blood pressure higher than 140 mmHg were considered hypertensive.[14]

After 8 weeks, the animals were anaesthetized with intraperitoneal injection of ketamin (100 mg/kg) and xylazine (5 mg/kg). Right femoral artery and vein were cannulated with polyethylene catheter (PE-50) for blood pressure and HR monitoring and blood withdrawal during shock induction, respectively. Then, the animals were given a 30-minute rest period for recovery. Afterwards, graded hemorrhagic shock was induced by blood withdrawal from venous catheter during 34 minutes in four steps separated by 8-minute intervals (totally 16 ml/kg) as shown in figure 1.[15] Blood samples were preserved in 1 ml (50 units) heparinized syringe at room temperature. After the induction of hemorrhagic shock, the animals were kept in the same condition for 120 minutes which was considered as the shock period. Then, they were resuscitated with withdrawn blood at a rate of approximately 1 ml/min. During the shock induction, shock period and resuscitation, arterial blood pressure was recorded continuously. Thereafter, catheters were removed and the incisions were sutured with 3-0 silk thread and rats were returned to cages for recovery. The survival rate was determined during the first 4 hours and every 12 hours up to 72 hours. Blood samples were taken before and after shock induction and at the end of the shock period. The blood collected was accounted in the total hemorrhage volume. Samples were centrifuged at 3000 rpm for 15 minutes and serums were stored at -70˚C.

Blood pressure and HR were recorded using a physiograph (Hugo Sachs Electronic, Germany) connected to a computer. Data was collected and analyzed with a Windows compatible software. Mean arterial pressure (MAP) and HR were determined at specific time points.

Serum nitrite measurement

Serum nitrite concentrations were determined by Griess reaction method using available reagents and kit (Promega, USA) with a detection limit of 2.5 μmol/lit.

Statistical analysis

The results are expressed as mean ± standard error. Data was compared between groups using repeated measured ANOVA and independent t-test. Pre-shock and post-shock values were analyzed using paired t-test. The survival rate was evaluated by Fischer exact test. A $p < 0.05$ was considered statistically significant.

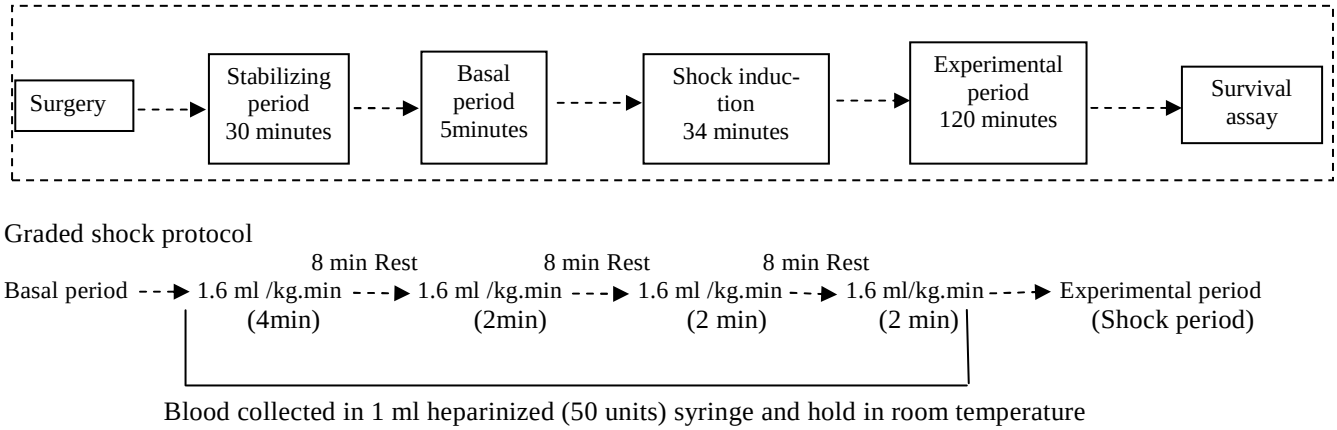

Figure 1. The protocol of inducing graded hemorrhagic shock

Results

Changes of hemodynamic parameters

Figure 2 illustrates the time course of MAP and HR changes during the shock period and resuscitation in experimental groups. As shown in Figure 2A, in baseline condition (before hemorrhage), MAP in the HT group was significantly higher than the NT group (122 ± 3.9 mmHg vs. 75.8 ± 3.23 mmHg; Systolic BP: 164.5 ± 2.7 mmHg vs. 102.83 ± 3.6 mmHg, respectively; $p < 0.01$). Graded hemorrhage caused significant decreases in MAP in the NT (75.83 ± 3.23 mmHg vs. 51.18 ± 4.54 mmHg; $p < 0.05$) and HT (122 ± 3.9 mmHg vs. 76.17 ± 5.13 mmHg; $p < 0.05$) groups. Hemorrhage also caused a significant decrease in HR in both the NT (323.9 ± 17.83 vs. 181.38 ± 13.6; $p < 0.05$) and HT (268.8 ± 16.42 vs. 205 ± 25.58; $p < 0.05$) groups. Although, the percentage of MAP reduction was not significantly different between the two groups, the magnitude of the decrease in blood pressure caused by hemorrhage in the HT group was higher than that in the NT group (45.83 ± 4.38 vs. 24.25 ± 5.89 mmHg, respectively; $p < 0.05$) (Figure 3A). In addition, the HT group showed a higher reduction in HR compared with the NT group (142.53 ± 11.98 vs. 63.83 ± 17.22 beat/min, respectively; $p < 0.05$) (Figure 3B).

Resuscitation increased MAP and HR in both groups. However, in the NT group, MAP reached closer to the baseline level compared with the HT group (Figure 2A). Moreover, resuscitation caused increased HR in both groups. However, the increases in the two groups were not significantly different ($p > 0.05$) (Figure 2B).

Serum nitrite concentration

Serum nitrite concentrations in the HT group were significantly lower than the NT group before the hemorrhagic shock (2.45 ± 0.18 vs. 3.35 ± 0.26 μmol/lit, respectively; $p < 0.05$). Graded hemorrhage caused significant increases in serum nitrite concentration in both groups ($p < 0.05$) (Figure 4). Although serum nitrite concentrations in the HT group were still lower than the NT group at the end of the shock period, the difference was not statistically significant (Figure 4).

Survival analysis

Figure 5 illustrates the survival rate of each group after resuscitation. One HT rat died during the shock period. Six out of seven (85.3%) in the NT and two out of five (40%) rats in the HT groups were alive during the first 12 hours after resuscitation. Five out of seven (71.4%) NT rats and only one out of six (16.7%) HT rats were alive 72 hours after the experiments.

Discussion

Hemorrhagic shock is associated with multiple organ damage which may increase poor outcomes and mortality in injured and traumatic patients.[16] HT patients have higher vascular resistance and sympathetic tone than NT subjects and may demonstrate aberrant responses

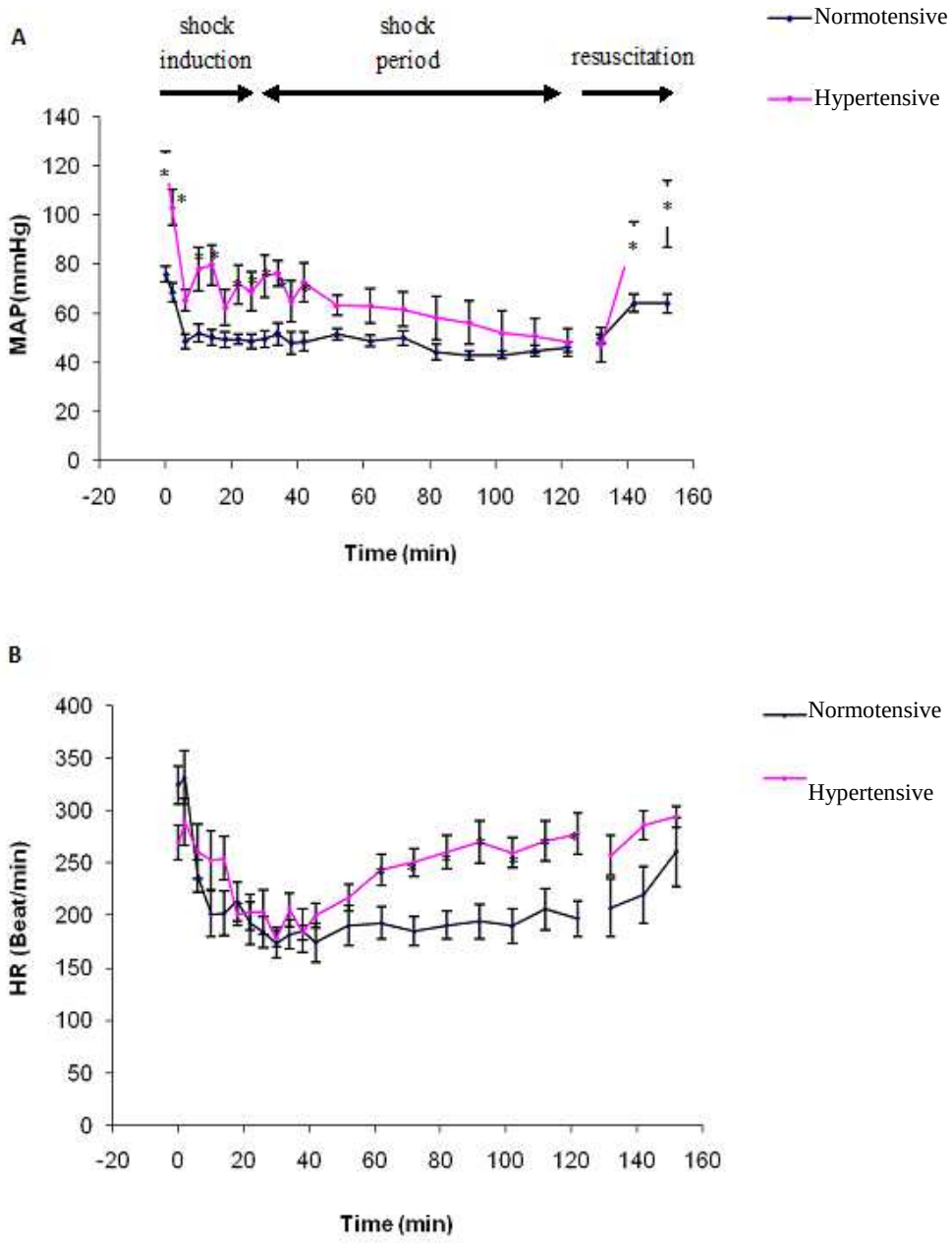

Figure 2. Time course of changes of MAP (A) and HR (B) values during hemorrhage, shock period and resuscitation in NT and HT groups.
* indicates a significant difference between the two groups.

to bleeding.[2] In the present study, we showed that HT animals had greater MAP and HR depression during hemorrhage and less improvement of MAP and HR during the shock period and resuscitation. In addition, we found that mortality rate of HT animals was higher than the NT group. In agreement with our results, it was shown that hemorrhagic shock induction with withdrawing 25% of the total blood volume in spontaneous HT rats caused significantly more MAP depression and acidosis than NT rats.[2] Moreover, the mortality rate

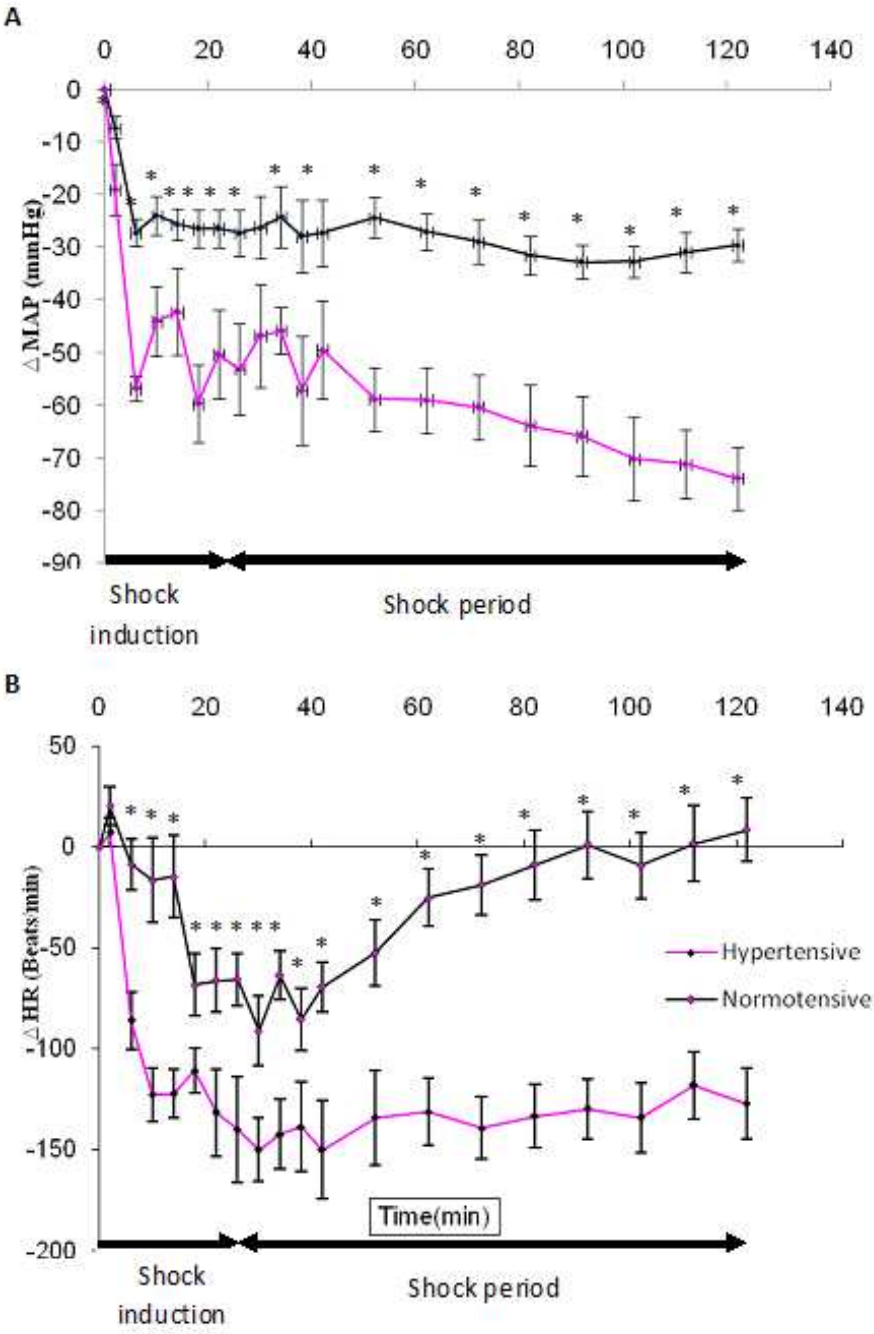

Figure 3. Changes of MAP and HR during shock induction and the shock period in NT and HT rats (n = 7 and 6, respectively). * indicates a significant difference between the two groups.

due to hemorrhage in HT rats was more than that of the normal group.[17] HT subjects have a defect in baroreflex response even in recovery phase[17] which may be important in their management. In addition, it has been demonstrated that tissue ischemia and organ failure in spontaneous HT rats were more than NT animals at the same blood pressure after hemorrhagic shock[17] which may explain higher mortality in HT animals.

Vascular hyporeactivity to vasoconstrictors and vasodilators during hemorrhagic and other kinds of shocks has been documented in several studies.[18-21] Many factors have been proposed to be involved in vascular hyporeactivity including NO.[19-22] NO has been impli-

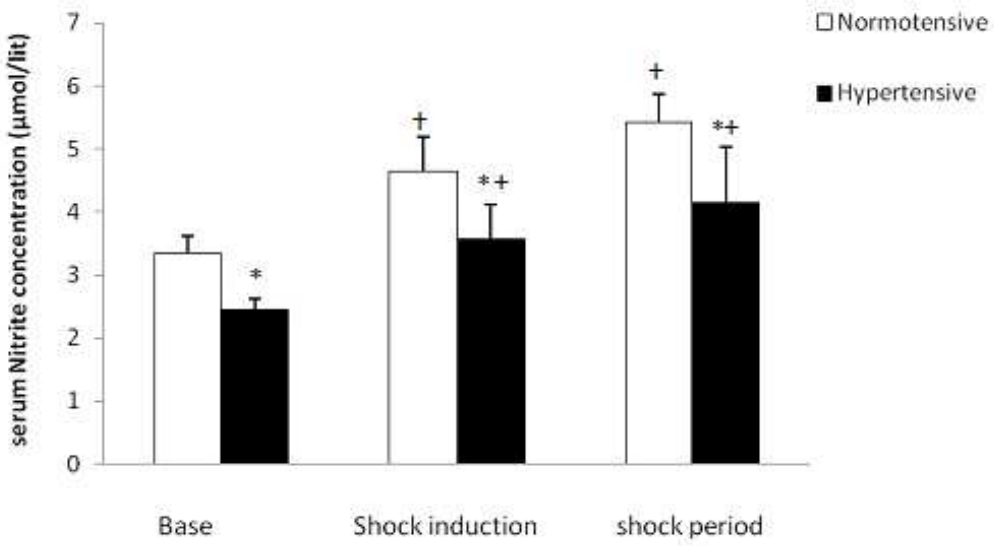

Figure 4. Serum nitrite level in NT and HT rats at baseline, after hemorrhage (34th minute) and at the end of the shock period (120 minutes after shock induction).
*: p ≤ 0.05 compared with NT. †: p ≤ 0.05 compared with baseline.

cated in pathogenesis of hemorrhagic and other types of shocks.[9-11] In the present study, the baseline level of serum NO in the HT group was lower than the NT group. Endothelial dysfunction has been indicated in conduit and resistance arteries of hypertensive animals.[23] Several mechanisms such as increased reactive oxygen species, impaired l-arginine uptake[24] or lowered endothelial nitric oxide synthase (eNOS) expression have been suggested to account for reduced availability of NO in hypertension.[25] We showed that serum NO concentration increased after and during hemorrhagic shock period in both NT and HT rats which supports the previous studies.[11, 26] Shirhan et al. indicated that serum nitrite/nitrate level increased in untreated hemorrhagic shock and inhibition of excessive NO formation

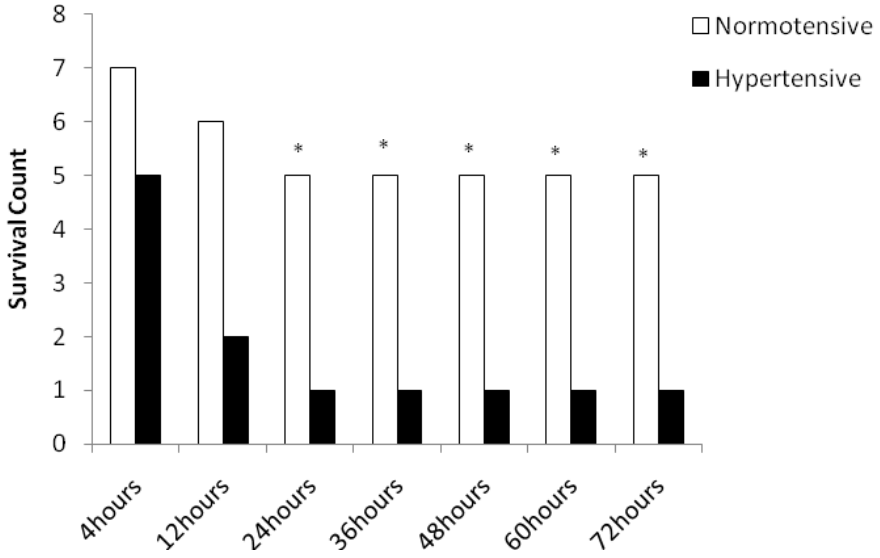

Figure 5. Survival counts in NT and HT groups after the experiment.
* indicates a significant difference between the two groups (p ≤ 0.05).

improved the vascular response to angiotensin II.[11] A recent study also found that hemorrhage is associated with vascular decompensation due to low NO bioavailability.[26] Furthermore, gene expression of inducible nitric oxide synthase (iNOS) and eNOS in selected vasculatures increased during hemorrhagic shock.[10] It is suggested that vascular hyporeactivity during hemorrhagic shock is related to different expressions of NOS and some cytokines after shock.[10] Increased activity of iNOS and constitutional NOS in liver, spleen, lung, and various organs during shock has been documented.[27] Excessive NO production and vascular hyporeactivity not only play an important role in development of shock, but also reduce the effectiveness of shock therapy by vasoactive agents. Moreover, excessive formation of NO may contribute to damage to multiple organs such as heart, lung and liver during shock.[11]

In conclusion, serum NO concentration was increased in NT and HT animals after hemorrhagic shock induction and during the shock period. More reduction of MAP after hemorrhagic shock, less improvement of MAP and HR after resuscitation and low survival rate in HT animals suggested the impairment of cardiovascular system adaptation of HT animals during blood loss and it should be considered in management of hypertensive subjects.

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Conflict of Interests
Authors have no conflict of interests.

Authors' Contributions
A N contributed in the study design. BB and M Kh involved in study design, conducting the experiments and writing the manuscript.

References
1. Radisavljevic Z. Hypertension-induced dysfunction of circulation in hemorrhagic shock. Am J Hypertens 1995; 8(7): 761-7.
2. 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(4): 318-26.
3. Moochhala S, Wu J, Lu J. Hemorrhagic shock: an overview of animal models. Front Biosci 2009; 14: 4631-9.
4. Balaszczuk AM, Arreche ND, Mc LM, Arranz C, Fellet AL. Nitric oxide synthases are involved in the modulation of cardiovascular adaptation in hemorrhaged rats. Vascul Pharmacol 2006; 44(6): 417-26.
5. Dutton RP. Current concepts in hemorrhagic shock. Anesthesiol Clin 2007; 25(1): 23-34, viii.
6. Johnson RA, Durante W, Craig T, Peyton KJ, Myers JG, Stewart RM, et al. Vascular arginase contributes to arteriolar endothelial dysfunction in a rat model of hemorrhagic shock. J Trauma 2010; 69(2): 384-91.
7. Sadeghi-Hashjin G. Role of nitric oxide in the plasma lipid profile in the rabbits. Archives of Medical Sciences 2009; 5(3): 308-12.
8. Forstermann U. Nitric oxide and oxidative stress in vascular disease. Pflugers Arch 2010; 459(6): 923-39.
9. Lange M, Enkhbaatar P, Nakano Y, Traber DL. Role of nitric oxide in shock: the large animal perspective. Front Biosci 2009; 14: 1979-89.
10. 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(2): 128-36.
11. 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(2): 221-9.
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(9): 1750-5.

13. 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(2 Part 2): 781-6.
14. Seifi B, Kadkhodaee M, Karimian SM, Zahmatkesh M, Xu J, Soleimani M. Evaluation of renal oxidative stress in the development of DOCA-salt induced hypertension and its renal damage. Clin Exp Hypertens 2010; 32(2): 90-7.
15. Blair ML, Jaworski RL, Want A, Piekut DT. Parabrachial nucleus modulates cardiovascular responses to blood loss. Am J Physiol Regul Integr Comp Physiol 2001; 280(4): R1141-R1148.
16. Angele MK, Schneider CP, Chaudry IH. Bench-to-bedside review: latest results in hemorrhagic shock. Crit Care 2008; 12(4): 218.
17. 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(8): 767-74.
18. Hasan A, McDonough KH. The effects of Escherichia coli sepsis and short-term ischemia on coronary vascular reactivity and myocardial function. Shock 1997; 8(4): 305-10.
19. Li S, Fan SX, McKenna TM. Role of nitric oxide in sepsis-induced hyporeactivity in isolated rat lungs. Shock 1996; 5(2): 122-9.
20. 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(3): 208-14.
21. Yaghi A, Paterson NA, McCormack DG. Vascular reactivity in sepsis: importance of controls and role of nitric oxide. Am J Respir Crit Care Med 1995; 151(3 Pt 1): 706-12.
22. 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(2): 362-8.
23. Tang EH, Vanhoutte PM. Endothelial dysfunction: a strategic target in the treatment of hypertension? Pflugers Arch 2010; 459(6): 995-1004.
24. Gkaliagkousi E, Douma S, Zamboulis C, Ferro A. Nitric oxide dysfunction in vascular endothelium and platelets: role in essential hypertension. J Hypertens 2009; 27(12): 2310-20.
25. 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.
26. Cabrales P, Tsai AG, Intaglietta M. Exogenous nitric oxide induces protection during hemorrhagic shock. Resuscitation 2009; 80(6): 707-12.
27. Rajnik M, Salkowski CA, Thomas KE, Li YY, Rollwagen FM, Vogel SN. Induction of early inflammatory gene expression in a murine model of nonresuscitated, fixed-volume hemorrhage. Shock 2002; 17(4): 322-8.