Hemodynamic responses and serum nitrite concentration during uncontrolled hemorrhagic shock in normotensive and hypertensive rats

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Background. We evaluated the effect of hypertension on hemodynamic responses and serum nitrite concentrations in normotensive (NT) and deoxycorticosteron acetate (DOCA)-Salt hypertensive (HT) rats.

Methods. Uncontrolled hemorrhagic shock was induced in NT and HT rats (n=7 each) by preliminary bleed of 25 ml/kg followed by a 75% tail amputation. The mean arterial pressure (MAP), heart rate and serum nitrite were measured pre-hemorrhage and during hemorrhage.

Results. Changes in time-averaged MAP after hemorrhage were significantly greater in HT group than NT. After resuscitation, the HT rats failed to restore MAP to baseline level. Serum nitrite level in both groups was significantly increased during shock period. Survival rate of HT animals was lower than NT group, although it was not statistically significant.

Conclusions. Marked reduction of MAP and less improvement after resuscitation suggested the less adaptation of cardiovascular system in HT animals which may interfere with management of these subjects during uncontrolled hemorrhagic shock.

Key words: hypertension, shock, hemorrhage, nitric oxide

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INTRODUCTION

Shock is a condition in which tissue perfusion is not capable to maintain normal tissue metabolism. Hemorrhagic shock is an emergency which can lead to hemodynamic instability, reduced tissue perfusion, cell hypoxia and finally death¹. Trauma and gastrointestinal bleeding are the most common causes of hemorrhagic shock¹. During hemorrhagic shock, heart rate (HR) increases followed by bradycardia and in severe hypotension causes tachycardia².

Hypertensive subjects are at risk of higher morbidity and mortality after hemorrhage^{3,5}. It is indicated that baroreflex response to hemorrhage is impaired in spontaneous hypertensive rats³. In addition, a defect in hemodynamic responses after gastrointestinal hemorrhage in hypertensive subjects was reported⁶. Several circulating factors such as Nitric Oxide (NO) are involved in pathogenesis of various shocks which modulate cardiovascular response to hemorrhagic shock⁷. NO is one of the most important endothelium-derived releasing factors which is synthesized by NO synthase⁸. There are three isoforms of NO synthase: constitutive, neuronal and inducible⁸. Excessive generation of NO is associated with vascular hyporeactivity to vasoconstrictive substances during shock^{7,9}.

Since knowledge regarding the responses of hypertensive subjects to hemorrhagic shock has important implications for management of these patients, in this study we used uncontrolled hemorrhagic shock model in normotensive (NT) and deoxycorticosterone acetate (DOCA)-Salt hypertensive (HT) rats to study the changes of the

hemodynamic responses and serum nitrite concentration, the main metabolite of NO, during uncontrolled hemorrhagic shock and resuscitation.

MATERIALS AND METHODS

Animal preparation

Fourteen male wistar rats weighting 280-320 g (age between 10-12 weeks) were purchased from Pasteur institute of Iran. The animals were kept on 12-h light/dark cycle with 20-25 °C temperature and received standard rat chow and water *ad libitum*. All experiments were performed in accordance with the guidelines of the Animal and Human Ethical Committee of Isfahan University of Medical Sciences.

The animals were randomly divided into two groups: HT and NT (n=7 each). Hypertension was induced by subcutaneous injection of DOCA dissolved in almond oil (30 mg/kg two times a week) in uninephrectomized rats and NaCl 1% and KCl 0.2% as drinking water^{10,11}. In NT group, solvent of DOCA was injected and tap water used for drinking. Systolic blood pressure was recorded by tail cuff method every week. The rats with systolic blood pressure higher than 140 mmHg were considered HT (ref.¹¹).

Shock protocol

After 8 weeks, the animals were anaesthetized with intraperitoneal injection of ketamin (100 mg/kg) and xylazine (5 mg/kg). A polyethylene catheter (PE-50) was inserted via right femoral artery for blood pressure and

Fig. 1. Uncontrolled hemorrhagic shock protocol.

heart rate (HR) monitoring. Another PE-50 catheter was inserted via right femoral vein for blood withdrawal and sampling. Then, the animals were allowed 30 min rest period for stabilizing from surgery. Hemorrhagic shock was induced by withdrawing blood from femoral vein (25 ml/kg) for 20 min followed by 10 min rest period (Fig. 1)(ref.^{3,12}). The shed blood was preserved for later re-infusion. Next, uncontrolled hemorrhage was added by amputation of 75% of the tail measured from the tip. The bleeding tail was directed into a container containing heparin to record cumulative hemorrhage volume. This phase was continued for 60 min (shock period). During this phase, ringer lactate was infused through femoral vein to maintain Mean Arterial Pressure (MAP) above 40 mmHg, if necessary (initial resuscitation phase). After shock period, tail wound was closed and initially shed blood re-infused during 15 min. This phase lasted 60 min and during this phase, MAP was maintained above 80 mmHg with infusion of ringer lactate if necessary (post resuscitation phase)(ref.¹³). Volume of ringer lactate infusion in initial resuscitation and post resuscitation periods were recorded. Blood pressure and HR were recorded with a physiograph (Hugosachs electronic, Germany) and data analyzed with a windows compatible software. MAP and HR were determined at specific time points. Blood samples were taken before hemorrhage, immediately post-hemorrhage and after resuscitation phase. Bloods were centrifuged and serums kept in -70°C for further nitrite

assay. At the end of experiment, catheters were removed and wounds closed. The animals were returned to their cages and allowed free access to water and food. Survival rate was determined every 12 h up to 72 h.

Serum nitrite measurement

Serum nitrite concentrations were determined by conventional griess reaction method using available reagent (Promega, USA, Detection limit: 2.5 μ mol) (ref.¹⁴).

Fig. 2. Systolic blood pressure and MAP in NT and HT groups. *: $P < 0.05$ compared with NT group. MAP: mean arterial pressure; NT: normotensive; HT: hypertensive.

Fig. 3. Changes of MAP and HR during hemorrhagic shock and resuscitation in experimental groups. *: $P < 0.05$ compared with other group. MAP: mean arterial pressure; HR: heart rate.

Fig. 4. Changes of MAP and HR after induction of uncontrolled hemorrhage (A) and resuscitation (B).

* $P < 0.05$ when compared with normotensive group. MAP: mean arterial pressure; HR: heart rate.

Statistical analysis

Repeated measure ANOVA followed by tukey's test was used for comparison of continuous variables. Comparison of data before and after shock was analyzed by paired t test. Data between two groups was analyzed by independent t test. Survival rate was evaluated by Chi square Fischer exact test. Bivariate correlation was calculated using pearson's correlation coefficient. $P \leq 0.05$ was considered statistically significant.

RESULTS

Hemodynamic changes

Systolic blood pressure and MAP of NT and HT groups were shown in Fig. 2. DOCA-Salt HT rats had significantly higher blood pressure than NT group ($P < 0.05$). Fig. 3 illustrates changes of MAP and HR of the NT and HT rats during hemorrhage and resuscitation. Blood withdrawal (25 ml/kg) via venous catheter for 20 min caused

Fig. 5. Serum nitrite levels before shock (base), after shock induction and during shock period in experimental groups.

*: $P < 0.05$ when compared with normotensive group.

†: $P < 0.05$ when compared with base and shock period.

Fig. 6. Comparison of survival count between groups during 72 hours survival assay.

a marked decrease in MAP in both NT and HT groups (47 ± 4.14 mmHg vs. 74 ± 4.22 mmHg, respectively; $P < 0.05$). HT animals experienced more reduction of MAP than NT group (Fig. 3,4A). During shock period, MAP was maintained around 40 mmHg usingringer lactate solution infusion (Fig. 2). After resuscitation, MAP was increased in both groups, however, it was closer to basal level (pre-hemorrhage) in NT than HT group ($P < 0.05$) (Fig. 4B).

Baseline HR was slightly higher in HT group compared with NT ($P > 0.05$). Hemorrhage initially caused HR reduction in both groups. Then, HR increased continuously during hemorrhage and resuscitation (Fig. 4A,B), however, there was no significant difference between groups.

Serum nitrite measurement

Fig. 5 illustrates serum nitrite concentration before hemorrhagic shock, after shock induction and at the end of shock period. Before hemorrhage, serum nitrite level in HT groups was significantly lower than NT ($P < 0.05$). Immediately after hemorrhage, serum nitrite was increased in both groups. At the end of shock period, serum nitrite concentration in both groups was higher than before hemorrhage ($P < 0.05$).

Volume of tail bleeding and saline infusion during early resuscitation

Volume of tail bleeding in HT group was higher than NT group, although it was not statistically significant (3.66 ± 0.59 vs. 2.07 ± 0.53 ml; $P < 0.05$). The initial fluid volume required to maintain MAP around 40 mmHg (early resuscitation phase) in HT rats was higher than NT rats (19.4 ± 2.58 vs. 15.21 ± 2.51 ml; $P < 0.05$). Fluid volume infused in addition to shed blood in late resuscitation phase were not different between HT and NT groups (5.50 ± 1.23 vs. 6.56 ± 1.49 ml; $P < 0.05$).

Survival analysis

Two out of seven HT rats were died during 4 h after experiment. Two out of seven HT and one out of seven NT rats were died during first 12h after resuscitation.

One out of seven NT rats was died after 48 h. The rest of animals in each group were alive until 72 h (Fig. 6). By correlation analysis, we found that there is no correlation between serum nitrite concentration after resuscitation and mortality rate after 72 h ($r = 0.10$).

DISCUSSION

This study investigated the effect of uncontrolled hemorrhagic shock on hemodynamic responses and serum nitrite concentration in NT and HT rats.

Shock is a state of tissue hypoperfusion with hemodynamic instability. Our data showed that immediately post-hemorrhage, HT rats experienced more MAP reduction than NT rats as evidenced by larger drop in MAP. Moreover, during the resuscitation phase, MAP almost completely returned to basal level in NT group, while, the HT rats failed to restore MAP to baseline level. We also found that blood loss from tail cut (uncontrolled hemorrhage phase) and the initial fluid volume required to maintain MAP during early resuscitation phase in HT group was higher than NT, although it was not statistically significant. This condition means a hypotensive state for HT animals and could place them at risk of tissue ischemia and failure and may increase mortality rate in these animals. Previous studies demonstrated that hypertensive subjects are at risk of higher morbidity and mortality after blood loss³⁻⁵. In the present study, we showed that the survival rate of HT animals was lower than NT group. Marked reduction of MAP and less improvement of blood pressure after resuscitation may be partly responsible for higher mortality in HT animals.

Our observations are in parallel with other findings that reported HT rats as compared with NT group, had higher mortality rate after the same vascular injury, however, post-hemorrhage blood pressure and base excess were not different between groups³. Another study also demonstrated that hypertensive animals experienced more profound hemorrhagic shock compared with nor-

motensive animals¹⁵. In this study, we used uncontrolled hemorrhagic shock model. In this model, in spite of catheter controlled hemorrhagic model, vascular injury (amputation of tail) results in continued blood loss³. Experimental and clinical studies indicated that there is a defect in hemodynamic and baroreflex responses after hemorrhage in hypertensive subjects⁶. Furthermore, hypertensive animals have greater tissue ischemia such as liver, skeletal muscles and brain than normotensive¹⁵.

Several circulating factors such as NO are involved in modulation of cardiovascular response to hemorrhagic shock⁷. Excessive formation of NO may contribute to pathogenesis of various shock and vascular hyporeactivity in various shocks^{7,16,17}. In the present study, serum nitrite level in HT animals was lower than NT group which may be the result of endothelial dysfunction in these animals^{18,19}. In this study, hemorrhagic shock increased serum nitrite concentration in both groups. Activating of NO synthase during shock may be responsible for excessive generation of NO (ref.²⁰). Furthermore, it is demonstrated that inducible form of NO synthase expression is up-regulated in different organs during shock^{21,22}. It was shown that inhibition of NO synthase in hemorrhagic shocked rats restored vasoconstrictive response to phenylephrine and angiotensin II (ref.^{20,23}). Therefore, excessive formation of nitrite may increase reactive oxygen species and PGE2 which might result in organ damages²⁴, although, in this study, we did not evaluate tissue ischemia during blood loss.

CONCLUSION

In conclusion, marked reduction of MAP during uncontrolled hemorrhagic shock. Less improvement of blood pressure after resuscitation, and higher fluid therapy during early resuscitation phase suggested the less adaptation of cardiovascular system in HT animals during blood loss which may interfere with management of these subjects. In addition, increased serum nitrite concentration during hemorrhagic shock may contribute to higher mortality of HT animals during severe hemorrhage.

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