Original Article

Effect of aminoguanidine on cardiovascular responses and survival time during blood loss: A study in normotensive and deoxycorticosterone acetate-salt hypertensive rats

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Abstract

Introduction: Hemorrhagic shock causes more circulatory disturbances and mortality in hypertensive than normotensive subjects. In the late phase of hemorrhagic shock, nitric oxide (NO) overproduction leads to vascular decompensation. In this study, we evaluated the effect of inducible NO synthase (iNOS) inhibitor, aminoguanidine (AG), on hemodynamic parameters and serum nitrite concentration in decompensated hemorrhagic shock model in normotensive and hypertensive male rats. **Materials and Methods:** Twenty-four male rats were divided into hypertensive and normotensive groups (n = 12 each). Hypertension was induced by subcutaneous injection of deoxycorticoesterone acetate (DOCA), 30 mg/kg in uninephrectomized rats. Decompensated hemorrhagic shock was induced by withdrawing blood until the mean arterial pressure (MAP) reached 40 mmHg. After 120 min, each group was assigned to aminguanidine (100 mg/kg) and control group. Hemodynamic parameters were monitored for next 60 min. Blood samples were taken before and after shock period and 60 min after treatment. Survival rate was monitored for 72 h. **Results:** Infusion of AG in normotensive animals caused a transient increase in MAP and increase of heart rate, whereas it did not affect those parameters in hypertensive animals. Hemorrhagic shock caused a significant rise in serum nitrite concentration in normotensive and hypertensive rats and infusion of AG did not significantly change it in both groups. No significant differences observed in survival rate between AG-treated and not treated groups. **Conclusion:** It seems that inhibition of iNOS with AG does not have beneficial effects on hemodynamatic parameters and survival rate during decompensated hemorrhagic shock in normotensive and hypertensive animals.

Key words: Hemorrhagic shock, hypertension, nitric oxide

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Introduction

Hypertensive patients have higher vascular resistance and sympathetic tone than normotensive ones and may demonstrate aberrant responses to bleeding.[1] Hemorrhagic shock induction with withdrawing 25% of total blood volume in spontaneous hypertensive rats causes more blood pressure (BP) depression and acidosis in hypertensive compared with normotensive animals.[2,3]

Nitric oxide (NO) over production after hemorrhagic shock has been documented.[4-8] NO has several cardiovascular effects including regulation of vascular tones, neurotransmission, heart rate (HR) and contractility, inhibition of platelets aggression and leukocyte adhesion and acts as antiatherogenic factor.[9-11] NO is synthesized by three NO synthase (NOS) isoforms: Endothelial, neuronal and inducible NOS (iNOS). Hemorrhage leads to iNOS induction in the late phase of hemorrhagic shock with NO overproduction that lead to vascular decompensation and more mortality rate.[5,12]

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In this study, we proposed that more circulatory disturbances and mortality in hypertensive animals during hemorrhagic shock may be due to more iNOS activation compare to normotensive ones and to evaluate the effect of an iNOS inhibitor, aminoguanidine (AG), on hemodynamic parameters, serum nitrite level and survival rate in decompensated hemorrhagic shock model.

Materials and Methods

The study was conducted after getting ethical approval from the University Ethical Committee.

Animals

The male Wistar rats (age: 10–12 weeks, weight: 220 ± 20 g) were purchased from Pasteur Institute of Iran and kept in the animal room, two per cages, with 12 h light/dark cycle and temperature between 20°C and 25°C. The ethical committee of the authors' university approved the experimental procedures. Hypertension was induced by subcutaneous injection of deoxycorticoesterone acetate (DOCA) (Irandaru Co.), 30 mg/kg dissolved in almond oil, twice a week for 8 weeks in uninephrectomized rats plus NaCl 1% and KCl 0.2% solution for drinking.[13] In normotensive group, solvent of DOCA was injected subcutaneously and tap water was used for drinking in uninephrectomized rats. Systolic BP (SBP) was recorded by tail cuff method every week. Rats with SBP higher than 140 mmHg were considered hypertensive.[13]

Decompensated hemorrhagic shock induction

The animals were anesthetized by ketamine (75 mg/kg; Sigma Co. USA) and xylazine (5 mg/kg; Sigma Co. USA). The body temperature was monitored by rectal thermometer and maintained around 37°C using a heating pad. Right and left femoral arteries were cannulated by PE-50 catheters for blood withdrawal and monitoring of mean arterial pressure (MAP) and HR during the experiment. Right femoral vein was cannulated for drug administration. After 30 min (stabilizing period), decompensated hemorrhagic shock was induced by withdrawing blood using a heparinized syringe (rate: 1 ml/4–5 min) until the MAP reached 40 mmHg during the total time of 20 min[14] and maintained at shock state and MAP around 40 mmHg for next 120 min by withdrawing or reinfusing shed blood as necessary (shock period). Blood pressure was recorded with a physiograph (Hugo-Sachs Electronik, Germany) and data analyzed with a windows compatible software.

Aminoguanidine administration

After shock period, AG (100 mg/kg; Sigma Co.) dissolved in normal saline (1 ml/kg) was infused during 15 min through femoral vein in hypertensive and normotensive rats and the animals were monitored for 1-h. In the control group, normal saline (1 ml/kg) was infused. After this period, the catheters were removed in survived animals. Incisions closed, and the animals returned to their cages. All the rats were returned to their cages where unlimited food and water were supplied. Mortality was recorded during the first 4 h and every 12 h for a total of 72 h.

Serum nitrite measurement

Blood samples were collected before hemorrhage, 2 h after shock induction and 1-h after AG infusion. Blood samples (0.3 ml) were centrifuged at 5000 c/s for 20 min. Serum samples were poured in eppendorf tubes and saved at - 70°C for further analysis of serum nitrite. Serum nitrite concentrations, the main metabolite of NO, were measured by griess reaction method using available reagents and kit (Promega Co, USA) with a detection limit of 2.5 µmol.

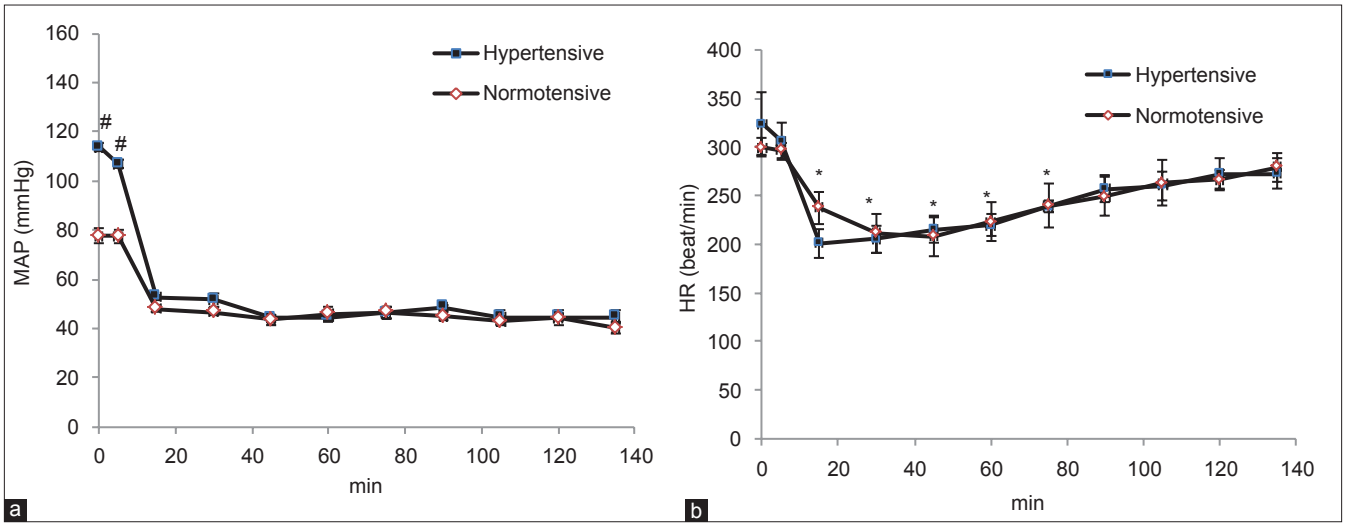

Figure 1: Changes of mean arterial pressure (a) and Heart rate (b) during the shock period in normotensive and hypertensive rats (a). *Significant difference compare to before experiment; #Significant difference compare to normotensive group.

Statistical analysis

The results are expressed as mean ± standard error. One-way ANOVA test was used for comparison of data between groups. Data were compared between two groups using an independent *t*-test. Pre and postshock values were analyzed by paired *t*-test. Survival rate was evaluated by Fischer exact test. $P < 0.05$ was considered to be statistically significant.

Results

Mean arterial pressure and heart rate

Deoxycorticoesterone acetate-salt hypertensive animals had higher BP compare to normotensive group (MAP: 122 ± 4 vs. 81 ± 2.7 mmHg; SBP: 160 ± 5.5. vs. 106 ± 9.7 mmHg, $P < 0.05$). Hemorrhage decreased MAP in two groups and maintained around 40 mmHg during the shock period [Figure 1a]. Hemorrhage caused a significant decrease of HR in normotensive and hypertensive animals that continued throughout of a shock period [Figure 1b], however, there was no significant difference between groups ($P > 0.05$).

Infusion of AG during the shock period in normotensive animals caused a transient increase in MAP, however, after 1-h it returned to the base level [Figure 2a]. In hypertensive animals, AG increased MAP, although it was not statistically significant [Figure 2b]. Administration of AG caused an increase of HR in normotensive and hypertensive animals ($P < 0.05$) [Figure 2c and d].

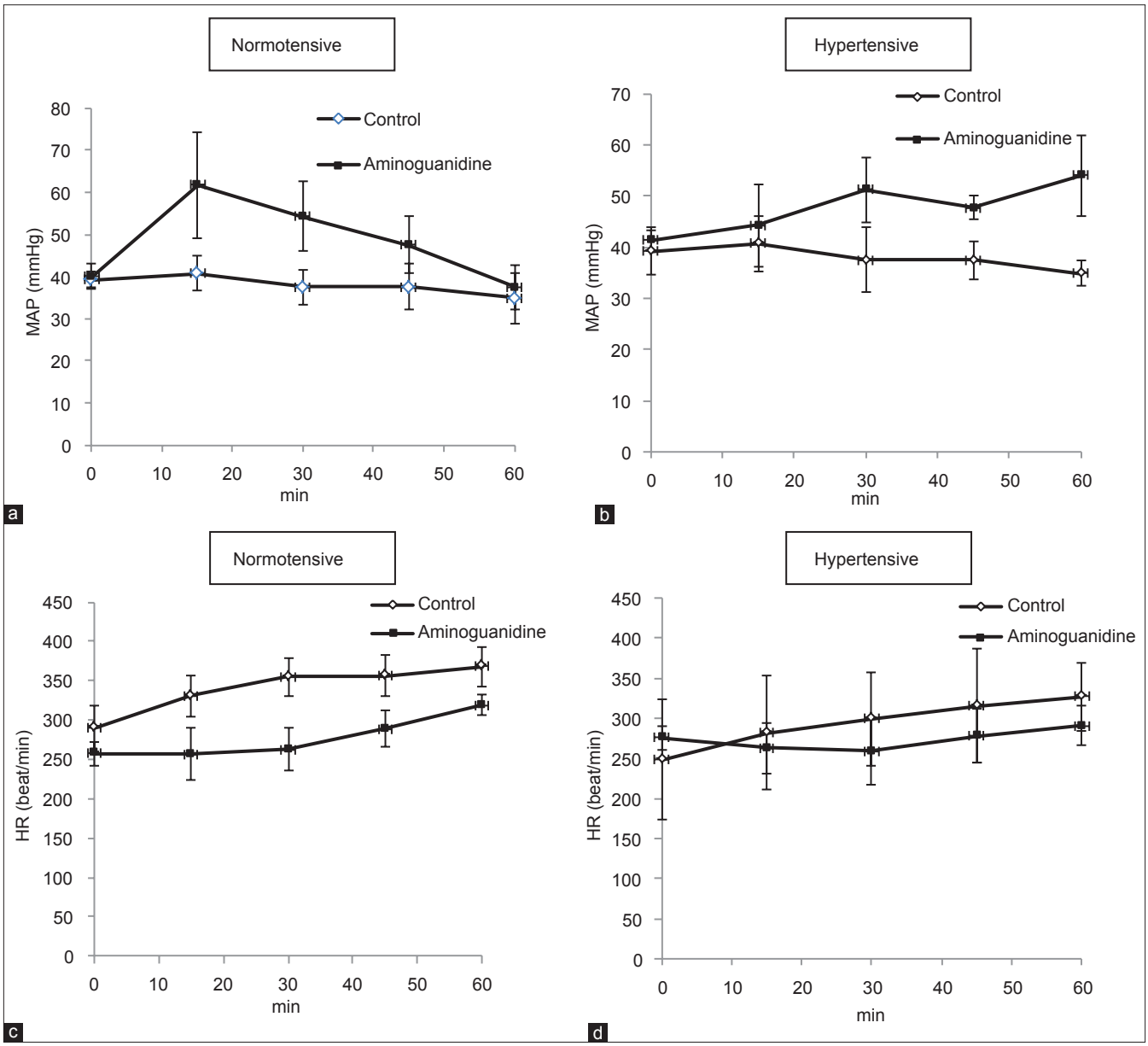

Figure 2: Effect of aminoguanidine on mean arterial pressure and heart rate in normotensive (a and c) and hypertensive (b and d) rats

Serum nitrite concentration

Basal level of serum nitrite concentration in the hypertensive group was lower than control (3.41 ± 0.17 vs. 3.97 ± 0.24 μmol/l, respectively). Hemorrhagic shock caused a significant rise in serum nitrite concentration in normotensive (5.17 ± 0.38 vs. 3.97 ± 0.24 μmol/l, $P < 0.01$) and hypertensive rats (4.87 ± 0.3 vs. 3.41 ± 0.17 μmol/l; $P < 0.05$) [Figure 3]. Infusion of AG reduced serum nitrite concentration in normotensive (4.92 ± 1.37 vs. 5.17 ± 0.38 μmol/l) and hypertensive (4.62 ± 0.3 vs. 4.87 ± 0.3. μmol/l) groups [Figure 3].

Survival rate

All normotensive and hypertensive rats were survived during experiment. After 4 h, two animals (33%) were died in the hypertensive group, while animals in the normotensive group were alive. After 72 h, the number of survived animals was the same in AG-treated and nontreated groups [Figure 4].

Discussion

Previous studies showed that the late phase of hemorrhagic shock is accompanied with NO overproduction and vascular decompensation.[12,14,15] In this study, we evaluate the effect of AG on hemodynamic responses, serum NO production and survival rate in decompensated hemorrhagic shock model. In this model, during the shock period, BP stabilizes at a low definite point to prevent from compensatory neural and hormonal responses.[14,15]

Prognosis of hemorrhagic shock primarily depends on the level of hypovolemia and hypotension that restricts tissue perfusion and causes oxidative stress and tissue damages. Furthermore, endothelial cell swelling due to ischemia worsen impaired perfusion of tissues. Hemorrhagic shock causes a general ischemia in the body that usually produces

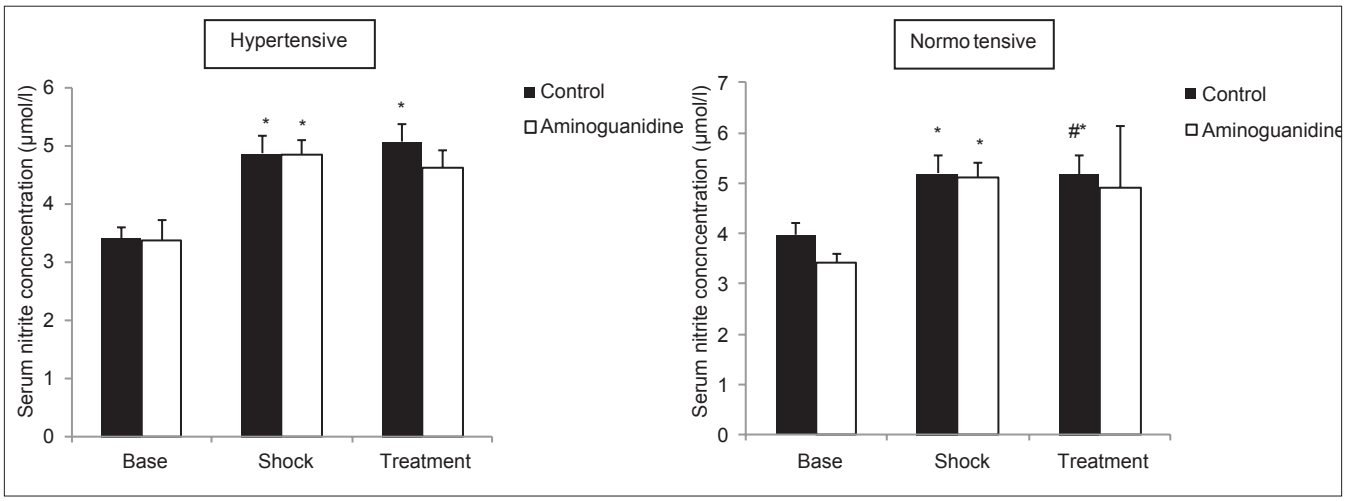

Figure 3: Comparison of serum nitrite concentrations during the shock period and after aminoguanidine (AG) treatment in normotensive and hypertensive groups. *$P < 0.05$ compare to base level. #$P < 0.05$ compare to AG-treated group

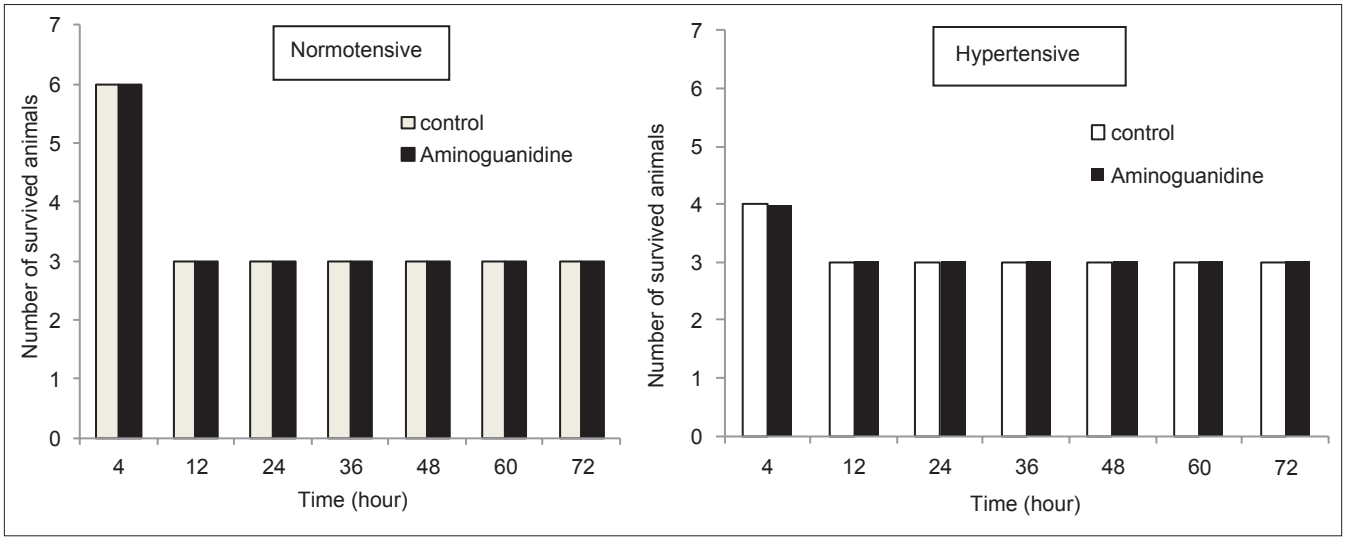

Figure 4: Survival rate of normotensive and hypertensive animals aftershock period in aminoguanidine-treated and nontreated groups

systemic inflammation and degree of inflammation depends on duration of shock.[16] Studies demonstrated higher morbidity and mortality during hemorrhagic shock in hypertensive subjects [1-3] which supported the results of this study. The factors which may be involved on lower survival count are vascular decompensation, baroreflex insufficiency, systemic inflammatory response and tissue damages.[17-20]

We also found that serum NO concentration in hypertensive animals was lower than normotensive, which may reflect endothelial dysfunction. Endothelial dysfunction in hypertensive subjects decreases NO bioavailability due to increased oxidative stress in the vessels.[21-23] Previous studies indicated increased NO production during hemorrhagic shock.[8] In the present study, we found increased serum NO concentration after shock period in normotensive and hypertensive groups. Hemorrhage induces inflammatory responses that lead to induction of iNOS particularly in the late phase of shock.[20,24] Increased serum NO level is accompanied with vascular decompensation.[25] We expected that administration of AG (iNOS inhibitor) decreases serum NO level and increases BP, however, we observed no significant effects on MAP, HR and serum NO level in normotensive and hypertensive groups. In contrast to our results, some studies demonstrated that iNOS inhibition caused a significant improvement of hemodynamic parameters and tissue injuries.[12,15,26,27] Hua and Moochhala indicated that NG-nitro-L-arginine methyl ester (L-NAME) and AG (1, 10, and 100 mg/kg) increased the survival time of shocked animals.[27] They also showed that L-arginine reversed the beneficial effects of L-NAME and AG and suggested the involvement of NO in the pathophysiology of hemorrhagic shock. Mercaptoguanidine, an iNOS inhibitor and scavenger of peroxynitrite, prevents vascular decompensation in the late phase of shock.[28] In agree to our results, an iNOS inhibitor, GW274150, used in the experimental model of hemorrhagic shock did not change hemodynamic response but improved tissue injuries and renal function and decreased production of nitrotyrosine in lung and liver.[29] In another study, 2 h hemorrhagic shock caused a time-dependent decrease of vascular response to norepinephrine. This decrease was inhibited by L-NAME administration that indicates NO overproduction by endothelial NOS. However, in the longer period of shock, hyporeactivity of blood vessels reversed by dexamethasone that implies to NO production by iNOS.[30] Based on our results, it seems that the main source of higher NO production during blood loss and shock period is not the iNOS and perhaps this is the reason that AG could not alter hemodynamic response, serum NO concentration and survival rate during blood loss.

Conclusion

Administration of AG during decompensated hemorrhagic shock could not improve hemodynamic responses, serum NO concentration and survival rate in normotensive and DOCA-salt hypertensive rats.

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