Physiology and Pharmacology

Physiol Pharmacol 19 (2015) 31-37 **www.phypha.ir/ppj**

Original Article

Reduction of hemorrhagic shock–induced acute kidney injury by lower limb ischemic preconditioning in rats

Parisa Ahghari[1], Mehri Kadkhodaee[2*], Behjat Seifi[2], Mina Ranjbaran[2], Zahra Sedaghat[3], Sedigheh Shams[4]

1. Department of Physiology, School of Medicine, Tehran University of Medical Sciences, International Campus (TUMS-IC), Tehran, Iran
2. Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Physiology and Pharmacology, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
4. Children Medical Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Introduction: During hemorrhagic shock (HS), the kidneys are one of the primary target organs involved. Oxidative stress is shown to be enhanced in different models of acute kidney injury (AKI). Remote ischemic preconditioning (RPC) by brief limb ischemia is considered to be a safe method to protect different organs from further damage. In this study, we investigated the effects of brief hind limb occlusion on protection against AKI and whether this protection is related to a reduction in oxidative stress.

Materials and Methods: Twenty one rats were divided into three groups of seven rats. Sham-operated animals underwent surgical procedures, without hemorrhage. HS was induced by bleeding from a femoral arterial catheter to remove 44% of total blood volume. In RPC group, four cycles of lower limb ischemic preconditioning (5 min ischemia followed by 5 min reperfusion) were performed immediately before HS. Three hours later, plasma and renal tissue samples were collected for renal function monitoring and oxidative stress assessment.

Results: Compared with the sham group, HS resulted in renal dysfunction, significantly increased blood urea nitrogen (BUN), plasma creatinine (Cr) and renal malondialdehyde (MDA) levels as well as decreased superoxide dismutase (SOD) activity in the kidneys ($P<0.05$). In the RPC group, renal function was significantly improved. Plasma Cr and BUN and renal MDA levels were significantly lower in RPC group comparing to HS group ($P<0.05$). Renal SOD activity was significantly higher in RPC group compared to HS group ($P<0.05$).

Conclusion: These results demonstrate that induction of brief periods of lower limb ischemic preconditioning improves kidney function, restores SOD activity and reduces oxidative stress injury caused by hemorrhagic shock.

Keywords:
Remote ischemic preconditioning;
Hemorrhagic shock;
Kidney;
Oxidative stress

Received:
12 Jun 2014
Accepted:
25 Sep 2014

***Correspondence to:**
M. Kadkhodaee

Tel:
00982188259862
Fax:
00982166419484

Email:
kadkhodm@tums.ac.ir

Introduction

A common outcome of HS is multiple organ failure, which may lead to hemodynamic instability and cause cellular hypoxia, organ damage and death (Yang et al., 2011). The kidneys are one of the most important organs that are particularly sensitive to HS. During

HS, compensatory mechanisms maintain blood flow in favor of vital organs such as brain and heart. These events lead to exacerbated renal damage by diminishing oxygen delivery (Hultstrom, 2013).

AKI is a serious complication among hospitalized patients. Despite improvements in the management, incidence of AKI is increasing and in some patients leads to chronic kidney disease and death (Mayeur et al., 2011). Most clinical ischemic AKI occurs as a result of renal hypo perfusion which develops in HS (Onen et al., 2003). In recent years, different animal models have been proposed to simulate and study HS conditions (Mayeur et al., 2011; Ronn et al., 2011). The goal is the development of interventions for restoration of tissue survival during ischemia (Mulier et al., 2012).

RPC, non-lethal short term episodes of ischemia – reperfusion (IR) in a remote organ, is thought to trigger pathways that confer protection to a target organ against a subsequent lethal prolonged ischemia (Przyklenk et al., 1993). Using transient ischemic upper and lower extremities (hands and feet), instead of more vital organs, we can create protective effects similar to local conditioning models. The limbs are resistant to ischemic damage and are easily accessible (Schmidt et al., 2007). In 2011, Jan et al. showed that bilateral lower limb IR could alleviate lung injury in hemorrhagic shock/resuscitation rats (Jan et al., 2011).

Free radicals are normal metabolic products that are continuously generated during consumption of oxygen in the body. Increases in the generation of free radicals that exceed the capacity of antioxidants, results in oxidative stress (Zheng et al., 2008). In different models of HS in rodents, the production of peroxynitrite ($ONOO^{\bullet-}$), a highly reactive product of nitric oxide (NO) and superoxide anion ($O2^{\bullet-}$), is shown. Peroxynitrite starts oxidative reactions and cause lipid peroxidation which leads to degradation of the cytoskeleton (Wang et al., 2012). MDA, a product of lipid peroxidation, is a well-known oxidative stress biomarker (Moore and Roberts, 1998). In normal conditions, reactive oxygen species (ROS) are eliminated by antioxidant defense system. Among them superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are very important (Scandalios, 2002).

The present study was conducted to evaluate the effects of RPC on the improvement of renal ischemic injury following hemorrhagic shock. We then measured the level of the oxidative stress markers in different groups.

Materials and methods

Twenty one male Wistar rats, weighing 250–300 g, were randomly selected. Rats were housed under standard conditions (12 h light–dark cycle; 20–22°C) and were allowed ad libitum access to food and water. All procedures were approved by the Animal Ethics Committee of Tehran University of Medical Sciences.

Experimental protocols

There were three groups (n = 7 in each): (i) Sham group (Anesthesia and surgery without induction of HS) (ii) Hemorrhagic shock group (iii) RPC group (RPC plus HS).

Rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally and maintained with ketamine (20 mg/kg). A sterile cut down was performed in left groin, and cannula was inserted into the left femoral artery. During these experiments, animals were kept warm using a heat pad. In hemorrhagic shock group, experimental animals were bled to 44% of total blood volume (animal weight [g] × 0.03 + 0.7 ml is equal to 50% of total blood volume) using syringes. Sham animals underwent anesthesia and surgery (groin incision and cannulation) without induction of HS. In RPC group, four cycles of lower limb ischemic preconditioning (IP) were performed by applying rubber band tourniquet high around right thigh for 5 min followed by 5 min reperfusion immediately before HS (Ahmadi-Yazdi et al., 2009; Wu et al., 2010).

Renal functional assessments

After three hours, rats were euthanized and blood samples were collected. Plasma creatinine (Cr) and blood urea nitrogen (BUN) were measured by colorimetric methods (Hitachi 704 auto-analyzer,

Japan).

Renal oxidative stress assessments

Kidneys were collected and then washed and dissected. Part of the kidney tissues were immediately snap-frozen in liquid nitrogen and stored at -70°c until further use.

Malondialdehyde levels

MDA levels were determined in kidney tissue samples according to the method of Esterbauer and Cheeseman (Esterbauer and Cheeseman, 1990). MDA reacts with thiobarbituric acid (TBA) to create a pink pigment that has maximum absorption at 532 nm.

Superoxide dismutase activity

Kidney tissue SOD activity was determined according to the method of Paoletti and Mocali (Paoletti and Mocali, 1988). In this assay, superoxide anion is produced from O_2 in the presence of EDTA, mercaptoethanol and manganese chloride. The rate of NADPH Oxidation is related to the availability of superoxide anions in the medium.

Statistical analysis

All results are expressed as the mean ± SEM. One-way ANOVA was used to compare mean values between groups followed by Tukey test. $P<0.05$ was considered significant.

Results

Effect of RPC on renal function

HS resulted in a significant increase in plasma Cr compared with that in the sham group (1.12 ± 0.09 vs 0.62 ± 0.01 mg/dl, respectively; $P<0.05$). RPC significantly decreased plasma Cr (0.9 ± 0.04 vs 1.12 ± 0.09 mg/dl, respectively; $P<0.05$) compared with the shock group (Fig.1).

Furthermore, HS resulted in significant increases in BUN (45.6 ± 2.3 vs 19 ± 0.61 mg/dl, respectively, $P<0.05$) compared with the sham group. However, RPC significantly decreased BUN level (37.16 ± 1.92 vs 45.6 ± 2.3 mg/dl, respectively; $P<0.05$) compared with that in the shock group (Fig. 2).

Effects of RPC on HS-induced oxidative stress in the kidney

HS resulted in a significant increase in MDA content compared with the sham group (5.16 ± 0.27 vs 1.98 ± 0.18 µmol/100 mg tissue, respectively; $P<0.05$). RPC significantly decreased MDA content (3.23 ± 0.24 vs 5.16 ± 0.27 µmol/100 mg tissue, respectively; $P<0.05$) compared with that in the shock group (Fig. 3). Compared with the sham group, HS resulted in

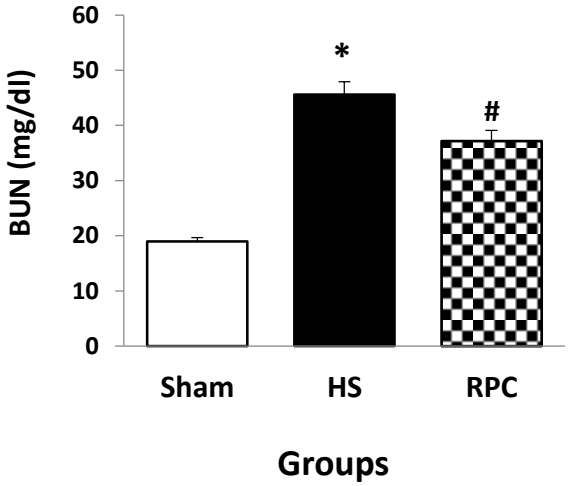

Fig 2: Effects of remote preconditioning (RPC) on plasma blood urea nitrogen (BUN). Data are the mean ± SEM (n = 7 in each). * P<0.05 compared with the sham group. # P<0.05 compared with the HS group (one-way ANOVA followed by Tukeys' test).

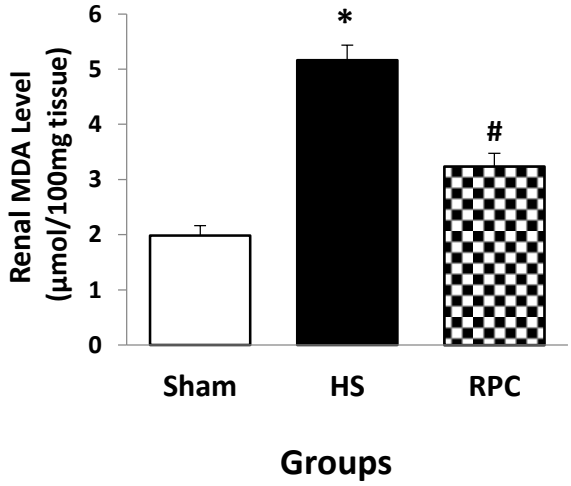

Fig 3: Effects of remote preconditioning (RPC) on Malondialdehyde content (MDA) in the kidney tissue. Data are the mean ± SEM (n = 7 in each). * P<0.05 compared with the sham group. # P<0.05 compared with the HS group (one-way ANOVA followed by Tukeys' test).

significant decreases in SOD activity (30.7 ± 1.87 vs 2.24 ± 0.53 U/g tissue, respectively; P<0.05). The activity of SOD was higher in the RPC group (22.34 ± 3.05 U/g tissue vs 2.24 ± 0.53 U/g tissue, respectively; P<0.05) compared with the shock group (Fig.4).

Discussion

In this study serum creatinine (Cr) and blood urea nitrogen (BUN) were increased as a result of the AKI induced by HS. These two values are considered as indices of renal function and their elevated plasma concentrations indicate decreased kidney function. In a study of hemorrhagic shock in rats, limb preconditioning before induction of HS mitigates lung injury (Jan et al., 2011). Similarly, in our study, the kidney function parameters were improved by applying RPC before induction of HS.

Circulatory shock can lead to heart failure and

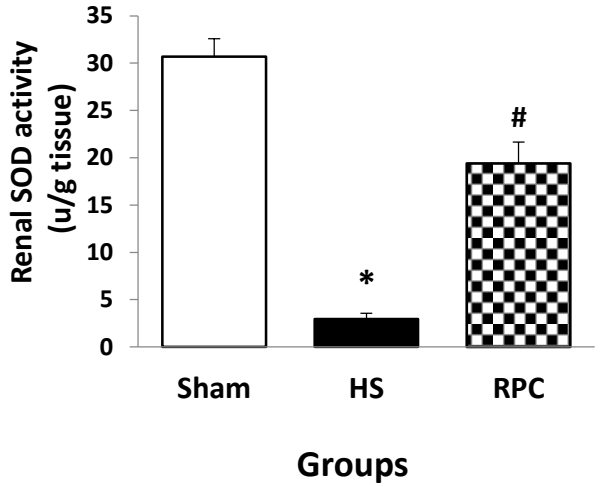

Fig 4: Effects of remote preconditioning (RPC) on superoxide dismutase (SOD) in the kidney tissues. Data are the mean ± SEM (n = 7 in each). * P<0.05 compared with the sham group. # P<0.05 compared with the HS group (one-way ANOVA followed by Tukeys' test).

multiple organ dysfunctions including damage of the liver, kidney, lung and spleen. HS is one of the biggest causes of death worldwide which is associated with reduced tissue perfusion, activation of immune responses, oxidative damage and cell death (Santry and Alam, 2010). Inadequate perfusion during ischemia plays an important role in the cellular damage after HS. Hypoperfusion diminishes nutrient and adenosine tri-phosphate (ATP) transport and affects intracellular processes such as trans-membrane potential and mitochondrial function. The liver and kidney are particularly sensitive to low energy levels (Zheng et al., 2008).

During IR damage to an organ, IP as a therapeutic strategy may be applied locally or in a place distant from the site of main damage (Hosseniakbari et al., 2008; Murry et al., 1986). For instance, beneficial effects of brief ischemia of liver on renal IR was demonstrated by Ates et al. in 2002 through biochemical, histopathological and ultra-structural indices (Ates et al., 2002). In another study, Lazaris et al. showed that a transient infrarenal aortic occlusion ameliorated renal IR injury (Lazaris et al., 2009). In 2013, Sedaghat et al. reported that the application of remote preconditioning, IR episodes of femoral artery applied at the beginning of renal ischemia, reduced renal injury (Sedaghat et al., 2013). Even in the clinical setting, induction of RPC by brief blood pressure (BP) cuff occlusion of hind limb lowered IR-induced kidney damage (Wever et al., 2011). Jan et al. in 2011, described that bilateral lower limb ischemic preconditioning attenuated acute lung injury induced by HS (Jan et al., 2011). However, the mechanism underlying this protection and its signaling pathways is not clear. Some circulatory humoral, neurogenic and cellular mediators / mechanisms have been implicated. Mediators such as adenosine, nitric oxide, TNF-α and bradykinin are discussed (Schmidt et al., 2007). High levels of oxygen radicals formation and oxidative stress is proposed as an underlying mechanism in these situations (Chandel and Budinger, 2007; Salom et al., 2007). As HS progresses, under -catalytic reaction of xanthine oxidase, superoxide anion occurs and is consequently transformed into hydrogen peroxide which then converts into highly reactive hydroxyl radicals. One of the destructive effects of these oxidative products is the activation of the neutrophils, releasing proteases and finally fatally damaging the cellular organelles (Kirpatovskii et al., 2007; Rodriguez et al., 2013).

Significant increase in tissue nitric oxide concentration occurs during ischemia. Nitric oxide in competition with oxygen may bind to cytochrome oxidase, favouring superoxide formation and inhibition of cellular respiration (Antico Arciuch et al.,

2009; Zheng et al., 2008). Nitric oxide reacts with superoxide ion to generate peroxynitrite that is a much more potent oxidant than nitric oxide and superoxide alone (Szabo., 2010). Formation of large amounts of free radicals causes detrimental effects, including lipid peroxidation, DNA and protein oxidative damage which leads to progressive injury to kidney tubular cells (Salom et al., 2007). Overproduction of free radicals and a considerable amount of oxidant stress, demonstrated by lipid peroxidation and increased MDA content, is shown in a study on the acute lung and kidney injury after hemorrhagic shock in rats (Lee et al., 2013). In the present study, we showed that HS increased MDA content in renal tissue and RPC prevented this effect. These data are in agreement with those of Jan et al. suggesting that RPC demonstrates its protective effects by reducing the production of MDA in the pulmonary tissues (Jan et al., 2011). Oxidative stress might result from decreased antioxidant capacity (Rodriguez et al., 2003). Peroxynitrite is able to inhibit a number of crucial molecules in the respiratory chain and antioxidant enzymes such as SOD. Any intervention that diminishes the ROS production induces beneficial effects in different organs during HS. These therapeutic interventions include administration of tocopherol, catalase and SOD, as well as inhibitors of peroxynitrite and nitric oxide synthase (Izumi et al., 2002). Wang et al. reported that SOD activity was decreased by 55% in HS group compared with the sham animals (Wang et al., 2012). Using ROS scavengers inhibited the production of reactive oxygen species and restored superoxide dismutase activities resulting in amelioration of renal dysfunction. In the present study, during hemorrhaging shock SOD activity is reduced and MDA content is elevated. These are two main factors which define the pathological consequences during oxidative stress. Application of RPC was able to reverse these processes.

These results demonstrate that induction of brief periods of lower limb ischemic preconditioning improves kidney function, restores SOD activity and declines oxidative stress injury caused by hemorrhagic shock.

Acknowledgments

This work was supported by a grant from the International Campus of Tehran University of Medical Sciences.

Conflict of interest

All authors declared that there is no conflict of interest.

References

Ahmadi-Yazdi C, Williams B, Oakes S, Francis D, Moore FD Jr: Attenuation of the effects of rat hemorrhagic shock with a reperfusion injury-inhibiting agent specific to mice. *Shock* 2009;32:295-301.

Antico Arciuch VG, Alippe Y, Carreras MC, Poderoso JJ: Mitochondrial kinases in cell signaling: Facts and perspectives. *Adv Drug Deliv Rev* 2009;61:1234-1249.

Ates E, Genc E, Erkasap N, Erkasap S, Akman S, Firat P, Emre S, Kiper H: Renal protection by brief liver ischemia in rats. Transplantation 2002;74:1247-1251.

Chandel NS, Budinger GR: The cellular basis for diverse responses to oxygen. *Free Radic Biol Med* 2007;42:165-174.

Esterbauer H, Cheeseman K: Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol* 1990;186:407-421.

Hosseniakbari HM, Rasoulian B, Mofid M, Noroozzadeh A, Noroozi M: The effect of short ischemic periods in reducing subsequent rat renal ischemic injury. *Physiol Pharmacol* 2008;12:149-157.

Hultstrom M: Neurohormonal interactions on the renal oxygen delivery and consumption in haemorrhagic shock-induced acute kidney injury. *Acta Physiol* (Oxf) 2013;209:11-25.

Izumi M, McDonald MC, Sharpe MA, Chatterjee PK, Thiemermann C: Superoxide dismutase mimetics with catalase activity reduce the organ injury in hemorrhagic shock. *Shock* 2002;18:230-235.

Jan WC, Chen CH, Tsai PS, Huang CJ: Limb ischemic preconditioning mitigates lung injury induced by haemorrhagic shock/resuscitation in rats. *Resuscitation* 2011;82:760-766.

Kirpatovskii VI, Kazachenko AV, Plotnikov EY, Kon'kova TA, Drozhzheva VV, Zorov DB: Effects of ischemic and hypoxic preconditioning on the state of mitochondria and function of ischemic kidneys. *Bull Exp Biol Med* 2007;143:105-109.

Lazaris AM, Maheras AN, Vasdekis SN, Karkaletsis KG, Charalambopoulos A, Kakisis JD, Martikos G, Patapis P, Giamarellos-Bourboulis EJ, Karatzas GM, Liakakos TD:

Protective effect of remote ischemic preconditioning in renal ischemia/reperfusion injury, in a model of thoracoabdominal aorta approach. *J Surg Res* 2009;154:267-273.

Lee JH, Jo YH, Kim K, Lee JH, Rim KP, Kwon WY, et al: Effect of N-acetyl cysteine (NAC) on acute lung injury and acute kidney injury in hemorrhagic shock. *Resuscitation* 2013;84:121-127.

Mayeur N, Minville V, Jaafar A, Allard J, Al Saati T, Guilbeau-Frugier C, et al: Morphologic and functional renal impact of acute kidney injury after prolonged hemorrhagic shock in mice. *Crit Care Med* 2011;39:2131-2138.

Moore K, Roberts LJ, 2nd: Measurement of lipid peroxidation. *Free Radic Res* 1998;28:659-671.

Mulier KE, Lexcen DR, Luzcek E, Greenberg JJ, Beilman GJ: Treatment with beta-hydroxybutyrate and melatonin is associated with improved survival in a porcine model of hemorrhagic shock. *Resuscitation* 2012;83:253-258.

Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-1136.

Onen A, Cigdem MK, Deveci E, Kaya S, Turhanoglu S, Yaldiz M: Effects of whole blood, crystalloid, and colloid resuscitation of hemorrhagic shock on renal damage in rats: An ultrastructural study. *J Pediatr Surg* 2003; 38: 1642-1649.

Paoletti F, Mocali A: Changes in cuzn-superoxide dismutase during induced differentiation of murine erythroleukemia cells. *Cancer Res* 1988;48:6674-6677.

Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P: Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993;87:893-899.

Rodriguez F, Bonacasa B, Fenoy FJ, Salom MG: Reactive oxygen and nitrogen species in the renal ischemia/reperfusion injury. Curr Pharm Des 2013;19:2776-2794.

Rodriguez J, Maloney RE, Rassaf T, Bryan NS, Feelisch M: Chemical nature of nitric oxide storage forms in rat vascular tissue. *Proc Natl Acad Sci U S A* 2003;100:336-341.

Ronn T, Lendemans S, de Groot H, Petrat F: A new model of severe hemorrhagic shock in rats. *Comp Med* 2011;61:419-426.

Salom MG, Ceron SN, Rodriguez F, Lopez B, Hernandez I, Martinez JG, Losa AM, Fenoy FJ: Heme oxygenase-1 induction improves ischemic renal failure: Role of nitric oxide and peroxynitrite. *Am J Physiol Heart Circ Physiol* 2007;293:H3542-3549.

Santry HP, Alam HB: Fluid resuscitation: Past, present, and the future. *Shock* 2010;33:229-241.

Scandalios JG: The rise of ros. *Trends Biochem* Sci 2002;27:483-486.

Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, et al: Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a katp-dependent mechanism: First demonstration of remote ischemic perconditioning. *Am J Physiol Heart Circ Physiol* 2007;292:H1883-1890.

Sedaghat Z, Kadkhodaee M, Seifi B, Salehi E, Najafi A, Dargahi L: Remote preconditioning reduces oxidative stress, downregulates cyclo-oxygenase-2 expression and attenuates ischaemia-reperfusion-induced acute kidney injury. *Clin Exp Pharmacol Physiol* 2013;40:97-103.

Szabo C, Modis K: Pathophysiological roles of peroxynitrite in circulatory shock. *Shock* 2010;34 Suppl 1:4-14.

Wang Y, Yan J, Xi L, Qian Z, Wang Z, Yang L: Protective effect of crocetin on hemorrhagic shock-induced acute renal failure in rats. *Shock* 2012;38;63-67.

Wever KE, Warle MC, Wagener FA, van der Hoorn JW, Masereeuw R, van der Vliet JA, et al: Remote ischaemic preconditioning by brief hind limb ischaemia protects against renal ischaemia-reperfusion injury: The role of adenosine. *Nephrol Dial Transplant* 2011;26:3108-3117.

Wu WT, Lin NT, Subeq YM, Lee RP, Chen IH, Hsu BG: Erythropoietin protects severe haemorrhagic shock-induced organ damage in conscious rats. *Injury* 2010;41:724-730.

Yang FL, Subeq YM, Lee CJ, Lee RP, Peng TC, Hsu BG: Melatonin ameliorates hemorrhagic shock-induced organ damage in rats. *J Surg Res* 2011;167:e315-321.

Zheng W, Huang LZ, Zhao L, Wang B, Xu HB, Wang GY, et al: Superoxide dismutase activity and malondialdehyde level in plasma and morphological evaluation of acute severe hemorrhagic shock in rats. *Am J Emerg Med* 2008;26:54-58.