

Hypertonic Saline Infusion Minimizes Bacterial Translocation and Degenerative Changes in Liver of Shocked Rats

¹Nouf Salem Mashyut ALjuaid , ²Abeer Nasser M ALghalbi

¹Faculty of Science , ²Faculty of Medicine , Taif University , Kingdom of Saudi Arabia (KSA)

Corresponding author: Nouf Salem Mashyut ALjuaid, Email: noof-salim@hotmail.com, mobile: 00966554800982

ABSTRACT

Background: The hypertonic saline (HTS) solutions have recently gained widespread acceptance in control of shock.

Aim of the work: The aim of this work is to study the use of hypertonic saline with the resuscitation fluids in rats with induced hemorrhagic shock to evaluate the impact of this solution on the extent of early bacterial translocation, blood pressure, and histopathological liver changes.

Materials and methods: sixty phenobarbital anesthetized rats were involved in this study where they were allowed to bleed. Arterial pressure was maintained below 50 mm Hg for 30 minutes. They were randomized into 2 groups, each of 30 rats. Resuscitation in group I was done by using Lactated Ringer's (LR) solution (60 ml/Kg) and in group II HTS 7.5% in a dose of 4 ml /kg body weight was added to Lactated Ringer's (LR) solution (60 ml/Kg). Regular monitoring of blood pressure was done and blood samples were withdrawn 1, 30, and 90 minutes and 6 hours after commencement of the resuscitation and sent for blood culture for both aerobic and anaerobic growths. After 24 hours the liver was resected and sent for histological examination. **Results:** The mean arterial blood pressure in group I before resuscitation was found to be about 48±11 mm Hg and it was raised to a mean of 90±8 mm Hg after 2 hours of resuscitation with LR alone. In group II, the mean arterial blood pressure was found to be 49±5 mm Hg and it was efficiently controlled by HTS with LR and 2 hours after commencement of resuscitation it was elevated to 114±10 mm Hg. The blood cultures were positive in 3.3% of the rats of group II and in 40% of rats of group I. Escherichia coli were the most commonly isolated organism. Histopathological findings showed inflammatory process and early severe hepatocyte degeneration of the liver of group I rats and these changes were absent or minimal in group II.

Conclusion: hypertonic saline was found to be effective for decreasing the rate of early bacterial translocation to blood and also for more efficient restoring of the mean arterial pressure in rats with induced hemorrhagic shock; in addition, HTS infusion minimizes the inflammatory and degenerative process in liver.

Keywords: Hypertonic saline, hepatocytes, bacterial translocation, rats.

INTRODUCTION

The gastrointestinal tract has been considered to be a potential source of sepsis from the bacterial translocation that may occur after shock, burns, and other major injuries ⁽¹⁾. Bacterial translocation (BT) occurs when the bacteria and/or their cell wall components pass across the intestinal wall to the mesenteric lymph nodes and in turn to peripheral blood ⁽²⁾. Factors which have been shown to predispose to BT include reduced splanchnic blood flow, intestinal mucosal damage, immune deficiency, parental nutrition, antibiotic therapy, and bacterial overgrowth ⁽³⁾. The route of entry of these bacterial products and other non-bacterial soluble factors into the systemic circulation is the mesenteric lymphatic and not the portal vein ⁽⁴⁾. Three basic mechanisms have been proposed to promote BT, which, include; intestinal mucosal injury by hypo-perfusion, decreased intracellular killing of bacteria and decrease of intracellular generation

of nitric oxide and super oxide with intestinal arteriolar vasoconstriction ⁽⁵⁾. Volume replacement alone seems to be not the only factor to prevent this process ⁽⁶⁻¹²⁾. Uncontrolled hemorrhagic shock is associated with host responses related to inflammation and apoptotic process and liver clearly plays an important role in metabolic processes following shock ⁽¹³⁾. Investigators have shown that crystalloids represent an effective and inexpensive means to restore intravascular volume and offer a survival advantage over colloids in the resuscitation of traumatic hemorrhagic shock ^(7, 9). More recently, small volume resuscitation with 4 mL of 7.5% Na Cl per kilogram of body weight of hypertonic saline (HTS) has been proposed to be very effective in the treatment of hemorrhagic shock ⁽⁵⁻⁹⁾. The aim of this work is to study the use of hypertonic saline (HTS) with the resuscitation fluids in rats with induced hemorrhagic shock to

evaluate the impact of this solution on the extent of early bacterial translocation, blood pressure, and histopathological liver changes.

The study was done after approval of ethical board of King Abdulaziz university.

MATERIALS AND METHODS

The study was approved by the local committee of animal research. The sixty rats involved in this study were anesthetized by intravenous injection of 30 mg Phenobarbital/ Kg and allowed to bleed. Arterial pressure was maintained below 50 mm Hg for 30 minutes. They were randomized into 2 groups, each of 30 rats. Resuscitation in group I was done by using Lactated Ringer's (LR) solution (60 ml/Kg) and in group II HTS 7.5% with dose of 4 ml /kg body weight was added to Lactated Ringer's (LR) solution (60 ml/Kg). Regular monitoring of blood pressure was done and blood samples were withdrawn 1, 30, and 90 minutes and 6 hours after commencement of the resuscitation and sent for blood culture for both aerobic and anaerobic growths. After 24 hours the liver was resected and sent for histological examination. The blood samples were then incubated for 48 hours in blood sheep agar, and Mc Conkey's agar, and in liquid thiogluconate for anaerobic culture. Colonizing samples were incubated with vitamin K hemin blood agar for another 48 hours at 37°C. The sample was considered to be positive for bacterial translocation if the bacterial count was higher than 100 colony forming units (CFU)/gram. Positive blood cultures were passed into blood agar for determining the morphology. The liver specimens was immediately fixed in 10% formalin phosphate buffer for 24 h, embedded in paraffin blocks, and cut in

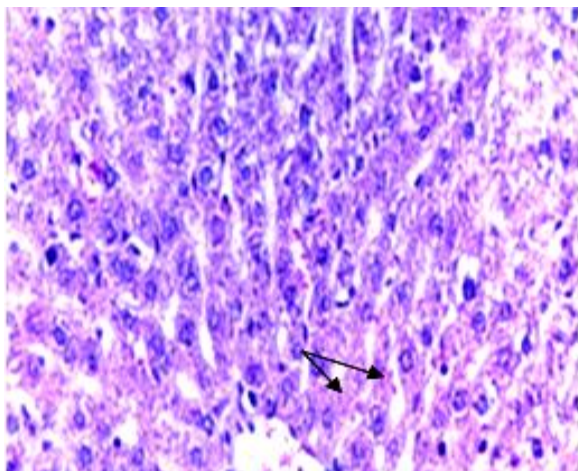


Figure 1: Shows minimal cloudy swelling (black arrows) with normally arranged hepatocytes (Hx & E X100)

transverse sections each of 4 μ m and the slides were stained with hematoxylin and eosin (H and E). **Statistical analysis** was performed using SPSS program version 20.5 (SPSS Inc., Chicago, IL, USA) and by the t-student test and Mann-Whitney tests were used. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Table I shows that before resuscitation the difference of blood pressure among both groups did not reach statistical significance ($P > 0.05$); however, the mean arterial blood pressure was significantly lower in group I than in group II after 2 hours of resuscitation. The blood cultures were positive only in the samples taken 90 minutes after commencement of resuscitation and the mean time from onset of trauma to detect the BT in peripheral blood was 3 hours. It was positive in 1/30 rats (3.3%) of group II and in 12/30 rats (40%) of group I. The differences among the groups were significant ($P < 0.05$) and bacterial translocation was significantly higher in group I (Table I).

Escherichia coli was the most commonly isolated organism followed by *Enterobacter*, *Bacteriodes*, *Pseudomonas aerogonasa*, *Staphylococcus aureus*, and the least was *Proteus*.

Liver cuts from group II revealed that hepatocytes were distributed equidistantly within sinusoidal space with few cells showing cloudy swelling (Figure 1). Histopathological evaluation of group I revealed the presence of erythrocytes in sinusoidal space associated with inflammatory infiltrate and hepatocytes show severe early degenerative changes in the form of hydropic degeneration (Figure 2).

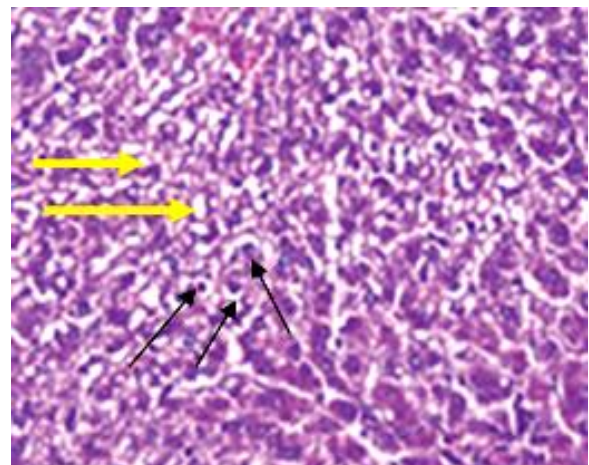


Figure 2: Shows inflammatory infiltrate (black arrows) and yellow arrows point to ballooned liver cells (Hx & E X100)

Table 1: Summary of the results

Finding	Group I	Group II	P-Value
Number of Rats	30	30	-----
Mean ABP (before resuscitation)	48±11 mm Hg	49±5 mm Hg	>0.05
Mean ABP (after resuscitation)	90±8 mm Hg	114±10 mm Hg.	<0.05
Positive blood cultures	40%	3.3%%	<0.05

ABP; Arterial Blood Pressure

DISCUSSION

The popularity of HTS has been increased in the last two decades showing a trend to increased post-traumatic survival both in the early phase and in the late phase^(6, 9). In the early phase (<12 hours after trauma), the effect of HTS is believed to be due to a functional increase in cardiac preload, primarily by inducing intravascular shift of the osmotic fluid from the cells and interstitium leading to an increase in systemic blood pressure and cardiac output as well as decrease in small vessel capacitance⁽⁹⁻¹²⁾.

The barrier function of the intestinal mucosa is deranged in animals subjected to severe injury leading to bacterial translocation which has been proved to play the major role in the late (days to weeks after trauma) post-traumatic morbidity and mortality^(9, 10). The late morbidity and mortality of traumatic hemorrhage have been attributed to the “second hit”; that is, a relatively less important inflammatory event as pneumonia, aspiration or minor surgery that occurs days to weeks after the injury and in presence of the translocated bacteria and abnormal neutrophil function it initiates progression to the systemic inflammatory response syndrome (SIRS), organ failure, and eventual death⁽⁹⁾.

The mechanisms to be proposed involve intestinal mucosal injury by hypo-perfusion, decrease in intracellular killing of bacteria and arteriolar vasoconstriction, the effects which had been proved to be reversed if hypertonic saline is used in resuscitation and it has been proved also that HTS has immune modulation of leukocytes, in particular neutrophils^(5, 8).

Alejander *et al.* suggested that resuscitation with hypertonic saline solutions present significant potential as an immunomodulator agent for hemorrhagic shock and trauma victims⁽²⁾.

Yada *et al.* found that translocation occurs in hemorrhagic shock and develops acutely within 1-4 hours, extending over the next several days⁽¹²⁾. In this study, bacterial translocation to the peripheral blood was seen about 3 hours (mean) after hemorrhagic shock and bacteria were grown in 40% of blood derived from the rats who did not receive HTS as part of their resuscitation fluid and only in 3.3% of rats who received HTS.

In this study, it was clearly evident that HTS decreased bacterial translocation in hemorrhagic shock. volume replacement itself is probably not very effective, considering that, rats of group I given LR had a high rate of translocation. Alejandra *et al.* demonstrated that small volume of hypertonic saline would be beneficial as the initial fluid replacement in hypodynamic sepsis and he found that the use of HTS minimized the need for other resuscitation fluids.

In this study it was found that addition of HTS to LR controlled blood pressure of the shocked rats in group II and the mean arterial pressure was significantly higher in this group than that recorded in group I when LR was used alone. Similar results are also seen by Hotchkiss and Karl, Oliveira *et al.* and Wade^(7, 9, 11).

In accordance with our findings, Alam, reported in a large controlled USA multicenter study that hypertonic saline as initial treatment of hemorrhagic shock had a significant improvement of arterial pressure with significant decrease in long term mortality rate in patients with an entry mean arterial pressure below 70 mmHg⁽¹⁾.

Assalia *et al.* concluded that HTS and LR showed significant reduction in bacterial translocation with efficient control of blood pressure⁽³⁾. In other studies, hypertonic saline was shown to induce hemodynamic improvement in hemorrhagic shock⁽¹⁾. Whereas isotonic fluid administration requires large

volumes, hypertonic resuscitation offers the advantages of ease of transport, speed of administration, and almost instantaneous hemodynamic effect, moreover, its value in reversal of intestinal arteriolar vasoconstriction and reduction of the extent of bacterial translocation^(1, 3, 7, 9). The host responses related to inflammation and apoptotic process if the shock is not properly controlled is associated with liver insult which clearly plays an important role in metabolic processes following shock⁽¹³⁾. The results of various studies showed morphological alterations in liver such as sinusoidal space inflammatory infiltrate with severe degenerative changes and these changes were minimized by the use of HTS as a part of resuscitation fluids^(1, 3, 7, 13, 14).

Damage resulting from uncontrolled shock initiates a SIRS as far as serious metabolic disturbances and systemic signs manifested in the first hours following shock are related to the enlarged systemic capillary permeability with protein escapement into the interstitial space^(1, 3, 7, 13-15). The current results are consistent with this stress response of liver after insufficiently controlled shock which was minimized when HTS was used for resuscitation; however, these evidences require additional research and careful evaluation in more clinical trials.

CONCLUSION

Hypertonic saline was found to be effective for decreasing the rate of early bacterial translocation to blood and also for more efficient restoring of the mean arterial pressure in rats with induced hemorrhagic shock; in addition, HTS infusion minimizes the inflammatory and degenerative process in liver.

REFERENCES

1. **Alam H B (2011):** Advances in Resuscitation Strategies. *Int J Surg.*, 9: 5–12.
2. **Alejandra D, Ruy J, Junior L, Francisco P and Mauricio R (2006):** Small volume of hypertonic saline as the initial fluid replacement in experimental hypodynamic sepsis. *Crit Care*, 10: R62.
3. **Assalia A, Bitterman H, Hirsh TM and Krausz MM (2001):** Influence of hypertonic saline on bacterial translocation in controlled hemorrhagic shock. *Patterns of Shock*, 15: 307-11.
4. **Deitch EA, Shi HP, Feketeova E, Hauser CJ and Xu DZ (2003):** Hypertonic saline resuscitation limits neutrophil activation after trauma-hemorrhagic shock. *Shock*, 19: 328-33.
5. **Frank MP, Van H, James S, Ray C, Mary L, Peter P, and Johannes G Van H (2011):** The effects of hypertonic fluid administration on the gene expression of inflammatory mediators in circulating leucocytes in patients with septic shock: a preliminary study. *Annals Intensive Care*, 1: 44-51.
6. **Gustavo A, Bartolomeu N, Mauricio F and Sandro R (2008).** Operating Room Use of Hypertonic Solutions: A Clinical Review. *Clinics*, 63: 833–840.
7. **Hotchkiss RS, Karl IE, (2003):** The Pathophysiology and treatment of sepsis. *N Engl J Med.*, 348: 138-50.
8. **Murao Y, Haa M, Ohnishi K, Okuchi - K, Nakajirna Y, Hiasa Y, Junger WG, Hoyt DB, Ohinishi T (2003):** Hypertonic saline resuscitation reduces apoptosis and tissue damage of the small intestine in a mouse model of hemorrhagic shock, 20: 23-8.
9. **Oliveira RP, Velasco I, Soriano F, Friedman G (2002):** Clinical review: Hypertonic saline resuscitation in sepsis. *Crit Care*, 6: 397-8.
10. **Pascual JL, Fern LE, Seely AJ, Campisi G, Chaudhury P, Giannias B, Evans DC et al. (2002):** Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage. *Ann Surg.*, 236: 634-42.
11. **Wade CE (2002):** Hypertonic saline resuscitation in sepsis. *Crit Care*, 6: 418-23.
12. **Yada Langui MM, Coimbra R, Lancellotti C. Mirnica I, Garcia C, Correia N Jr, Rocha E Saliva M (2000):** Hypertonic saline prevents lung injury and bacterial translocation after hemorrhagic shock. *Shock*, 14: 594-8.
13. **Müller T, Kirsch M, & Petrat F (2017):** Attenuation of Multiple Organ Damage by Continuous Low-Dose Hypertonic Saline Infusions after Severe Hemorrhagic Shock in Rats. *Nutrients*, 9:889-93.
14. **Petrat F, de Groot H (2011):** Protection Against Severe Intestinal Ischemia/Reperfusion Injury in Rats. *J. Surg. Res.*, 167: E145–E155.
15. **Stern S.A.** Low-volume fluid resuscitation for presumed hemorrhagic shock: Helpful or harmful? *Curr. Opin. Crit. Care*, 7:422–30.