The effectiveness of hypertonic saline and pentoxifylline (HTS-PTX) resuscitation in hemorrhagic shock and sepsis tissue injury: Comparison with LR, HES, and LR-PTX treatments

Ho Jung Kim

The Graduate School
Yonsei University
Department of Medicine

The effectiveness of hypertonic saline and pentoxifylline (HTS-PTX) resuscitation in hemorrhagic shock and sepsis tissue injury: Comparison with LR, HES, and LR-PTX treatments

A Dissertation Submitted to the Department of Medicine

and the Graduate School of Yonsei University

in partial fulfillment of the requirements

for the degree of Doctor of Medicine

Ho Jung Kim

August 2012

This certifies that the Doctoral Dissertation of Ho Jung Kim is approved.

Thesis Supervisor: Kang Hyun Lee

Thesis Committee Member: Sung Oh Hwang

Thesis Committee Member: Gum Seok Bae

Thesis Committee Member: Hyun Kim

Thesis Committee Member: Jun Hwi Cho

The Graduate School

Yonsei University

August 2012

Acknowledgements

역사를 만드는 것이 얼마나 많은 노력과 시간이 필요한 것인지 한번 더 생각나게합니다. 더욱 성실하고 많은 노력을 통해 의학의 발전을 위해 노력해야겠다는 각오를 하게 됩니다.

학위를 준비는 동안 영감을 주시고 귀감이 되어 주신 이강현 교수님께 감사드립니다. 그리고 전공의 시절부터 많은 가르침과 표본을 보여주신 황성오 선생님께도 감사드립니다. 기꺼이 가르침에 동참해주신 존경하는 배금석, 김현, 조준휘 선생님께도 깊은 감사를 드립니다.

항상 버팀목이 되어주는 사랑하는 아내와 하준, 하윤에게 이 논문을 드립니다. 제 삶의 이유가 되시는 하나님께 모든 영광을 돌립니다.

저자 드림

Table of contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The effectiveness of hypertonic saline and pentoxifyline(HSPTX) resuscitation in hemorrhagic shock and sepsis tissue injury comparison with the LR, HES-HTS, LR-PTX treatments

Purpose: to compare the organs (lung and liver) injury and laboratory results of hemorrhagic shock and sepsis models with various treatments; HTSPTX, RLPTX, HESHTS and RL.

Methods: Male Sprague-Dawley rats (200 - 290g, Charles-River, St. Constant, Canada) were used this study and they were randomly assigned to one of the four groups (n = 16 per group) to receive the following treatments: (1) lactated Ringer's solution group (LR); (2) 7.5% hypertonic saline with hydroxyethyl starch group (HTS-HES); (3) lactated Ringer's (LR) solution with PTX group (LR-PTX); and (4) 7.5% hypertonic saline with PTX group (HTS-PTX) and each group was divided to one of the two following event models; (1) hemorrhagic shock (n = 8); (2) sepsis (n = 8). The venous catheter was utilized for injection of resuscitative fluids, and the arterial catheter was used to withdraw blood and monitor the mean arterial pressure (MAP) by MacLab® (PowerMac, AD Instruments, Australia). Organ (lung and liver) histologiy study, Bronchoalveolar lavage (BAL) and Cytokine test were performed.

Results: Mean lung injury score is 1.7. Total leukocyte count in the BAL 24 h after treatment was significantly higher in LR treated sepsis model (10 x 106 ±0.8) as compared to other sepsis treatment models (HTS-HES; 6 x 106 ± 1.2, LR-PTX; 5 x 106 ± 1.5, HTS-PTX; 5 x 106 ± 0.6)($p<0.05$). The higher total leukocyte count in the LR sepsis model (17 ±1.5 %) is due to an increased number of neutrophils, as compared to other sepsis treatment models (HTS-HES; 6 ± 0.8 %, LR-PTX; 10 ± 1.3 %, HTS-PTX; 5 ± 0.4 %%). The

total hepatic injury score in sepsis model was significantly greater in the LR group (9.9 ± 0.5) than either the other treatment groups (HTS-HES; 6.7 ± 0.8, LR-PTX; 5.6 ± 0.7, HTS-PTX; 3.1 ± 0.9, respectively; $p < 0.05$). Also, in shock model, LR group (10.6 ± 2.1) was significantly higer (HTS-HES; 5.8 ± 0.9, LR-PTX; 7.3 ±0.9, HTS-PTX; 3.5 ± 0.9, respectively; $p < 0.05$). HTSPTX resuscitation resulted in a 49% decrease in TNF-α, a 29% decrease in IL-1β, and a 58% decrease in IL-6 at 24 hours when compared with RL in shock model ($p < 0.05$) and, in sepsis model, in a 45% derease in TNF-α, a 24% decrease in IL-1β, and a 35% decrease in IL-6 at 24 hours when compared with LR ($p < 0.05$).

Conclusion: HTS-PTX may have most advantage over other proposed resuscitation strategies and LR-PTX or HTS-HES was better results than LR therapy.

Key Word: Shock, Sepsis, Hypertonic, Hydroxyethyl starch, Pentoxifyline, Cytokine

The effectiveness of hypertonic saline and pentoxifyline(HSPTX) resuscitation in hemorrhagic shock or sepsis tissue injury comparison with the LR, HES-HTS, LR-PTX treatments

Ho Jung Kim

Department of Medicine
(Directed by Professor Kang Hyun Lee)

The Graduate School, Yonsei University

I . INTRODUCTION

Pentoxifylline (PTX), a methylxanthine derivative and nonspecific phosphodiesterase inhibitor, has recently gained attentionas an immune modulator in sepsis and hemorrhage. It has the capacity to attenuate neutrophil activation and lung injury in endotoxemia and to prevent endothelial damage, neutrophil adhesion, and mortality in animal models of ischemia-reperfusion[1-4].

PTX alone is not capable of volume expansion and must be administered with fluid in the treatment of shock. Hemorrhagic shock results in peripheral tissue hypoxia and generalized ischemia. Although fluids are capable of restoring adequate tissue perfusion, they are implicated in the pathogenesis of ischemia-reperfusion injury, which results in systemic neutrophil activation and proinflammatory mediator production[5,6]. If inflammation continues unabated, it can result in end organ injury involving the liver, intestine, and, most notably, the lung.

The hemorheologic effects of PTX are reported that due to increased red blood cell deformability and to reduced blood viscosity. Sepsis has been studied experimentally by infusion of lipopolysaccharide (LPS) and dead or live bacteria, and the cecal ligation and puncture (CLP) procedure in order to mimic features of the septic state in humans7). Animals develop progressive bacteremia, with the release of multiple cytokines and chemokines into plasma, hypermetabolism, fever/ hypothermia, and other clinical features similar to those found in humans with sepsis[7].

The administration of racemic lactated Ringer's (LR), the current standard of fluid resuscitation, has been shown to enhance neutrophil activation, inflammation, and organ injury in both animal and human studies of ischemia-reperfusion injury[8-10]. Also, Gao et al. reported Hydroxyethyl starch (HES/ voluven®, Fresenius-Kabi, Germany) with hypertonic saline (HTS) solutions were more effective than no fluid resuscitation and lactated Ringer's solution in reducing the detrimental effects of hemorrhagic shock and infection

on the lungs, as seen by the significantly lower pulmonary microvascular permeability and wet to dry lung weight ratio, the improved arterial blood gases and lower levels of TGF-beta1and Smad2 expression in lung tissues[11]. Alternative resuscitation strategies designed to restore perfusion and down-regulate inflammation may ultimately reduce the incidence and severity of hepatic dysfunction after severe hemorrhage and sepsis. Two novel methods of resuscitation that may potentially aid in the attenuation of ischemia-reperfusion injury are the use of small volume hypertonic agents such as hypertonic saline (7.5% NaCl; HTS) and use of anti-inflammatory pharmacologic adjuncts such as the phosphodiesterase inhibitor pentoxifylline (1-[5-oxohexyl]-3,7-dimethylxanthine; PTX). the combined administration of hypertonic saline and pentoxifylline (HTS-PTX) may serve as a potent low volume anti-inflammatory fluid thatis capable of reducing resuscitation-induced organ dysfunction due to the inhibition of inflammatory cytokines, such as TNF-α and IL-6. Recently, lactated Ringer's (LR) combinded with HTS-PTX and HTS with HES was reported as a effective treatment in sepsis model[12-14]. However, until now, each alternative and combination therapy was not compared with all shock and sepsis models.

Therefore, The aim of this study is to compare the LR-PTX, HES-HTS, LR treatments with HTS-PTX treatments at the organs (lung and liver) injury and laboratory results of hemorrhagic shock or sepsis models.

Ⅱ. MATERIALS AND METHODS

1. Animal group of experimental design

The experiment was approved by the University of Soonchunhyang Animal Subjects Committee and in accordance with guidelines established by the National Institutes of Health.

Male Sprague-Dawley rats (200 - 290g, Charles-River, St. Constant, Canada) were used this study and they were randomly assigned to one of the four groups (n = 16 per group) to receive the following treatments: (1) lactated Ringer's solution group (LR); (2) 7.5% hypertonic saline with hydroxyethyl starch group (HTS-HES); (3) lactated Ringer's (LR) solution with PTX group (LR-PTX); and (4) 7.5% hypertonic saline with PTX group (HTS-PTX) and each group was divided to one of the two following event models; (1) hemorrhagic shock (n = 8); (2) sepsis (n = 8).

2. Animal preparation

Hemorrhagic shock model was entailed massive hemorrhage by tail amputation maintaining a mean arterial pressure of 35 mm Hg to 40 mm Hg for 60 minutes with withdrawal or reinfusion of blood as necessary and sepsis model was by intratracheal administration of lipopolysaccharide 2 mg/kg. For all rats, a 12-hour light and dark cycle was instituted, and food and water were provided ad libitum. Animals were anesthetized with Zoletil® (Tiletamine/ Zolazepam, Virbac, France) by intramuscular injection after pretreatment with atropine. A right inguinal incision was performed, and the femoral artery and

vein were cannulated with polyethylene catheters (PE no 10). The venous catheter was utilized for injection of resuscitative fluids, and the arterial catheter was used to withdraw blood and monitor the mean arterial pressure (MAP) by MacLab® (PowerMac, AD Instruments, Australia). HTS-PTX was infused with 4mL/kg of 7.5%NaCl+25mg/kg of PTX (Sigma, St. Louis, MO). LR-PTX was infused with 32mg/kg of LR+25mg/kg of PTX (Sigma, St. Louis, MO). PTX was dissolved in HTS or LR without the infusion of any additional fluid. HES-HTS was infused with 7.5ml/kg of voluven®+4mL/kg of 7.5%NaCl. After resuscitation with the fluid, shed blood was reinfused. The body temperature of the animals was maintained at 37°C throughout the experiment.

3. Lung histopathological examination

Lung specimens (each one at both lung lower lobes) in half of each event group were randomly collected and sampled, fixed in 10% paraformaldehyde and stained with hematoxylin and eosin. A pathologist blinded to the groups subjected the lungs to histopathological examination. A scoring system to grade the degree of lung injury was devised based on the following histologic features: 1) Degree of intra-alveolar macrophage in filtration, 2) septal edema, 3) congestion, 4) degree of neutrophil infiltration. 5) septal mononuclear cell infiltration, 6) alveolar hemorrhage, and 7) alveolar edema. A scale from 0 to 4 for each feature was used (0 = normal, 4 = most severe). A total score was obtained by adding values for each feature from every animal. Mean scores and standard errors were calculated for each group of animals[15].

4. Bronchoalveolar Lavage Collection

At the end of volume resuscitation, the catheters were removed, the incision was closed, and the animals were returned to their cages. The animals were killed via cardiac puncture 24 hours after the completion of resuscitation. Immediately after death, the trachea of each animal was accessed through a tracheotomy. The lungs were instilled with 10 mL of sterile normal saline under direct vision to avoid overdistension. The BALF was obtained and centrifuged at 250 g for 10 minutes. The supernatant was subsequently collected and stored at -80°C.

5. Hepatic Injury

Hepatic tissue from animals killed 24 hours after resuscitation was embedded in paraffin. Sections 5-μm thick were transferred onto glass slides and stained with hematoxylin and eosin (Richard Allen Scientific, Kalamazoo, MI). A pathologist, blinded to the treatment groups, subjected the slides to microscopic examination and scored the sections for injury according to the system developed by Sneed et al[16]. from 0 (normal) to 4 (severe) in the following four categories :necrosis, hemorrhage, hepatic parenchymal inflammatory infiltrate, and sinusoidal inflammatory infiltrate. The score from each category was summed to produce the total hepatic injury score. Serum alanine aminotransferase (ALT) were determined through previously described techniques[17].

6. Proinflammatory Cytokine Levels

The concentration of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 was measured from serum after resuscitation using commercially available sandwich enzyme immunoassay techniques (enzyme-linked immunosorbent assay kit, R&D Systems, Minneapolis, MN). Results are expressed in pg/mL.

7. Statistical analysis

All values are expressed as the mean ± standard error (SE) of n observations, where n represents the number of animals in each group. Each assay was performed in duplicate or triplicate where appropriate. Statistical significance of differences among groups was determined by analysis of variance with Bonferroni correction. A p value <0.05 was considered statistically significant.

Ⅲ. RESULTS

1. Lung injury score

Mean lung injury score is 1.7. HES-HTS (sepsis 4.7 ± 0.9/ shock 7.7 ± 0.8), LR-PTX (sepsis 6.2 ± 0.9/ shock 4.4 ± 0.9) and HTS-PTX (sepsis 4.7 ± 0.9/ shock 5.3 ± 0.9) significantly reduced lung injury as compared to LR (sepsis 12.6 ± 0.9/ shock 11.6 ± 1.1) group in both event models ($p<0.05$). The LR animals consistently demonstrated more intra-alveolar hemorrhage and congestion in sepsis but more interstitial and alveolar edema in shock **(Table 1)**.

Table 1. Average lung injury score for each estimated feature per each event model (n = 4) in each treatment group (n = 8).

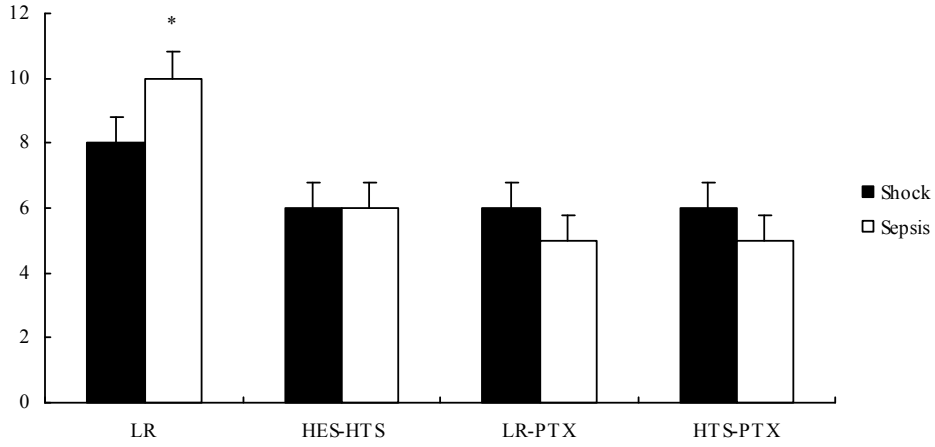
	LR		HTS-HES		LR-PTX		HTS-PTX	
	Sepsis	Shock	Sepsis	Shock	Sepsis	Shock	Sepsis	Shock
Alveolar macrophage	1.5	1.3	1	1	1.2	1.1	1	1
Septal edema	1.3	2.3	1.2	1.2	1	1	0.9	1.1
Congestion	2.3	1.8	0.6	0.6	0.5	0.7	0.8	0.6
PMN	1.9	1.1	0.4	0.8	0.9	0.3	0.6	0.7
Septal Mononuclear cell	1.9	2	0.7	0.7	0.8	0.6	0.4	0.6
Alveolar hemorrhage	2.4	1	0.1	0.5	0.9	0.2	0.6	0.7
Alveolar edema	1.5	2.3	0.7	0.7	0.9	0.5	0.4	0.6
Total	12.6 ± 0.9*	11.6 ± 1.1*	4.7 ± 0.9	7.7 ± 0.8	6.2 ± 0.9	4.4 ± 0.9	4.7 ± 0.9	5.3 ± 0.9

Individual scores range from 0 to 4 (0=normal; 4=most severe)

*;$p<0.05$ in LR compared with HTS-PTX.

2. Total leukocyte count and Percentage of neutrophil in the BAL

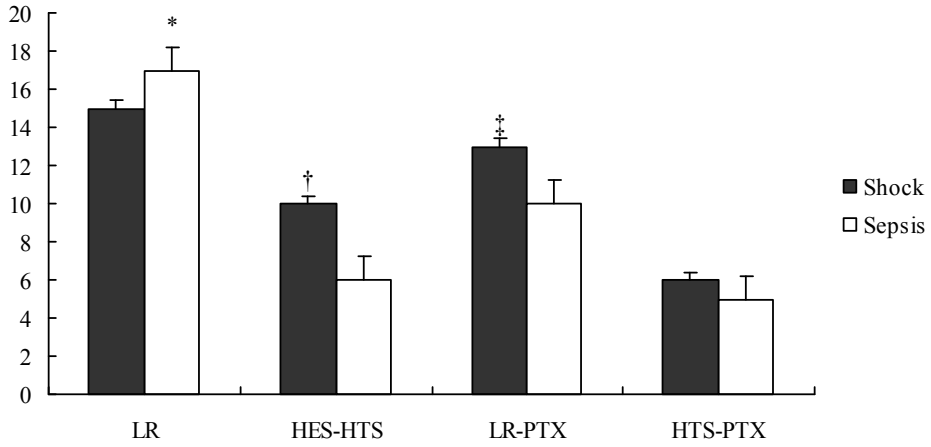
Total leukocyte count in the BAL 24 h after treatment was significantly higher in LR treated sepsis model (10 x 10^6±0.8) as compared to other sepsis treatment models (HTS-HES; 6x10^6±1.2, LR-PTX; 5x10^6±1.5, HTS-PTX; 5x10^6±0.6) ($p<0.05$). LR treated shock model (8x10^6±1.2) was also higher than other sepsis treatment models (HTS-HES; 6x10^6±0.9, LR-PTX; 6x10^6±1.6, HTS-PTX; 6x10^6±0.7) but there was not statistically significant **(Fig.1)**. The higher total neutrophil count in the LR sepsis model (17±1.5%) is due to an increased number of neutrophils, as compared to other sepsis treatment models (HTS-HES; 6±0.8%, LR-PTX; 10±1.3%, HTS-PTX; 5±0.4%). Also, in shock, HES-HTS (10±0.5%) or LR-PTX (13±0.4%) was significantly higher than HTS-PTX (6±0.3%) **(Fig.2)**.



*;$p<0.05$ in LR compared with HTS-PTX.

Fig. 1. Total leukocyte count in the BAL. LR-Treated animals in sepsis model

had a significant higher total leukocyte count in the BAL when compared to other treatment group but, in shock model, there was not statistically significant.



*;p<0.05 in LR compared with HTS-PTX, † ;p<0.05 in HES-HTS compared with HTS-PTX, ‡ ;p<0.05 in LR-PTX compared with HTS-PTX

Fig. 2. Percentage of neutrophil in the BAL was significantly higher for the LR group as compared to HSPTX in sepsis. HES-HTS or LR-PTX was higher than each level of HTS-PTX in shock.

3. Hepatic injury score and plasma ALT levels (IU/S)

Animals treated with standard LR resuscitation developed significant histologic changes including neutrophil infiltration, necrosis, and subcapsular hemorrhage. In contrast, the HSPTX infusion reduced these histologic changes after shock or sepsis. The total hepatic injury score was significantly greater in the LR group (sepsis 9.9 ± 0.5/ shock 10.6 ± 2.1). Also, sepsis (6.7 ± 0.8) in HTS-HES or shock in LR-PTX was significantly higher than HTS-PTX (sepsis 3.1 ± 0.9 / shock 3.5 ± 0.9) **(Table 2)**.

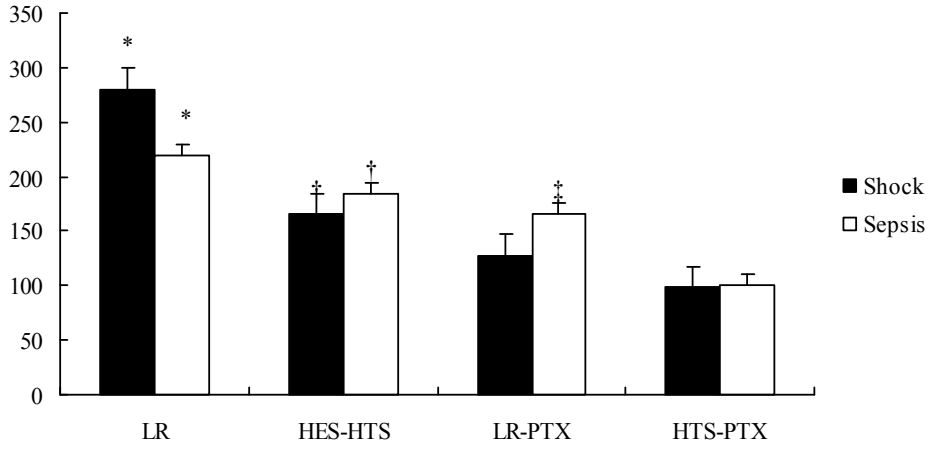
Table 2. Average liver injury score for each estimated feature per all treatment group.

	LR		HTS-HES		LR-PTX		HTS-PTX	
	Sepsis	Shock	Sepsis	Shock	Sepsis	Shock	Sepsis	Shock
Necrosis	2.9	2.1	1.6	1.2	2.1	1.8	0.9	1.1
Hemorrhage	2.4	2.9	1.8	1.9	0.8	1.7	1	1.1
Hepatic parenchymal inflammatory infiltrate	2.1	2.9	1.6	1.9	1.7	1.9	0.6	0.6
Sinusoidal inflammatory infiltrate	2.5	2.7	1.7	0.8	1	1.9	0.6	0.7
Total	9.9 ± 0.5*	10.6 ± 2.1*	6.7 ± 0.8†	5.8 ± 0.9	5.6 ± 0.7	7.3 ± 0.9‡	3.1 ± 0.9	3.5 ± 0.9

Individual scores range from 0 to 4 (0=normal; 4=most severe)

*;p<0.05 in LR compared with HTS-PTX, †;p<0.05 in HES-HTS compared with HTS-PTX, ‡;p<0.05 in LR-PTX compared with HTS-PTX

Hepatic injury was also estimated by determining the concentration of serum ALT at 24 hours (Fig. 4). Resuscitation with HTS-PTX resulted in markedly lower levels of ALT when compared with both models of RL (shock 98 ± 14 vs. 280 ± 31 IU/L; $p<0.05$ / sepsis 101 ± 11 vs. 220 ± 21 IU/L; $p < 0.05$) or HES-HTS (shock 98 ± 14 vs. 165 ± 18 IU/L; $p<0.05$ / sepsis 101±11 vs. 185±29 IU /L; $p< 0.05$). However, sepsis of LR-PTX (166 ± 12 IU/L) was significantly higher than HTS-PTX (101 ± 11) ($p< 0.05$) **(Fig. 3)**.

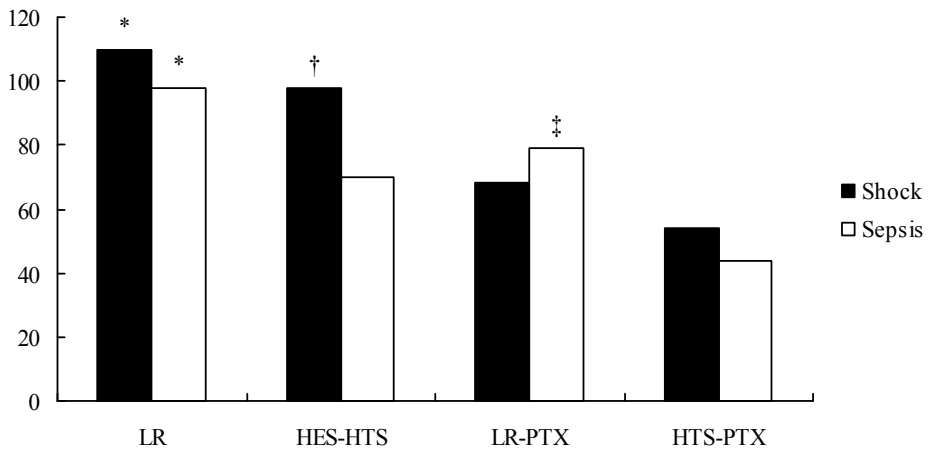


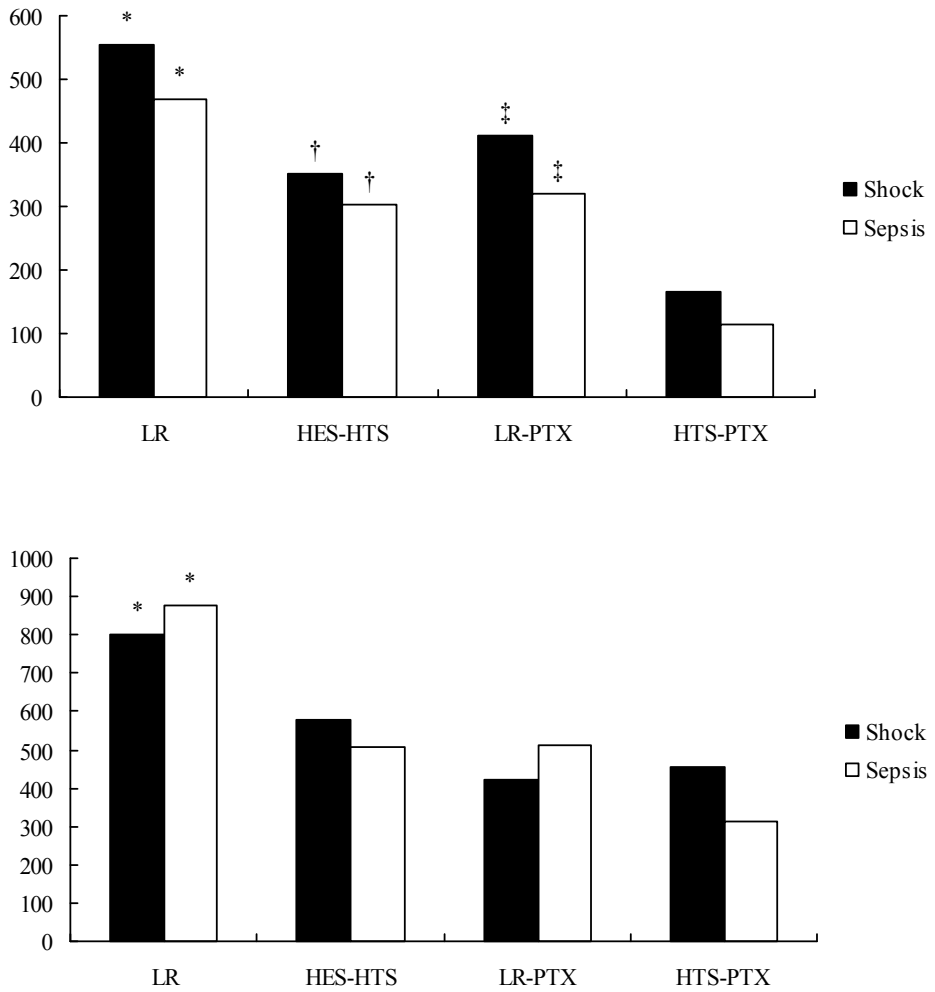
*;p<0.05 in LR compared with HTS-PTX, † ;p<0.05 in HES-HTS compared with HTS-PTX, ‡ ;p<0.05 in LR-PTX compared with HTS-PTX

Fig. 3. Serum ALT levels. LR or HES-HTS was higher level compared with HTS-PTX in both models but LR-PTX was higher just in sepsis compared with HTS-PTX.

4. Cytokine (TNF-α, IL-1β, IL-6 level) with all treatments

HTS-PTX resuscitation resulted in a 49% decrease in TNF-α, a 29% decrease in IL-1β, and a 58% decrease in IL-6 at 24 hours when compared with RL in shock model ($p < 0.05$) and, in sepsis model, in a 45% derease in TNF-α, a 24% decrease in IL-1β, and a 35% decrease in IL-6 at 24 hours when compared with LR ($p < 0.05$). HES-HTS and LR-PTX were statistically similar results each other and decreased compared with RL in both event models. However, in TNF- α, HTS-PTX was significantly lower than the shock of HES-HTX or sepsis of LR-PTX and was also lower than the both models of HES-HTX or LR-PTX in IL-1 β ($p<0.05$) **(Fig. 4)**.





*;p<0.05 in LR compared with HTS-PTX, † ;p<0.05 in HES-HTS compared with HTS-PTX, ‡ ;p<0.05 in LR-PTX compared with HTS-PTX

Fig. 4. Effects of resuscitation strategy on hepatic cytokine levels. TNF- α of HTS-PTX decreased at both of LR or shock of HES-HTS or sepsis of LR-PTX. IL-1β of HTS-PTX decreased at both of each groups. IL-6 of HTS-PTX was decreased only at both of LR.

Ⅳ. DISCUSSION

Shock and Sepsis were very important considerations in emergency treatments because they were occurred in much variable events and could be led to a fetal situations. But, they were initially treated with fluid which were known before or newly trials.

The primary clinical endpoint of fluid resuscitation after traumatic injury or sepsis is the restoration of intravascular volume and normalization of hemodynamic parameters. During the past decade, conventional fluid resuscitation with LR has been increasingly challenged by numerous studies documenting its proinflammatory nature18-20). In addition to its well-documented effects on neutrophils and endothelial cells, an increase in the number of iNOS mRNA transcripts has been observed after hemorrhage and LR administration21). The proinflammatory properties of racemic LR have been attributed to the D-form of lactate, an isomernot normally encountered in humans. This theory is supported by the observation that neutrophil activation does not occur upon exposure to LR strictly composed of the L-isomer22). Resuscitation with LR strictly composed of the L-isomer, however, does not circumvent the other major disadvantages of isotonic fluid resuscitation that are the large infusion volumes necessary to achieve adequate perfusion and the time-consuming process of infusing these large volumes. Both HTS and the addition of PTX to conventional LR resuscitation have been independently proposed as unique reperfusion strategie scapable of attenuating the post-shock inflammatory response.

Within about 10 years, HTS-PTX was most reported and reviewed for the treatments of sepsis and shock. Both regimens have been shown to independently ameliorate hepatic injury, neutrophil activation, endothelial dysfunction, and lung injury after hemorrhagic shock23-25). The mechanism by which HTS-PTX reduces reperfusion injury is dependent on the action of its two constituents. Both HTS and PTX have been shown to improve microcirculatory blood flow in hemorrhagic shock23,24). PTX is well known for its hemorheologic properties and has been shown to restore cardiac indices in an invivo model of shock when infused as an adjunct to LR25). More recently, PTX, through nonspecific phosphodiesterase inhibition and attenuation of TNF-α ,has been investigated for its broada nti-inflammatory potentialin septic and hemorrhagic shock secondary to its effect on neutrophil degranulation, adhesion, andoxidative burst21).

The use of PTX as an adjunct to standard LR resuscitation after shock has been studied by our laboratory and has resulted insimilar finding observed with HTS-PTX in this study. The disadvantage of large-volume resuscitation resulting from the simple combination of PTX and LR still remains. Therefore, intravascular volume expansion with a small volume of HTS with PTX is a more attractive alternative.

In animal hemorrhagic shock model, HTS-HES treatment significantly reduced the increase of IL-1β and IL-2 but not that of the other cytokines studied. Despite the strong effects of HTS and HES on cytokine production, both treatments had little effect on plasma lactate, base excess (BE), white blood cell (WBC) count, myeloperoxidase (MPO) content, and the wet / dry weight ratio of the lungs. Moreover, on day 7 after shock, the survival rate in rats treated with HTS was markedly, but not significantly, lower than that of Normal Saline-treated animals (47 % vs. 63 %, respectively)26). LR-PTX was

also compared with LR in hemorrhagic shock model and decreased lung injury and less neutrophil infiltration27).

The liver plays a major role in regulating biochemical, immunologic, and metabolic functions of the host. In the setting of trauma, it has been reported that approximately 20% of patients admitted in hypovolemic shock exhibit some evidence of liver dysfunction28,29). Although it is most often associated with hemorrhage, hepatic ischemia-reperfusion injury can also be a consequence of elective liver resection, transplantation, and other forms of shock. Therefore, strategies aimed at effectively limiting the severity of hepatic ischemia-reperfusion injury by either shortening the period of hypoperfusion or by ameliorating reperfusion-induced inflammation resulted are warranted. HTS-PTX alleviated hepatic injury, the induction of iNOS, and the excessive production of NO, in contrast to RL. Hierholzer et al. has previously reported that later events in the inflammatory cascade including the activation of NF-κB and STAT3 and the production of proinflammatory cytokines are dependent on the induction of iNOS30). Therefore, we sought to determine whether HTS-PTX also had an effect on these down stream events as well. Indeed, HTS-PTX effectively ameliorated LR-associated increasesin NF-κ Band STAT3 activation and the subsequent production of TNF-α, IL-1β, and IL-6. These findings are not surprising since TNF-α and IL-1β contain regulatory binding sites for NF-κB in their promoter regions and that STAT3 activation is a critical step in the production of IL-631,32). Linking the lesser known proximal with the well documented distal events of ischemia-reperfusion injury has been the focus of avast amount of recent research. Hierholzer et al. have proposed that acritical step between the initial cellular damage and activation of the inflammatory cascade is the release of damage associated molecular pattern signals from is chemic cells30). Therefore, it is plausible that

resuscitation HTS-PTX previously shown by Roche e Silva et al. to synergistically improve gastric oxygenation when compared with standard RL treatment 48 may decrease hepatocyte death and the subsequent induction of iNOS and iNOS-mediated events through a perfusion dependent mechanism. Further investigation is necessary to determine whether these events are indeed related. Both HTS-PTX are well-known modulators of late occurring events in inflammation such as neutrophil activation33).

When compared with LR. HTS-PTX resuscitation has been shown to markedly reduce neutrophil degranulation and adhesion molecule expression, two parameters of neutrophil function. Therefore, because of its effects on both early and late steps in the inflammatory cascade, HTS-PTX may have an advantage over other proposed resuscitation strategies that target only one specific portion of the inflammatory cascade. We compared with 4 treatment groups in organ injuries, cytokine, BAL of sepsis and shock models. HTS-PTX was better than the others in all factors and, especially, HTS-PTX was significantly different with LR in almost factors that was similar with past reports. But HTS-HES and LR-PTX were entity similar with each other and they were shown better than LR. At the neutrophil percentage in the BAL, LR-PTX was higher than HTS-HES on sepsis and shock models which was not different but there was significantly higher when each level of shock was compared with that of HTS-PTX.

Also, in serum ALT level, All treatment were over normal level. HTS-HES was higher than LR-PTX which was not different but HTS-PTX was significantly lower in both models of HES-HTS and sepsis of LR-PTX. All cytokine levels of combinded groups were decreased compared with LR but HTS-PTX was consistently lower than the others. Therefore, we proposed that HTS-PTX was better than HES-HTS or LR-PTX or LR in shock or sepsis

treatment and the proceed of lung injury in shock and liver injury in sepsis will be inhibited with HTS-PTX. Although the advantages of HTS-PTX in sepsis and shock was documented with comparison of variable treatments at this time there are some limitations to this study, which deserve mention. In our model, we did not divided the volume of HTS. In past reports, low volume of HTS was more effective in sepsis or shock. But we just focused combination with well known materials. In addition to this, HTS-PTX was not compared with control group which was basement of animal study but, during about 10 years, a lot of animal studies were reported with control and gold standard, especifically L-isomer LR which is now commercially available. Therefore, we regarded LR as control group.

Ⅴ. CONCLUSION

In an animal model of hemorrhagic shock and sepsis, HTS-PTX was better than HES-HTS or LR-PTX or LR treatment at the organs (lung and liver) injury and laboratory results .

REFERENCES

1. Deitch EA, Morrison J, Berg R, Specian RD. Effect of hemorrhagic shock on bacterial translocation, intestinal morphology, and intestinal permeability in conventional and antibiotic-decontaminated rats. Crit Care Med. 1990 May;18(5):529-36.

2. Deitch EA, Bridges W, Berg R, Specian RD, Granger DN. Hemorrhagic shock-induced bacterial translocation: the role of neutrophils and hydroxyl radicals. J Trauma. 1990 Aug;30(8):942-51; discussion 951-2.

3. Reed LL, Manglano R, Martin M, Hochman M, Kocka F, Barrett J. The effect of hypertonic saline resuscitation on bacterial translocation after hemorrhagic shock in rats. Surgery. 1991 Oct;110(4):685-8; discussion 688-90.

4. Rush BF Jr, Sori AJ, Murphy TF, Smith S, Flanagan JJ Jr, Machiedo GW. Endotoxemia and bacteremia during hemorrhagic shock. The link between trauma and sepsis? Ann Surg. 1988 May;207(5):549-54.

5. Stephan RN, Kupper TS, Geha AS, Baue AE, Chaudry IH. Hemorrhage Without Tissue Trauma Produces Immunosuppression and Enhances Susceptibility to Sepsis. Arch Surg. 1987;122(1):62-68.

6. Schmand JF, Ayala A, Chaudry IH. Effects of trauma, duration of hypotension, and resuscitation regimen on cellular immunity after hemorrhagic shock. Critical Care Medicine 1994, 22(7):1076-83.

7. Alves-Filho JC, Tavares-Murta BM, Barja-Fidalgo C, Benjamim CF, Basile-Filho A, Arraes SM, et al. Neutrophil function in severe sepsis. Endocr Metab Immune Disord Drug Targets 2006; 6: 151-158.

8. Rhee P, Burris D, Kaufmann C, Pikoulis M, Austin B, Ling G, et al. Lactated Ringer's solution resuscitation causes neutrophil activation after hemorrhagic shock. J Trauma. 1998;44:313 - 319.

9. Savage S, Fitzpatrick CM, Kashyap VS, Clouse WD, Kerby JD. Endothelial

dysfunction after Lactated Ringer's solution resuscitation for hemorrhagic shock. J Trauma. 2005;59:284 - 290.

10. Rhee P, Wang D, Ruff P, Austin B, DeBraux S, Wolcott K, et al. Human neutrophil activation and increased adhesion by various resuscitation fluids. Crit Care Med. 1998;28:74 - 78.

11. Gao J, Zhao WX, Xue FS, Zhou LJ, Yu YH, Zhou HB. Effects of different resuscitation fluids on acute lung injury in a rat model of uncontrolled hemorrhagic shock and infection. J Trauma. 2009 Dec;67(6):1213-9.

12. Park KH, Lee KH, Kim H, Hwang SO. The Effect on Acute Lung Injury When 7.5% Hypertonic Saline-Hydroxyethyl Starch for Resuscitated Rats after Hemorrhagic Shock. J Korean Soc Emerg Med 2009;20:272-9.

13. Allison KP, Gosling P, Jones S, Pallister I, Porter KM. Randomized trial of hydroxyethyl starch versus gelatin for trauma resuscitation. J Trauma 1999;47:1114-21.

14. Vollmar B, Lang G, Menger MD, Messmer K. Hypertonic hydroxyethyl starch restores hepatic microvascular perfusion in hemorrhagic shock. Am J Physiol 1994;266: H1927-34.

15. Deree J, Loomis WH, Wolf P, Coimbra R. Hepatic Transcription Factor Activation and Proinflammatory Mediator Production is Attenuated by Hypertonic Saline and Pentoxifylline Resuscitation After Hemorrhagic Shock. J Trauma. 2008 May;64(5):1230-8; discussion 1238-9.

16. Sneed RA, Grimes SD, Schultze AE, Brown AP, Ganey PE. Bacterial endotoxin enhances the hepatotoxicity of allyl alcohol. Toxicol Appl Pharmacol. 1997;144:77 - 87.

17. Coimbra R, Porcides RD, Melbostad H, Loomis W, Tobar M, Hoyt DB, et al. Nonspecific phosphodiesterase inhibition attenuates liver injury in acute endotoxemia. Surg Infect. 2005;6:73 - 85.

18. Alam HB, Stanton K, Koustova E, Burris D, Rich N, Rhee P. Effect of

different resuscitation strategies on neutrophil activation in a swine model of hemorrhagic shock. Resuscitation. 2004;60:91 - 99.

19. Alam HB, Sun L, Ruff P, Austin B, Burris D, Rhee P. E- and P-selectin expression depends on the resuscitation fluid used in hemorrhaged rats. J Surg Res. 2000;94:145 - 152.

20. Savage SA, Fitzpatrick CM, Kashyap VS, Clouse WD, Kerby JD. Endothelial dysfunction after lactated ringer's solution resuscitation for hemorrhagic shock. J Trauma. 2005;59:284 - 290.

21. Alam HB, Stegalkina S, Rhee P, Koustova E. cDNA array analysis of gene expression following hemorrhagic shock and resuscitation in rats. Resuscitation. 2002;54:195 - 206.

22. Koustova E, Stanton K, Gushchin V, Alam HB, Stegalkina S, Rhee PM. Effects of lactated ringer's solutions on human leukocytes. J Trauma. 2002;52:872 - 878.

23. Pascual JL, Ferri LE, Seely AJ, Campisi G, Chaudhury P, Giannias B, et al. Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduced in vivo vascular leakage. Ann Surg. 2002;236:634 - 642.

24. Coe DA, Freischlag JA, Johnson D, Mudaliar JH, Kosciesza SA, Traul DK, et al. Pentoxifylline prevents endothelial damage due to ischemia and reperfusion injury. J Surg Res. 1997;67:21 - 25.

25. Deree J, Loomis WH, Putnam JG, Wolf P, Coimbra R. Pentoxifylline-supplemented resuscitation attenuates both early and late mediators of hepatic inflammation after hemorrhagic shock. J Organ Dysfunct. In press.

26. Bahrami S, Zimmermann K, Szelényi Z, Hamar J, Scheiflinger F, Redl H, et al. Small-Volume Fluid Resuscitation With Hypertonic Saline Prevents Inflammation But Not Mortality in A Rat Model of Hemorrhagic Shock. Shock

2006;25:283-9.

27. Yada-Langui MM, Coimbra R, Lancellotti C, Mimica I, Garcia C, Correia N Jr, Rocha e Silva M.Hypertonic saline and pentoxifylline prevent lung injury and bacterial translocation after hemorrhagic shock. Shock. 2000 Dec;14(6):594-8.

28. Heckbert SR, Vedder NB, Hoffmann W, Winn RK, Hudson LD, Jurkovich GJ, et al. Outcome after hemorrhagic shock in trauma patients. Am J Physiol. 1998;45:545 - 549.

29. Helling TS. The liver and hemorrhagic shock. J Am Coll Surg. 2005;201:774 - 783.

30. Hierholzer C, Billiar TR, Tweardy DJ, Harbrecht B. Reduced hepatic transcription factor activation and expression of IL-6 and ICAM-1 after hemorrhage by NO scavenging. Arch Orthop Trauma Surg. 2003;123:55 - 59.

31. Ndengele MM, Muscoli C, Wang ZQ, Doyle TM, Matuschak GM, Salvemini D. Superoxide potentiates NF-kB activation and modulates endotoxin-induced cytokine production in alveolar macrophages. Shock. 2005;23:186 - 193.

32. Song M, Kellum JA. Interleukin-6. Crit Care Med. 2005;33:S463 - S465.

33. Coimbra R, Loomis WH, Melbostad H, Tobar M, Porcides RD, Lall R, et al. Role of hypertonic saline and pentoxifylline on neutrophil activation and tumor necrosis factor-_ synthesis: a novel resuscitation strategy. J Trauma. 2005;59:257 - 265.

국 문 요 약

출혈성 쇽과 폐혈증 동물모델에서 Lactate ringer (LR)액, Hydroethyl starch (HES)-Hypertonic saline (HTS), LR-Pentoxifyline (PTX) 치료법과 비교된 고장용액과 펜톡시필린 혼합(HTS-PTX)치료의 효과

배경: 출혈성 쇽과 폐혈증 동물모델에서 LR, HES-HTS, LR-PTX 치료법과 비교된 고장용액과 펜톡시필린 혼합(HTS-PTX)치료의 주요장기(간, 폐) 및 검사실 소견의 비교를 통해 HTS-PTX의 효과를 알아보고자 하였다.

방법: 64 마리의 Sparague-Dawley 쥐(200-290 g, male)가 연구에 사용되었다. 연구는 Lactate ringer (LR)액, Hydroethyl starch (HES)-Hypertonic saline (HTS), LR-Pentoxifyline (PTX) 치료법, 그리고 고장용액과 펜톡시필린 혼합(HTS-PTX)와 같이 총 4가지 치료군으로 분류하였는데 각 군마다 16마리를 배정하였고 각 군을 다시 출혈성 쇽과 폐혈증 모델로 분류하여 각 8마리씩 배정하였다. 각 군을 간과 폐의 조직학적 검사, Bronchoalveolar lavage (BAL) 그리고 Cytokine검사를 시행하여 각 군을 비교하였다.

결과: 평균 폐 손상점수는 1.7이었다. BAL에서 총 백혈구수는 폐혈증의 LR (10 x 106 ± 0.8)이 다른 군 (HTS-HES; 6 x 106 ± 1.2, LR-PTX; 5 x 106 ± 1.5, HTS-PTX; 5 x 106 ± 0.6)에 비해서 높았다($p<0.05$). 그리고 폐혈증에서 총 호중구수도 LR(17 ± 1.5 %) 에서 높았다. 쇽에서도 HES-HTS (10 ± 0.5 %) 또는 LR-PTX (13 ± 0.4 %)도 HTS-PTX (6 ± 0.3 %)에 비해 높았다. 간손상 점수는 폐혈증의 HTS-HES (6.7 ± 0.8) 또는 쇽의 LR-PTX가 HTS-PTX (폐혈증 3.1 ± 0.9/ 쇽 3.5 ± 0.9)에 비해 높았다. ALT수치에서도 HTS-PTX는 LR과 HES-PTX군에게는 쇽과 폐혈증에서 모두 낮게 나타났고 LR-PTX군에서는 폐혈증만 의미있게 낮았다(101 ± 11 vs. 166 ± 12 IU/L; $p<0.05$). 24시간째 Cytokine의 결과는 HTS-PTX가 쇽의 LR에 비해서 TNF-α는 49%, IL-1β는 29%, 그리고 IL-6는 58% 낮게 나타났다($p < 0.05$). 그리고 쇽에서도 LR에 비해서 TNF-α는

45%, IL-1β는 24%, 그리고 IL-6는 35% 낮게 나타났다($p < 0.05$). HES-HTS와 LR-PTX는 LRQHEKSMS 낮았으나 두군간에는 차이가 없었다. 그리고 TNF-α에서 HTS-PTX는 쇽의 HES-PTX 또는 폐혈증의 LR-PTX보다 통계적으로 낮았고 IL-1β에서는 쇽과 폐혈증 모두에서 낮았다($p<0.05$).

결론: HTS-PTX는 쇽과 폐혈증 동물 모델에서 LR, HES-HTS, LR-PTX 치료법에 비해 장기의 손상수치과 실험실결과 등에서 좋은 결과를 보였다.

핵심 되는 말 : Sepsis, Hypertonic, Hydroxyethyl starch, Pentoxifyline, Cytokine