Original Article
Effect of different fluid resuscitation methods on the lung injury and hemorrhagic shock

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Abstract: Objective: To observe the efficacy of limited fluid resuscitation on the early lung injury of hemorrhagic shock rats. Methods: Seventy SPF mature Sprague-Dawley (SD) rats were randomly divided into the sham group (n=10), model group (n=20), regular fluid resuscitation group (n=20) and limited fluid resuscitation group (n=20). In two fluid resuscitation groups, fluid resuscitation was carried out using the Ringer lactate solution in combination with the bleeding amount of rat (1:1). For rats in the regular fluid resuscitation group and limited fluid resuscitation group, the arterial pressure was sustained between 80 and 85 mmHg and between 50 and 55 mmHg, respectively. Results: Statistical significance was identified in the differences of the wet/dry weight ratio of the lung as well as the scores of pathological injuries of rat lungs among four groups ($p<0.05$) at two hours following fluid resuscitation. In the meantime, comparisons of the levels of TNF-α and IL-10 in lung tissues of rat among four groups showed statistically significant differences ($p<0.05$). Likewise, SOD and MDA levels in four groups also showed statistically significant differences at 2 hours after fluid resuscitation ($p<0.05$). Two hours following the fluid resuscitation, Bax and Bcl-2 levels of four groups also showed statistically significant differences ($p<0.05$). Two hours after fluid resuscitation, the survival rates of the rats in four groups were 100% in the control group, 15.0% in the model group, 75.0% in the regular fluid resuscitation group and 90.0% in the limited fluid resuscitation group ($p<0.05$). Conclusion: In comparison with the regular fluid resuscitation, limited fluid resuscitation can improve the survival of rats through ameliorating the hemorrhagic shock, inflammatory responses and oxidative stress of lung tissues, and mitigating the apoptosis of lung cells.

Keywords: Limited fluid resuscitation, hemorrhagic shock, lung injury, rat

Introduction

Hemorrhagic shock is refers to the excessive fluid loss caused by trauma-induced blood loss, vomiting or diarrhea, resulting in a rapid, massive reduction of blood volume, and particularly, the early-stage hypotension, or organ failure in case of delayed treatment, which are also considered as the major cause for the death of patients [1-3]. With the development of society and economy as well as the progress in traffic and heavy industry, the incidence rate of trauma keeps increasing with an augmentation in the incidence of hemorrhagic shock caused by trauma. Besides, hemorrhagic shock is also one of the hot topics in the clinical treatment and research [4, 5].

To restore the blood pressure and the blood volume has been considered as the major goal in treatment of the hemorrhagic shock patients, while fluid resuscitation is one of the methods to reach this goal. However, in light of the complicated pathophysiological mechanism of hemorrhagic shock, controversy remains in choice of the proper fluid resuscitation method for hemorrhagic shock [6, 7]. Currently, in clinical practice, rapid administration of fluid to restore the blood pressure has been frequently adopted, but the recent clinical or animal studies showed that regular fluid resuscitation results in the acidosis, destructing the hemostasis and leading to the coagulation disorders, or even aggravating the bleeding, reducing the success rate of resuscitation, and in some

cases, it may cause death [8, 9]. Limited fluid resuscitation, a novel method for treatment of hemorrhagic shock, can sustain the blood pressure at a relatively low level through limited infusion of fluid to guarantee the blood perfusion of organs, mitigate the load and increase the success rate of resuscitation. Lung injury is a very severe complication among the hemorrhagic shock patients, and usually gives to the respiratory dysfunction, e.g. respiratory distress syndrome, or even death [2, 10]. In recent years, limited fluid resuscitation has been scarcely reported in the studies involving the lung injury of patients with hemorrhagic shock.

In this study, we aimed to investigate how limited fluid resuscitation affects the lung injury of rat by establishing the hemorrhagic shock models in rats.

Materials and methods

Subjects

A total of 70 specific pathogen free (SPF) mature Sprague Dawley (SD) rats were randomized into four groups, i.e. the sham group (n=10), model group (n=20), regular fluid resuscitation group (n=20) and limited fluid resuscitation group (n=20). All rats were provided by the Experiment Animal Center of Guangzhou University of Chinese Medicine [SCXK (Yue) 2013-0034]. Rats were raised at a humidified chamber (45-55%) and 18-25°C at a 12/12 light/dark cycle, and the noise strength was controlled within 60 db. Ventilation was performed at frequency of 15 time/hour, and the cage was cleaned twice per week. Rats were fed with the complete food and sterilized acidized water at pH 2.5 to 3. Following one week of adaptive feeding, model establishment and treatment were performed.

Model establishment and treatment

Eight hours prior to the surgery, sterilization was performed at the abdomen of rats, followed by intraperitoneal injection of 10% chloral hydrate (300 μg/g, Shanghai Shifeng Biological Technology Co., Ltd.). Neck hair was removed and the skin and fascia were exposed sequentially, and the carotid artery was isolated bluntly. Unilateral intubation with the 22# anticoagulant arterio-venous catheter (Shanghai Zhangdong Medical Science And Technology

Co., Ltd.), during which the blood pressure was monitored by connecting the BIOPAC multileads (16-lead) physiology recorder (Upwards Teksystems Co., Ltd.) with the catheter through T-branch pipe. Rats in the sham group received no treatment, while for those in the remaining three groups, the catheter was opened at 10 min after intubation to drainage the blood which was later preserved for infusion, during which the arterial pressures of rats in the model group were maintained within 40 and 45 mmHg, and those in the regular and limited fluid resuscitation groups administrated the Ringer lactate solution (Darfun Biological Test Center) referring to the bleeding amount of rat (1:1) for fluid resuscitation. For regular fluid resuscitation, the arterial pressure of rats was sustained within 80 and 85 mmHg, and for limited fluid resuscitation, the pressure was sustained within 50 and 55 mmHg.

Observation indexes

Rats survived from the surgeries were sacrificed two hours following treatment, and those who died within 2 hours were prepared for tissue collection. For all rats, wet/dry ratio of lung tissues was determined, and the evaluation of the lung injury was carried out by pathological score. In addition, we also measured the levels of inflammatory cytokines [tumor necrosis factor α (TNF-α) and interleukin-10 (IL-10)], oxidative stress-related indexes [malondialdehyde (MDA) and superoxide dismutase (SOD)], and apoptosis-related indexes (Bax and Bcl-2), as well as the 2-hour survival rate of rats.

Detection methods

Wet/dry ratio of lung tissues: The wet weight and dry weight were measured using the electronic analytic scale (Shanghai Meiyu Equipment Co., Ltd.) with lung tissue that had been dried at 80°C for 12 hours. Wet/dry ratio = Wet weight (g)/dry weight (g).

Scoring of the pathological injury: For the integrity and edema of pulmonary alveoli, infiltration of inflammatory cells and interstitial edema, the score was given within 0 to 4 points (0 for normal).

Detection of inflammatory cytokines and apoptosis-related indexes: TNF-α, IL-10, Bax and Bcl-2 were evaluated by ELISA kit. In this experi-

Table 1. General data of rats

	Sham group	Model group	Regular liquid resuscitation group	Limited fluid resuscitation group	χ^2/t	p-value
Number	10	20	20	20		
Sex [n,(%)]					0.975	0.807
Male	5 (50.0)	11 (55.0)	12 (60.0)	9 (45.0)		
Female	5 (50.0)	9 (45.0)	8 (40.0)	11 (55.0)		
Age (day)	59.32±3.25	60.44±3.41	58.96±3.18	61.39±3.29	2.084	0.111
Weight (g)	254.25±11.66	261.73±12.54	258.67±11.83	262.41±12.49	1.212	0.312
Total blood volume (mL)	16.72±1.02	16.58±1.03	16.49±1.01	16.61±1.02	0.120	0.948
Blood loss volume (mL)		9.09±0.58	9.11±0.57	9.05±0.56	0.057	0.944
Before the bleeding MAP (mmHg)	113.33±6.41	115.72±6.93	119.41±6.82	116.59±6.77	2.028	0.119
Recovery after 2 h MAP (mmHg)	108.27±6.83	74.34±8.69[*,$]	83.94±11.83[*,#,$]	96.33±12.96[*,#,&,$]	16.272	<0.001

Note: [*]p<0.001 *vs.* control group; [#]p<0.001 *vs.* model group; [&]p<0.001 *vs.* regular fluid resuscitation group; [$]p<0.001 *vs.* the level before bleeding.

ment, three replicate wells were set for each sample, and this experiment was conducted in triplicate. ELISA kits for TNF-α, IL-10, Bax and Bcl-2 were provided by Shanghai Jingkang Biological Engineering Co., Ltd.

Measurement of the oxidative stress-related indexes, including SOD and MDA, was carried out using the colorimetric method in strict accordance with the instructions of kit. The SOD level was reflected by the absorbance at wavelength of 469 nm, and MDA at 535 nm. Measurement was performed within 3 min, and each sample was measured in triplicate. SOD kit and MDA kit were purchased from Promocell Biological Technology Co., Ltd.

Statistical analysis

SPSS 19.0 software (Asia Analytics Formerly SPSS China) was utilized to perform statistical analysis. Enumeration data were presented as ratio, and compared with chi-square test, while measurement data were presented as mean ± standard deviation. Analysis of variance was adopted for intergroup comparisons, while LSD test for pairwise comparison. Survival curve was prepared using Kaplan-Meier method, and the differences among the curves of four groups were tested by Log Rank. *P*<0.05 suggested that the difference had statistical significance.

Results

Model establishment

Seventy SD rats were randomized into 4 groups, and the comparisons over the age (P=0.807)
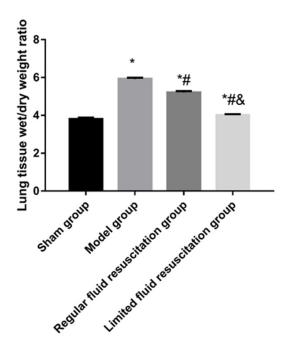
and weight (P=0.312) among four groups showed no differences with statistical significance. Also, the total blood volume (P=0.948), bleeding amount (P=0.944) and mean arterial pressure before bleeding (P=0.119) were compared, and no statistically significant difference was identified. Two hours after fluid resuscitation, we found that differences in MAP among four groups showed statistical significance (*p*<0.001), in which the MAP levels in the model group, regular fluid resuscitation group and the limited fluid resuscitation group were all lower than that in the control group (*p*<0.001). Rats in two resuscitation groups had a higher MAP level than those in the model group (*p*<0.001), and the limited resuscitation group was higher than that in the regular group (*p*=0.003); MAP levels in these three groups at 2 hours after resuscitation were also lower than those before bleeding (*p*<0.001; **Table 1**).

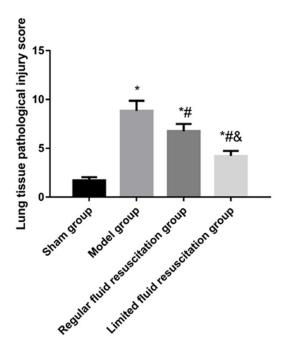*Wet/dry weight ratio of lung tissues*

Two hours following the fluid resuscitation, statistical significance was identified in the differences of the wet/dry weight ratio of the lung among four groups (*p*<0.001), and the ratios in the model group, regular fluid resuscitation group and limited fluid resuscitation group were all higher than those in the control group (*p*<0.001). Ratio in the model group was the highest, followed by regular and limited fluid resuscitation groups, sequentially (*p*<0.001; **Figure 1**).

Pathological score of the lung injuries

Two hours after fluid resuscitation, differences with statistical significance were found in com-
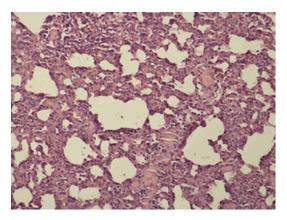
Figure 1. Analysis of the wet/dry weight ratio of lung tissues. $^*p<0.001$ *vs.* sham group; $^\#p<0.001$ *vs.* model group; $^\&p<0.05$ *vs.* regular fluid resuscitation group.
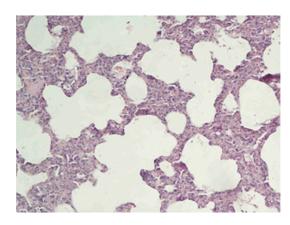

Figure 2. Analysis of the pathological score of lung injury. $^*p<0.05$ vs. sham group; $^\#p<0.001$ vs. model group; $^\&p<0.001$ *vs.* regular fluid resuscitation group.

Figure 3. The inflammatory process of leukocyte infiltration and alveolar space, edema, alveolar deformation and alveolar wall thickening.

Figure 4. The degree of injury was improved, the infiltration of inflammatory cells in the interstitial lung and alveolar was reduced, and the interstitial edema and alveolar hemorrhage were reduced.

parisons of the scores of pathological injuries of rat lungs at two hours following fluid resuscitation ($p<0.001$). The score of pathological injury of rat lungs in the model group, regular fluid resuscitation group and the limited fluid resuscitation group were all higher than that in the control group ($p<0.001$), and the highest score was found in the model group, followed by limited fluid resuscitation group and regular fluid resuscitation group ($p<0.001$; **Figures 2**-**4**).

Measurement of inflammatory cytokines

Following 2 hours of fluid resuscitation, comparisons of the levels of TNF-α and IL-10 in lung tissues of rat among four groups showed statistically significant differences ($p<0.001$), among which the levels in the model group were the highest, sequentially followed by model group, limited fluid resuscitation group, regular fluid
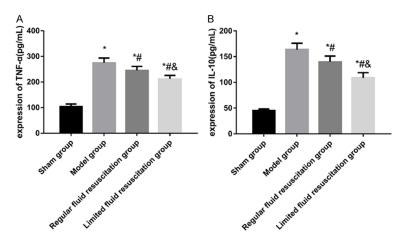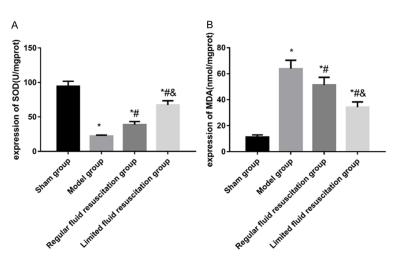
Figure 5. A: TNF-α level in lung tissues determined by ELISA kit. B: IL-10 level in lung tissues determined by ELISA kit. *p<0.05 vs. sham group; #p<0.001 vs. model group; &p<0.001 vs. regular fluid resuscitation group.

Figure 6. A: SOD level in lung tissues. B: MDA level in lung tissues. *p<0.001 vs. sham group; #p<0.001 vs. model group; &p<0.001 vs. regular fluid resuscitation group.

lungs were significantly elevated when compared with the level in the model group (p<0.001), while the MDA levels were lower than that in the model group (p<0.001). In the limited fluid resuscitation group, the SOD level was higher than that in the regular fluid resuscitation group (p<0.001), but on the contrary, the level of MDA was lower than the regular fluid resuscitation group (p<0.001; **Figure 6**).
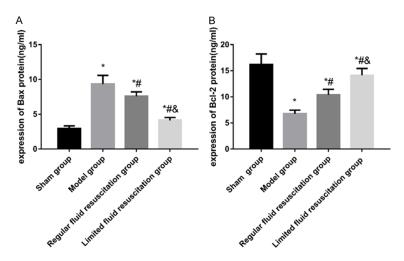
Measurement of the apoptosis-related indexes

Two hours following the fluid resuscitation, Bax and Bcl-2 levels of four groups also showed statistically significant differences (p<0.001): The levels of Bcl-2 in the model group, regular fluid resuscitation group and the limited resuscitation group were significantly lower than that in the control group (p<0.001), while Bax showed the definitely different trend (p<0.001); in the regular and limited fluid resuscitation group, the Bcl-2 levels in the rats were significantly higher than those in the model group (p<0.001); while the Bax was lower (p<0.001); in the limited 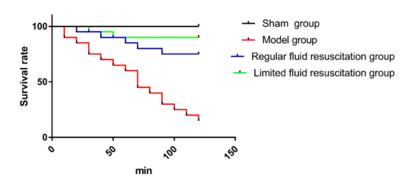fluid resuscitation group, the Bcl-2 level of rat was higher than that in the regular fluid resuscitation group (p<0.001) with a lower level of Bax (p<0.001; **Figure 7**).

resuscitation group and the control group (p<0.001; **Figure 5**).

Measurement of the oxidative stress-related indexes

Likewise, SOD and MDA levels in four groups also showed statistically significant differences at 2 hours after fluid resuscitation (p<0.001), and the levels of SOD in the rat lungs of model group, regular fluid resuscitation group and the limited fluid resuscitation group were all lower than that in the control group, but, conversely, MDA level in the control group was the lowest (p<0.001). In the regular and limited fluid resuscitation groups, SOD levels in the rat

Two-hour survival rate of rats in four groups

Two hours after fluid resuscitation, the survival rates of the rats in four groups were 100% in the control group (10/10), 15.0% in the model group (3/20), 75.0% in the regular fluid resuscitation group (15/20) and 90.0% in the limited fluid resuscitation group (18/20) (p<0.001). Comparisons of the survival rates of rats among model group, regular fluid resuscitation group and limited fluid resuscitation group showed differences with statistical significance

Figure 7. A: Bax level in lung tissues determined by ELISA kit. B: Bcl-2 level in lung tissues determined by ELISA kit. *p<0.001 vs. sham group; #p<0.001 vs. model group; &p<0.001 vs. regular fluid resuscitation group.

Figure 8. Analysis of the 2-hour survival rate of rats in four groups. Comparisons of the survival rates of rats among model group, regular fluid resuscitation group and limited fluid resuscitation group showed differences with statistical significance (p<0.001): The survival rates of rats in two resuscitation groups were higher than that in the model group (p<0.001), while the survival rate of the limited fluid resuscitation group was higher than that in the regular fluid resuscitation group (p<0.001).

(p<0.001): The survival rates of rats in two resuscitation groups were higher than that in the model group (p<0.001), while the survival rate of the limited fluid resuscitation group was higher than that in the regular fluid resuscitation group (p<0.001; **Figure 8**).

Discussion

In hemorrhagic shock, massive bleeding can rapid decrease the effective circulating blood volume, resulting in insufficient blood supply to the key organs, and cell apoptosis. Lung tissues has been considered as the major organs mostly affected by the hemorrhagic shock [11-

13]. In this study, we evaluated the effect of different fluid resuscitation methods on the hemorrhagic shock and lung injuries.

In this study, rats in the model groups showed an acute decrease in MAP, damaged pulmonary alveoli and increased pathological score, indicating the lung injuries, which are consistent with the previous reports involving the hemorrhagic shock animals [14]. Next, we determined the levels of Bax and Bcl-2, proteins that reflect the apoptosis [15]. In the model group, rats had an elevated Bax in comparison with those in the control group, with a decreased Bcl-2, indicating that the apoptosis in hemorrhagic shock rats was enhanced; after fluid resuscitation, phenomenon above was reversed, implicating that cell apoptosis in lung tissues was somehow ameliorated with increased pathological score. This suggested the amelioration in cell apoptosis in lung tissues, together with increased pathological score and integrity of pulmonary alveoli.

The wet/dry weight ratio of lung tissue reflects the water content of lung tissues [16]. Our results showed that the wet/dry weight ratio of lung tissue in the model group was significantly higher than that in the control group, indicating the imbalance of edema elimination in lung tissues of hemorrhagic shock rats, with the symptoms of tissue edema and increased pathological scores, which indicated the alveoli edema and intestinal edema. So maintenance of the liquid generation and elimination in lung tissues is crucial for the treatment of the lung injury in hemorrhagic shock [17]. Following the fluid resuscitation for hemorrhagic shock rats, we found an evident decrease in wet/dry weight ratio of lung tissues, especially in those that

received the limited fluid resuscitation, showing that limited fluid resuscitation works better in improving the balance between the generation and elimination of liquid in lung tissues to reduce the edema. Apart from those above, inflammation and oxidative stress response are also the major factors contributing to the lung injuries to hemorrhagic shock rats [18, 19]. We selected two indicators in close association with the inflammation and two indexes that precisely reflect the oxidative stress response. Previous studies showed inflammation can induce an acute increase of TNF-α and IL-10 [20, 21]. SOD can catalyze the disproportionated reaction of oxide radicals to antagonize the oxidative stress injuries [22]. MDA can induce the cross bonding among proteins, thereby aggravating the cell injuries [22]. In our study, hemorrhagic shock evidently caused the increases of TNF-α and IL-10 in lung tissues, and, with the decreased SOD and increased MDA, the oxidative stress response was exacerbated, which is consist with the previous report [23-25]. HE staining of the lung tissues also indicated an increased score of pathological score of lung and aggravated inflammatory cells. Following the fluid resuscitation, ameliorations in varying degrees were seen in levels of TNF-α, IL-10, SOD and MDA, suggesting that inflammatory response, oxidative stress response and the infiltration of inflammatory cells are also improved. Likewise, rats that received the limited fluid resuscitation gained better efficacy. Survival analysis also showed that a significant elevation of 2-hour survival rate was identified in rats that received the fluid resuscitation, and limited fluid resuscitation manifested a better efficiency in improving the survival rate of rats, suggesting that in comparison with the regular fluid resuscitation, limited fluid resuscitation can also improve the survival rate of hemorrhagic shock rats in a more efficient way, consistent with the previous reports [26]. Previous results suggested that due to the small volume of fluid, limited fluid resuscitation can hardly dilute the platelet with little effect on coagulation function, let alone the aggravation of bleeding event; meanwhile, small dosage cannot trigger a sudden increase of blood pressure, while maintain the blood perfusion of key organs and reduce the ischemia reperfusion injury [24, 27], which are the potential factors contributing to the positive results of the limited fluid resuscitation in this study. However,

some inevitable flaws in this study also exist. Generally, crystalloid solution and colloidal solution are often selected in fluid resuscitation, but we, in this study, only adopted the Ringer lactate solution, a kind of crystalloid solution, while the efficacy of other solutions remains unknown. Besides, we only evaluated the 2-hour efficacy of resuscitation, while the long-term efficacy requires further studies. We hope that our results can raise the interest of other scholars on the efficacy of limited fluid resuscitation on the hemorrhagic shock and its protective effect on lung tissues.

In conclusion, in comparison with the regular fluid resuscitation, limited fluid resuscitation can improve the survival of rats through ameliorating the hemorrhagic shock, inflammatory responses and oxidative stress of lung tissues, and mitigating the apoptosis of lung cells.

Disclosure of conflict of interest

None.

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