Reduction of contractility and reactivity in isolated lymphatics from hemorrhagic shock rats with resuscitation[1]

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ABSTRACT

PURPOSE: To evaluate the changes of contractility and reactivity in isolated lymphatics from hemorrhagic shock rats with resuscitation.

METHODS: Six rats in the shock group suffered hypotension for 90 min by hemorrhage, and resuscitation with shed blood and equal ringer's solution. Then, the contractility of lymphatics, obtained from thoracic ducts in rats of the shock and sham groups, were evaluated with an isolated lymphatic perfusion system using the indices of contractile frequency (CF), tonic index (TI), contractile amplitude (CA) and fractional pump flow (FPF). The lymphatic reactivity to substance P (SP) was evaluated with the different volume of CF, CA, TI and FPF between pre- and post-treatment of SP at different concentrations.

RESULTS: The CF, FPF, and TI of lymphatics obtained from the shocked rats were significantly decreased than that of the sham group. After SP stimulation, the ΔCF (1×10^{-8}, 3×10^{-8}, 1×10^{-7}, 3×10^{-7} mol/L), ΔFPF (1×10^{-8}, 3×10^{-8}, 1×10^{-7} mol/L), and ΔTI (1×10^{-8} mol/L) of lymphatics in the shock group were also obviously lower compared with the sham group. In addition, there were no statistical differences in CA and ΔCA between two groups.

CONCLUSION: Lymphatic contractility and reactivity to substance P appears reduction following hemorrhagic shock with resuscitation.

Key words: Shock. Lymphatic Vessels. Substance P. Isotonic Contraction. Rats.

Introduction

The lymphatic circulation is an important component of the circulatory system, which have the ability to contract and transport liquid and protein from tissue spaces to the intravascular space via collecting lymphatic vessels[1]. After acute hemorrhage, *in vivo*, lymphatic pumping function was enhanced in the early stage, which plays an important role in blood volume and protein restitution[2,3]; however, the contractility of lymphatic vessels was decreased in the late stage, which is the one of reasons leaded to severe shock and death[4]. In addition, there were biphasic changes in contractility and reactivity to norepinephrine (NE) and substance P (SP) of isolated lymphatics obtained from thoracic ducts of hemorrhagic shocked rats without resuscitation[5,6]. Hence, lymphatic circulation plays an important role during hemorrhagic shock. However, the changes in contractility and reactivity of isolated lymphatics after hemorrhage and resuscitation are unknown. Therefore, to address the relationship between lymphatic pump function and hemorrhagic shock, in the present study, we investigated the contractility and reactivity to SP in isolated lymphatics form hemorrhagic shock rats with resuscitation.

Methods

Research approved by the Institutional Animal Care and Use Committee of Hebei North University and conformed to National Institutes of Health guidelines. All efforts were made to minimize suffering of animals.

Twelve adult male Wistar rats (180–230 g, purchased from the Laboratory Animal Breeding Center of the Chinese Academy of Medical Sciences, Beijing, China) were used in this study. Before experimentation, the rats were fasted for 12 h, but were allowed free access to water. These rats were randomly divide into sham and shock groups (n=6 rats/group).

Hemorrhagic shock model

After anesthetization with 1% pentobarbital sodium (50 mg/kg, Merck, Germany), all of the rats received femoral operations to separate the right femoral vein for anticoagulation and bilateral femoral arteries for hemorrhage, and monitoring of mean arterial pressure (MAP) as previously reported[6]. After 30 min-equilibrium period, the rats in the shock group, establishment of hemorrhagic shock model was performed with the method of hemorrhage through the left femoral artery, and the MAP was maintained at a level of 40 mmHg for 90 min by withdrawing or reinfusing shed blood. Afterwards, the rats received resuscitation with shed blood and equal ringer's solution with a speed of 30 ml/h in 30 min. Rats in the sham group were anesthetized and cannulated identically to the shock rats, but no blood was withdrawn.

Preparation of isolated lymphatics

At 30 min after surgical operation in the sham group or at 3 h after resuscitation finished in the shock group, the lymphatic vessel was obtained from thoracic duct under a SZ2-ILST stereomicroscope (Olympus, Tokyo, Japan) as previously described[6].

Measurement of lymphatic contractility and reactivity

Isolated lymphatic vessels were transferred to the organ chamber of a pressure myograph system (110P, DMT, Denmark) containing physiological saline solution (PSS) containing the following (in mmol/L): 118.99 NaCl, 4.69 KCl, 2.50 $CaCl_2$, 1.17 $MgSO_4$, 1.18 KH_2PO_4, 25.0 $NaHCO_3$, 5.50 glucose, 0.03 EDTA•Na_2, pH 7.3-7.4, which was oxygenated by bubbling with 95% O_2/5% CO_2. Then, the lymphatic vessels were fixed onto the ends of two glass pipettes, and stabilized with a pressure servo system as previously described[6]. Once spontaneous contractions had begun to occur, the lymphatic vessels were allowed to equilibrate at 3 cmH_2O for 10 min. Then, lymphatic contraction frequency (CF), end-diastolic diameter (EDD), and end-systolic diameter (ESD) were measured as previously described[5].

Subsequently, the lymphatics were treated with concentration gradient of SP (Alexis Inc, Lausen, Switzerland) of final concentrations of 1×10^{-8}, 3×10^{-8}, 1×10^{-7} and 3×10^{-7} mol/L in that sequence, and the CF, EDD and ESD were recorded within 1 min after SP treatment. At the end of observation of lymphatic reactivity, the PSS in the chamber was replaced by calcium-free PSS (in mmol/L: 118.99 NaCl, 4.69 KCl, 2.50 $CaCl_2$, 1.17 $MgSO_4$, 1.18 KH_2PO_4, 25.0 $NaHCO_3$, 5.50 glucose, 3.0 EDTA•Na_2, pH 7.3-7.4) for 15 min for the measurement of maximum passive diameter (PD).

Finally, the contractility of isolated lymphatics was evaluated with the following indices as described previously[7-9]. Tonic index (TI = (PD-EDD) / PD × 100%), contraction amplitude (CA = (EDD-ESD) / PD × 100%), and fractional pump flow (FPF = (EDD^2 - ESD^2) / EDD^2 × CF). The reactivity to SP of isolated lymphatics was evaluated with the different volume of CF, CA, TI and FPF between pre- and post-treatment of SP at different concentrations.

Statistical analysis

Data in this study are presented as the mean±SE and were analyzed using SPSS version 16.0 software. The Independent-Samples *t* test was used to identify differences between the sham and shock groups. Values of $p<0.05$ were considered to be statistically significant.

Results

Change of contractility in isolated lymphatics obtained from hemorrhagic shock rats with resuscitation

The data of EDD, ESD and PD in isolated lymphatics before SP stimulation are shown in Table 1, and the captured images of lymphatic contractility are shown in Figure 1. Results from the Figure 2 demonstrated that the CF, FPF, and TI of lymphatics in the shock group was significantly decreased than that of the sham group ($p<0.05$), and there was no statistics difference in CA between the shock and sham group ($p>0.05$).

TABLE 1 - The data of end-diastolic diameter (EDD), end-systolic diameter (ESD) and maximum passive diameter (PD) of lymphatics in hemorrhagic shock rats with resuscitation and sham groups (*n*=6/group).

Group	Pre-SP		1×10^{-8} (SP, mol/L)		3×10^{-8} (SP, mol/L)		1×10^{-7} (SP, mol/L)		3×10^{-7} (SP, mol/L)		Ca^{2+}-free PSS
	EDD	ESD	EDD	ESD	EDD	ESD	EDD	ESD	EDD	ESD	PD
Sham	550±29	421±35	500±31	381±30	431±40	331±38	365±23	292±20	291±24	245±22	659±30
Shock	601±29	482±24	589±30	475±25	535±32	442±30	419±47	402±27	331±20	287±13	616±28

Data are presented as the mean±SE. SP: substance P; PSS: physiological saline solution.

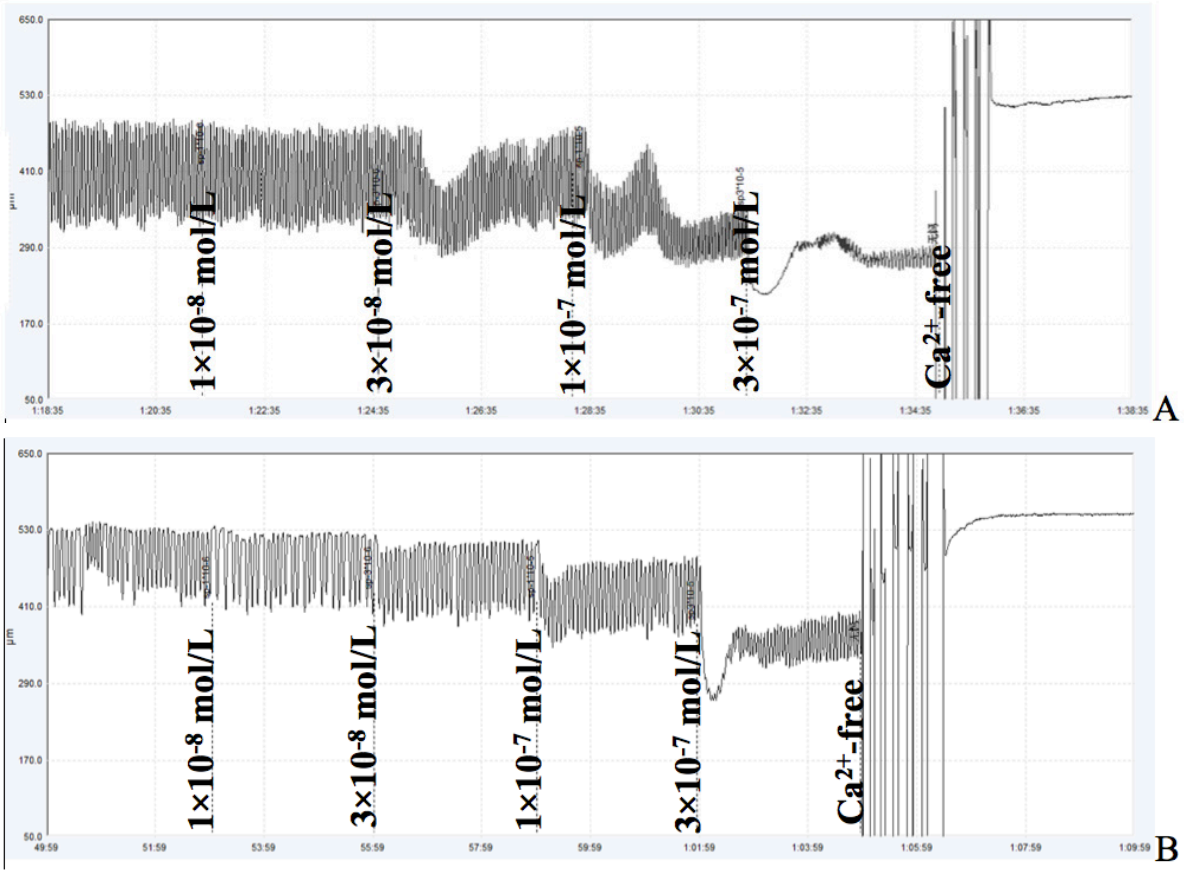

FIGURE 1 –The images of lymphatic contractility and reactivity to substance P (SP) were captured using an isolated lymphatic perfusion system. **A)** Sham group. **B)** Shock group.

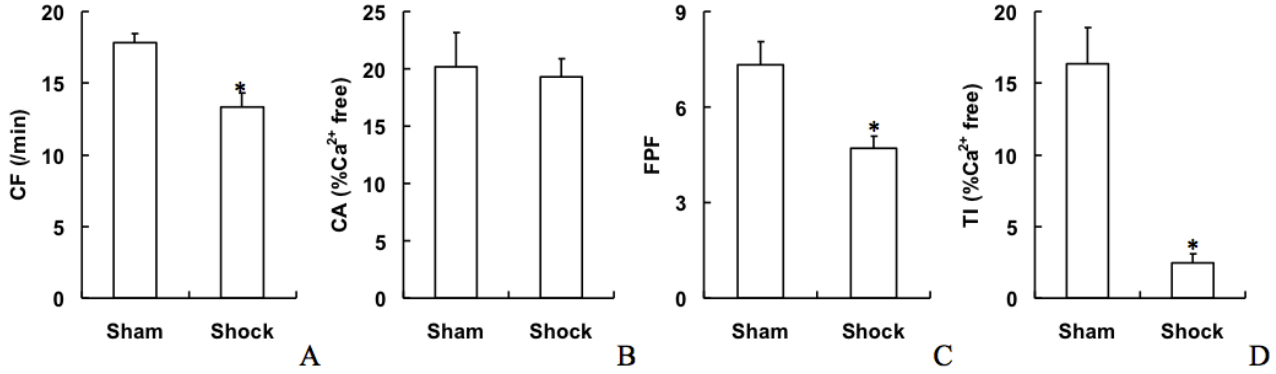

FIGURE 2 – Change of contractility in isolated lymphatics obtained from hemorrhagic shock rats with resuscitation. Data are presented as the mean±SE (n=6). **A)**: contractile frequency (CF); **B)**: contractile amplitude (CA); **C)**: fractional pump flow (FPF); **D)**: tonic index (TI). *p<0.05 *vs* sham group.

Change of reactivity to SP in isolated lymphatics obtained from hemorrhagic shock rats with resuscitation

The data of EDD, ESD and PD in isolated lymphatics after SP stimulation, and the images of lymphatic reactivity to SP are shown in Table 1 and Figure 1, respectively. Meanwhile, the values of increased CF in the shock group were significantly higher than those in the sham group at SP concentrations of 1×10^{-8}, 3×10^{-8}, 1×10^{-7} and 3×10^{-7} mol/L (Figure 3) ($p<0.05$). In addition, the ΔFPF after SP stimulation at concentrations of 1×10^{-8}, 3×10^{-8}, and 1×10^{-7} mol/L, and the ΔTI after SP stimulation with a concentration of 1×10^{-8} mol/L, were obviously depressed compared with the sham group ($p<0.05$). Moreover, there was no statistics difference in ΔCA between the shock and sham group ($p>0.05$).

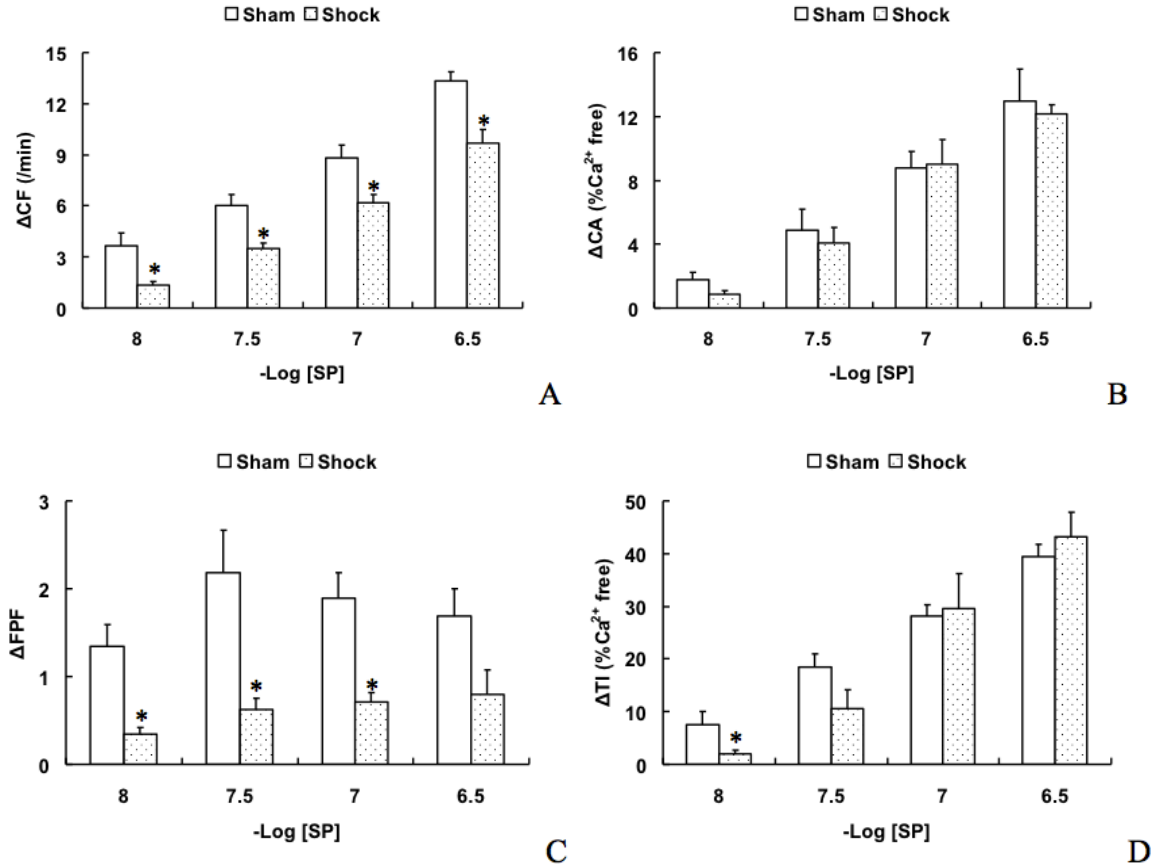

FIGURE 3 – Change of reactivity to p substance (SP) in isolated lymphatics obtained from hemorrhagic shock rats with resuscitation. Data are presented as the mean±SE (n=6). **A)**: different of contractile frequency (△CF); **B)**: different of contractile amplitude (△CA); **C)**: different of fractional pump flow (△FPF); **D)**: different of tonic index (△TI). *p<0.05 *vs* sham group.

Discussion

In the present research, thoracic duct were harvested and prepared for the measurement of lymphatic contractility and reactivity with the perfusion technology of isolated lymphatics with a pressure myograph system, and the present results demonstrated that there was decreased lymphatic pump function after hemorrhagic shock with resuscitation *in vitro*.

In the present experiment, we recorded the lymphatic CF, which refers to the number of contractions per minute, EDD, which refers to the diameter before the beginning of lymphatic contraction, ESD, which refers to the diameter at the end of lymphatic systole, and PD, which refers to the diameter of lymphatic vessels have been exposed to nominally calcium-free PSS for 15 min for the standardization of lymphatic diameter. Subsequently, we calculated these indices for the assessment of isolated lymphatic contractility as described previously[7-9], including the TI, which refers to the preparing status of lymphatic subsequent contraction, CA, which refers to the normalized intensity of lymphatic contraction, FPF, which refers to the capacity of lymph transport. We found that hemorrhagic shock lead a decrease in CF, TI, and FPF of isolated lymphatics from thoracic duct, suggesting a reduction of lymphatic contractility.

Previous studies demonstrated that lymphatic reactivity to vasoactive substances is associated with lymphatic contractile activity[5,6,10,11]. Therefore, in this study, furthermore, we tested the isolated lymphatic contractile response to gradient concentration of SP, and found that the difference value of indexes of CF, TI and FPF decreased at certain concentrations of SP, significantly. These findings indicated that the lymphatic hypo-reactivity was appeared after hemorrhagic shock and resuscitation.

It should be pointed out that there were no statistical alterations in CA and △CA of isolated lymphatics, suggesting there was no obvious change in every contractile cycle between the shock and sham groups. We thought that its reason might be related to the compensatory induced by acute hemorrhage and resuscitation, and/or the short observation time. Hence, further investigation is needed in the future.

These results of lymphatic contractility and reactivity following hemorrhage and resuscitation were similar with that of lymphatics from hemorrhagic shock without resuscitation in previous report[6]. Because lymphatic pumping plays a vital role in the regulation of interstitial fluid volume[12], therefore, the treatment of enhancing lymphatic pump function may be beneficial to hemorrhagic shock.

Conclusion

Lymphatic contractility and reactivity to substance P appears reduction following hemorrhagic shock with resuscitation.

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