Resuscitative Effect of Hyperoxia Fluid on High-Altitude Hemorrhagic Shock in Rats and Antishock Mechanisms

Qiquan Zhou · Yongjun Luo · Fuyu Liu · Yuqi Gao · Yi He · Bihai Zheng · Dingzhou Yang · Suzhi Li · Liangming Liu

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Abstract Pathophysiological characteristics of hemorrhagic shock at high altitude are different from that at plain which involve severe injury, high mortality, difficult treatment and compromised liquid tolerance. High-altitude pulmonary/cerebral edema and multiple-organ dysfunction render the conventional treatment ineffective. Herein, we evaluated the resuscitation effects of hyperoxia solution on high-altitude hemorrhagic shock in rats. For this purpose, a rat model of high-altitude (3,658 m) hemorrhagic shock was established on the plateau and hyperoxia solution (4 ml/kg) was infused through external jugular vein for resuscitation at 60 min post-hemorrhage. Blood pressure, blood gas, left and right ventricular pressure, lung and brain water content, survival time, survival rate at 2 h, levels of inflammatory cytokines and free oxygen radicals in blood and tissue were determined. After resuscitation with hyperoxia solution, blood pressure, arterial oxygen partial pressure, left and right ventricular systolic pressure, ±dp/dt max, survival time and rate were significantly increased. Lung and brain water content were unchanged, malondialdehyde activity in lung, brain and plasma and levels of TNF-α, IL-1, IL-6, and endothelin were significantly decreased. Besides, CGRP was elevated with reduced injury and improved lung and kidney functions. Concludingly, resuscitation with hyperoxia solution is feasible and more effective than other classical liquids, making it the first choice of treatment for high-altitude hemorrhagic shock.

Keywords High-altitude hemorrhagic shock · Hyperoxia fluid · Resuscitation · Rat

Introduction

Traumatic hemorrhagic shock is commonly observed in wartime and daily clinical practice. It was reported that approximately 32.6–59.5% patients died of hemorrhagic shock during wartime [1]. Effective fluid resuscitation for hemorrhagic shock is critical to the subsequent treatment. Thus, a number of studies on resuscitation of traumatic hemorrhagic shock at sea level have been reported [2–4]. However, traumatic hemorrhagic shock at high altitude, which has its pathophysiological characteristics different from that at sea level, is not well studied. Under hypoxic environment, once the traumatic hemorrhagic shock occurs, the injury condition may become critical. The treatment of high-altitude traumatic hemorrhagic shock is difficult and involves high mortality with compromised liquid tolerance. Furthermore, incorrect management of high-altitude traumatic hemorrhagic shock may lead to high-altitude pulmonary edema (HAPE) and/or high-altitude cerebral edema (HACE) as well as concomitant multiple-organ dysfunction syndrome (MODS). Due to such complications, the treatment of high-altitude traumatic hemorrhagic shock is very
difficult and high mortality rates have been reported [5]. Therefore, the treatment strategies [6, 7] for high-altitude traumatic hemorrhagic shock need to be improved. Once the treatment regimen for traumatic hemorrhagic shock at high altitude has been established, the efficacy may be profoundly improved. Recently, with the overall opening of high-altitude areas of development and construction, the number of people inhabiting the highland is increasing. Traumatic hemorrhagic shock at high altitude occurs frequently as a result of traffic accidents or severe trauma, with a relatively high mortality. Therefore, finding an effective treatment strategy is pivotal to the achievement of reduced mortality from high-altitude hemorrhagic shock. In this rat model study, we have evaluated the effects of hyperoxia solution on the hemorrhagic shock at an altitude of 3,658 m.

Materials and Methods

Blood gas analyzer (NOVO biomedical company MAO 2454VSH type, USA), 8-channel physiological recorder (ADInstruments Pty Ltd company ML118 type, Australia), dry box (Shanghai, China), hyperoxia liquid preparation instrument (Xi’an, China), XL2000-010 type ultrasonic cell disrupter (Microson, USA), refrigerated centrifuge (Eppendorf, Germany), common centrifuge (Beijing, China), medical oxygen, surgical instruments, rat platform, normal saline (Sichuan, China), Dextran-40 injection (Sichuan, China), 7.5% hypertonic saline (prepared immediately before use), hyperoxia liquid (prepared immediately before use).

A total of 72 adult male Sprague–Dawley rats, weighing 236.88 ± 32.54 g, were procured from the Experimental Animal Center of Surgery Institute, Third Military Medical University and were flown to Lhasa (3,658 m). The animals were randomly and equally divided into six groups including simple shock (SS), iso-osmotic saline (IS), hyper-osmotic saline (HS), Dextran (DX), hyper-osmotic hyper-colloid solution (HH), and hyperoxia solution (HO) groups. After anesthesia by intraperitoneal injection of 10% urethane (at the rate of 1 ml/100 g body weight), high-altitude hemorrhagic shock was induced following Weigger’s method [8]. The left common carotid artery was connected with an eight channel physiological recorder to monitor mean arterial blood pressure, intraventricular pressure, and blood gas were measured before, at, and after shock as well as at 30, 60, 120, and 240 min after shock treatment. Rats were anatomized 2 h after treatment, whole lungs and kidneys were weighed, and gross morphology was recorded. Then the left lung, renal cortex, and medulla were washed with physiological saline, fixed with 10% formalin, dehydrated, embedded in liquid paraffin, stained by H&E, and tissue samples were examined by light microscopy. Pathological grading (0–IV) was performed on the basis of severity of changes as follows: 0 no disease, I minor, II mild, III medium, and IV severe damage to the lung histology.

Preparation of Hyperoxic Solution

In a closed environment, different liquids were poured into special brushed glass funnels of quartz and pure oxygen was injected at a rate of 1 l/min for 10 min. Compared between post-oxygenated and un-oxygenated fluids, the oxygen concentration was elevated by 4.3–5.9 times (average 5.1 times). The highest oxygen concentration in the liquid could be maintained for 30 min.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 13.0 statistical software version (SPSS Inc, Chicago, IL), and the results are presented as mean ± SD values. One-way ANOVA with Bonferroni test was used for multiple comparisons between means, except for survival rates which were compared using Chi-square ($\chi^2$)-test. All $P$ values > 0.05 were considered as statistically significant.

Results

Survival Time and Survival Rates in Shock Animals

The survival time of rats in HO group was significantly ($P < 0.01$) longer than those in control groups, while it was
two times longer than that of rats in SS group. Two hours after the treatment, the survival rate in HO group was as high as 100% (Table 1), but the rats in SS group had no survival. The arterial blood pressure, survival time and survival rate in other groups were also higher than those of SS group, while the effectiveness of the treatment in all groups was far less than that observed in HO group. Most of the animals died within 2 h, as in the surviving animals, arterial blood pressure could only be maintained at a low level with poor stability and the 2-h survival rate was 33–67% in other groups.

Changes in Arterial Blood Pressure

The arterial blood pressure of rats in HO group was significantly higher ($P < 0.01$) than that of rats in other groups and was found to be stable at 40 mmHg without profound fluctuation within 2 h (Table 1). Although the arterial blood pressure in other groups was also high, it was not stable. The arterial blood pressure in HH group was also increased but it was unstable and lower than that of HO group within 2 h, and was accompanied by a low survival rate.

Lung and Brain Water Content

The water content of lung and brain did not increase after infusion with appropriate dose range of resuscitation fluid. We found that the resuscitation fluid dose of 4 ml/kg was feasible for the treatment of high-altitude hemorrhagic shock. Moreover, the hypoxic solution did not enhance water content of the lung and brain and thus did not deteriorate the tissue injury.

Table 1  Arterial pressure, survival time, and survival rate after resuscitation ($\bar{x} \pm SD$)

<table>
<thead>
<tr>
<th>Group</th>
<th>$N$</th>
<th>Mean arterial pressure (mmHg)</th>
<th>BP (pre-execute) (mmHg)</th>
<th>Survival time (min)</th>
<th>2-h Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>33.14 ± 3.36$^a$</td>
<td>7.11 ± 9.80$^a$</td>
<td>60.22 ± 9.79$^a$</td>
<td>0$^b$</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>41.43 ± 7.41$^b$</td>
<td>25.11 ± 7.84$^b$</td>
<td>75.00 ± 9.23$^f$</td>
<td>30$^e$</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>38.17 ± 6.26$^c$</td>
<td>25.51 ± 9.48$^b$</td>
<td>88.89 ± 7.79$^g$</td>
<td>50$^i$</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>42.99 ± 4.19$^d$</td>
<td>28.22 ± 5.41$^b$</td>
<td>104.44 ± 6.28$^b$</td>
<td>60$^j$</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>45.05 ± 4.09$^e$</td>
<td>25.56 ± 7.84$^b$</td>
<td>95.33 ± 7.83$^b$</td>
<td>40$^i$</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>54.36 ± 3.87</td>
<td>38.20 ± 5.91</td>
<td>120.21 ± 3.11</td>
<td>100$^j$</td>
</tr>
</tbody>
</table>

$^a$ $P < 0.05$ versus IS group, HS group, DX group, HH group, HO group  
$^b$ $P < 0.01$ versus HO group  
$^c$ $P < 0.01$ DX group, HH group, HO group  
$^d$ $P < 0.01$ versus HO group  
$^e$ $P < 0.01$ versus HO group  
$^f$ $P < 0.01$ versus SS group, DX group, HH group, HO group  
$^g$ $P < 0.01$ versus DX group, HO group  
$^h$ $P < 0.05$ versus HS group, DX group, HO group  
$^i$ $P < 0.05$ versus HO group  
$^j$ $P < 0.05$ versus HO group

PaO2 and PaCO2

As shown in Table 2, hypoxic solution significantly increased PaO2 and slightly decreased PaCO2 which was not caused by hyperventilation. Although PaO2 was increased also in other groups, it was still lower than that observed in HO group ($P < 0.05$).

Left and Right Intraventricular Pressure and the Maximum Rate of Change in Ventricular Pressure

Our data show that different kinds of resuscitation fluids could increase the left ventricular systolic pressure (LVSP), and also improved maximal systolic and maximal diastolic speeds of ventricular pressure. Hyperoxic solution improved the maximum rate of change ($\pm dp/dt_{max}$) in the left and right ventricular pressure. These findings suggest that hyperoxic solution had protective effects on the left and right cardiac function (Tables 3, 4).

Plasma Cytokines

Hyperoxic resuscitation solution decreased the levels of TNF-α, IL-1, IL-6 and ET, and increased the level of CGRP. This effect was more pronounced in HS and IS groups. Besides, the increase in CGRP was more significant in HS group (Table 5).

MDA and SOD Levels

Hyperoxic solution significantly decreased ($P < 0.01$) MDA levels in plasma, lung and brain, and simultaneously...
increased SOD levels. In HO group, MDA reduction levels were more remarkable in plasma and brain. However, the reduction of MDA levels in the lung of HO group was less remarkable than that of blank control, IS, and HS groups but MDA content in lung of HO group were higher than that of IS and HS groups, while MDA levels in brain were markedly lower in HO group ($P < 0.01$) (Table 6). Post-treatment monitoring data show that at 30 min

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Water content of the lung and brain, and oxygen partial pressure after resuscitation ($\bar{x} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>N</td>
</tr>
<tr>
<td>SS</td>
<td>10</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
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<tr>
<td>HS</td>
<td>10</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ $P < 0.05$ versus HS group, DX group
$^b$ $P < 0.01$ versus HS group, DX group, HH group, HO group
$^c$ $P < 0.05$ versus IS group, DX group
$^d$ $P < 0.05$ versus HS group, DX group, HH group, HO group
$^e$ $P < 0.01$ versus DX group
$^f$ $P < 0.01$ versus IS group, HS group, DX group, HH group, HO group
$^g$ $P < 0.01$ versus HS group, DX group, HH group, HO group
$^h$ $P < 0.01$ versus IS group, DX group, HH group, HO group
$^i$ $P < 0.01$ versus IS group, DX group, HH group, HO group
$^j$ $P < 0.05$ versus HS group, DX group, HH group, HO group

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Left and right intraventricular pressure after resuscitation (mm Hg; $\bar{x} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>10</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ $P < 0.01$ versus SS group, HS group, DX group, HH group, HO group
$^b$ $P < 0.01$ versus HS group, HO group
$^c$ $P < 0.01$ versus HH group, HO group
$^d$ $P < 0.01$ versus HO group
$^e$ $P < 0.05$ versus DX group
$^f$ $P < 0.01$ versus IS group, HH group, HO group
$^g$ $P < 0.01$ versus HS group, DX group
$^h$ $P < 0.01$ versus HH group, HO group
$^i$ $P < 0.05$ versus HH group, HO group
$^j$ $P < 0.05$ versus DX group
$^k$ $P < 0.05$ versus DX group, HO group
After the treatment, plasma MDA levels in HO group (38.156 ± 2.183 nmol/ml) were significantly lower ($P < 0.05$) than those of SS group (45.146 ± 5.118 nmol/ml) and IS group (44.164 ± 3.134 nmol/ml), while in other groups, plasma MDA levels were significantly higher. Likewise, at 4 h after the treatment, plasma MDA levels in HO group (39.172 ± 3.135 nmol/ml) were significantly lower ($P < 0.01$) than those of SS group (49.177 ± 3.123 nmol/ml) and IS group (43.107 ± 2.161 nmol/ml).

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Left ventricular function</th>
<th>Right ventricular function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+dp/dt max</td>
<td>−dp/dt max</td>
</tr>
<tr>
<td>SS</td>
<td>10</td>
<td>4,534.4 ± 522.9a</td>
<td>1,876.5 ± 523.1c</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>5,418.3 ± 649.9</td>
<td>3,017.7 ± 915.3e</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>5,126.6 ± 552.2b</td>
<td>2,354.8 ± 548.7g</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>5,793.9 ± 728.8c</td>
<td>3,053.8 ± 471.1</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>4,963.2 ± 569.6d</td>
<td>3,056.4 ± 472.5</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>5,836.2 ± 805.2</td>
<td>3,464.7 ± 534.8</td>
</tr>
</tbody>
</table>

*a* $P < 0.05$ versus IS group, DX group, HO group  
*b* $P < 0.05$ versus SS group  
*c* $P < 0.05$ versus HH group  
*d* $P < 0.05$ versus HO group  
*e* $P < 0.05$ versus IS group, HS group, DX group, HH group, HO group  
*f* $P < 0.05$ versus IS group, HS group, DX group, HH group, HO group  
*g* $P < 0.05$ versus IS group, HS group, DX group, HH group, HO group  
*h* $P < 0.05$ versus HH group  
*i* $P < 0.05$ versus HH group  
*j* $P < 0.05$ versus HH group, HO group  
*k* $P < 0.05$ versus HH group  
*l* $P < 0.05$ versus HO group  

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>TNF-α (ng/ml)</th>
<th>IL-1 (ng/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>Endothelin (pg/ml)</th>
<th>Calcitonin gene-related peptide (CGRP; pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>3.97 ± 0.55a</td>
<td>15.82 ± 0.62a</td>
<td>547.79 ± 14.24a</td>
<td>202.75 ± 11.75j</td>
<td>26.88 ± 3.36a</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>0.94 ± 0.54b</td>
<td>0.76 ± 0.45f</td>
<td>132.47 ± 14.01b</td>
<td>234.68 ± 15.59j</td>
<td>66.45 ± 8.96b</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>1.95 ± 0.13c</td>
<td>0.98 ± 0.46f</td>
<td>292.41 ± 12.60g</td>
<td>76.77 ± 16.03</td>
<td>204.78 ± 12.31c</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>1.86 ± 0.59d</td>
<td>0.24 ± 0.12g</td>
<td>167.37 ± 18.41d</td>
<td>167.16 ± 11.71k</td>
<td>164.24 ± 10.32d</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>1.54 ± 0.61c</td>
<td>1.35 ± 0.76b</td>
<td>349.25 ± 22.52c</td>
<td>49.24 ± 9.63j</td>
<td>185.15 ± 12.97c</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>2.22 ± 0.43</td>
<td>0.98 ± 0.46</td>
<td>201.55 ± 23.29</td>
<td>159.98 ± 12.07</td>
<td>113.84 ± 5.58</td>
</tr>
</tbody>
</table>

*a* $P < 0.01$ versus IS group, HS group, DX group, HH group, HO group  
*b* $P < 0.01$ versus SS group  
*c* $P < 0.05$ versus HS group, DX group, HH group, HO group  
*d* $P < 0.01$ versus HH group  
*e* $P < 0.01$ versus IS group  
*f* $P < 0.05$ versus DX group, HH group  
*g* $P < 0.01$ versus HH group, HO group  
*h* $P < 0.01$ versus HO group  
*i* $P < 0.01$ versus HH group  
*j* $P < 0.01$ versus HH group  
*k* $P < 0.01$ versus HH group  
*l* $P < 0.05$ versus HO group
Protective Effects on Lung and Kidney

After resuscitation with hyperoxic solution, the lung dry-to-wet ratio, lung weight-to-body weight ratio and water content in the lung were dramatically reduced, while the hyperemia, hemorrhage and edema in alveolar space were improved (Fig. 1a). Notably, the degree of tissue injury was only of grade I–II. Pulmonary diffusion function was also significantly improved. On the other hand, hyperemia of alveolar wall, edema of alveolar space, hemorrhage, confluent bronchopneumonia and microabscess were observed in control (SS) group (Fig. 1b), and the degree of tissue injury was of grade III–IV (Table 7). Furthermore, resuscitation with hyperoxic solution improved the microcirculation and hemoperfusion in the kidney which was accompanied by significantly increased urine volume. Importantly, blood urea nitrogen (BUN), serum creatinine (Cr), fractional excretion of filtered sodium (FENa) and renal failure index (RFI) were also significantly improved (Table 8). Renal uropoiesis improved and renal tissue injury decreased after resuscitation with hyperoxic solution. The swelling of glomerular capillary endothelial cells was reduced, and there were no red blood cells (RBC) siltation in the renal interstitial vessels (Fig. 1c). On the contrary, in control group, profound swelling of endothelial cells was observed which was also accompanied by RBC siltation and renal tubular obstruction (Fig. 1d). As well, BUN, Cr, FENa, and RFI were markedly deteriorated. These findings suggest that hyperoxic solution could effectively protect the kidney against the injury induced by shock.

Discussion

It is essential to choose the most suitable resuscitation fluid for treatment of high-altitude hemorrhagic shock and to ensure that the patients are tolerant to the selected fluid therapy. Otherwise, they may develop pulmonary and cerebral edema as well as right heart failure, especially if the treatment is performed as in the plain area [5, 9]. The resuscitation fluid which produces persistent effects at relatively low doses is desirable. In general, a balanced salt solution (BSS) or lactated Ringer’s solution is the first choice treatment, followed by dextran-70 and dextran-40. Hyperosmotic saline solution has been used for resuscitation over the past decade. Initially, hyperosmotic saline solution referred to hyperosmotic NaCl solution. Nowadays, it is a mixed fluid with hyperosmotic saline solution and medium molecular dextran/dextran-40 [10] or hydroxyethyl starch [11, 12]. In addition, hyperosmotic NaCl solution with 2.5% natrium aceticum and medium molecular dextran/dextran-40 was also reported [13, 14]. It was also suggested [3] that during hemorrhagic shock, surplus water could be transferred from the swollen endothelial cells and intercellular space into the blood vessels after administration of hyperosmotic saline solution which resulted in increased blood volume, improved peripheral tissue perfusion and reduced tissue edema. Transfusion of hyperosmotic saline solution can also dilate capillaries, enhance myocardial contractility and improve arteriolar vasoconstriction, resulting in improved blood rheology and hemodynamics. Application of hyperosmotic saline solution to resuscitate animals with hemorrhagic

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Table 6  Superoxide dismutase, malondialdehyde, and nitric oxide levels after resuscitation

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Plasma SOD (U/ml)</th>
<th>Plasma MDA (nmol/ml)</th>
<th>Plasma NO (μmol/L)</th>
<th>Brain MDA (nmol/mg prot)</th>
<th>Lung MDA (nmol/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>53.27 ± 3.08a</td>
<td>1.83 ± 0.13a</td>
<td>14.09 ± 3.27a</td>
<td>13.21 ± 2.35i</td>
<td>10.94 ± 1.24a</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>45.46 ± 4.2b</td>
<td>1.73 ± 0.36b</td>
<td>16.17 ± 1.52b</td>
<td>11.65 ± 2.66c</td>
<td>3.05 ± 1.09c</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>92.89 ± 5.9c</td>
<td>1.24 ± 0.37f</td>
<td>17.89 ± 1.92c</td>
<td>12.48 ± 2.75c</td>
<td>4.11 ± 0.93c</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>60.33 ± 3.77d</td>
<td>1.13 ± 0.21</td>
<td>13.05 ± 1.62d</td>
<td>10.62 ± 2.92d</td>
<td>4.42 ± 1.29c</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>86.15 ± 4.31e</td>
<td>1.17 ± 0.24g</td>
<td>15.55 ± 1.11</td>
<td>9.55 ± 1.68</td>
<td>4.58 ± 0.75e</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>61.75 ± 2.61</td>
<td>1.07 ± 0.15</td>
<td>16.42 ± 3.21</td>
<td>9.12 ± 1.35</td>
<td>7.55 ± 1.66</td>
</tr>
</tbody>
</table>

a  P < 0.01 versus IS group, HS group, DX group, HH group, HO group
b  P < 0.01 versus HS group, DX group, HH group, HO group
c  P < 0.05 versus DX group, HH group, HO group
d  P < 0.01 versus HH group
e  P < 0.01 versus HO group
f  P < 0.01 versus DX group, HO group
g  P < 0.01 versus HO group
h  P < 0.01 versus HS group, DX group
i  P < 0.01 versus IS group, DX group, HH group, HO group

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shock on the plain has met with favorable outcome [15]. Thereafter, it was also used to cure hemorrhagic shock at high altitudes [16, 17]. However, the hyperosmotic hypercolloid solution is a more frequently used resuscitation fluid [6]. In this regard, Yin et al. [18] showed that after administration of hyperosmotic hypercolloid solution, recovery of blood pressure and steady maintenance over a long period and blood gas index were significantly improved. The use of hyperosmotic- hypertonic solution confers acceptable safety and involves fewer complications. It was

**Fig. 1** Protective effects of oxygenated fluid resuscitation on lung and kidney tissues. **a** In hyperoxia solution (HO) group, after resuscitation with oxygenated fluid for hemorrhagic shock at high altitude, pulmonary congestion and hemorrhage were significantly reduced, alveolar edema fluid was lacking and only the mild degree of pulmonary interstitial inflammation was observed. The degree of tissue injury was of grade I–II (H&E staining; ×400); **b** On the contrary, in simple shock group, a high degree of alveolar wall capillary congestion was observed with severe alveolar bleeding, alveolar edema, and a severe degree of pulmonary interstitial inflammatory cell infiltration. The degree of tissue injury was only of grade III to IV (H&E staining; ×400); **c** In HO group, after hyperoxia fluid resuscitation, renal vasospasm was absent and the renal tissue showed more blue dye particles and microsphere distribution with blue-stained glomeruli. The renal tubular epithelial cell injury was attenuated and the tubular blue dye revealed rare, homogeneous protein casts (H&E staining; ×240); **d** In SS group, renal microvessels contraction was observed with no blue dye microsphere distribution. The renal tubular epithelial cell degeneration and necrosis were observed with RBC siltation and renal tubular obstruction (H&E staining; ×240)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Pulmonary congestion</th>
<th>Pulmonary hemorrhage</th>
<th>Alveolar edema</th>
<th>Interstitial lung inflammation</th>
<th>Pulmonary abscess</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>++++</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>+++++</td>
<td>+++</td>
<td>+++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ Grade I  
++ Grade II  
+++ Grade III  
++++ Grade IV

**Table 7** Lung tissue damage after resuscitation with different liquids in hemorrhagic shock animals
also shown that supplementing the thyrotropin-releasing hormone (TRH) into 5% NaCl, 2.5% natrium aceticum, and 6% dextran solution improved the lung vascular leakage and minimized the transfusion complications [19]. The present study conducted on the plateau (at an altitude of 4,700 m) showed that oxygenated hyperosmotic hypercolloid solution induced blood pressure stabilization and significantly prolonged the survival time with a low dose. The efficacy of oxygenated hyperosmotic hypercolloid solution for treating high-altitude hemorrhagic shock was found to be superior to non-oxygenated solution, which is also corroborated by the previous study [20].

Of note, the patients with high-altitude traumatic hemorrhagic shock are intolerant to large fluid volumes, and thus, the amount of resuscitation fluid needs to be strictly controlled which is about one-third to half of the fluid volume used to treat the hemorrhagic shock on the plain. As shown previously [21], the infusion of BSS in amounts 1–1.5 times higher the amount of blood loss could achieve favorable outcome in a rat model of hemorrhagic shock with improved hemodynamics, prolonged survival time and no deterioration of water content in the brain or lung. Nevertheless, infusion of fluid volumes greater than two times of the amount of blood loss on the plateau caused pulmonary edema, aggravated shock and decreased the survival rate [6, 22]. On the other hand, minimizing the amount of resuscitation fluid significantly improved the prognosis and reduced the mortality in rats subjected to hemorrhagic shock for 72 h [23]. Previously, we used hypertonic solution (at the rate of 6–8 ml/kg body weight) in the treatment of plateau hemorrhagic shock in rabbits and achieved favorable outcome with no evidence of lung and brain edema [24]. In the present rat model study of high-altitude hemorrhagic shock, the animals were found to be intolerant to resuscitation fluid at the rate of 8 ml/kg body weight and, therefore, the resuscitation dose was reduced to 4 ml/kg. These results suggest that the liquid tolerance varies across different species. Interestingly, Yin et al. [25] showed that the liquid tolerance of the native Tibetan on the plateau was significantly higher as compared with Han immigrant population, and the Han population having been on the plateau for more than 3 months had markedly increased tolerance to liquid as compared with newly migrated population to plateau. It implies that the amount of liquid required for resuscitation of traumatic hemorrhagic shock in different population groups would change on the plateau. Besides, hematocrit level can be considered as an auxiliary indicator in resuscitation therapy. It is generally agreed that liquid crystal is superior to blood transfusion which may cause hypercoagulability in microcirculation at the early shock stage. Once the blood pressure is stabilized after administration of liquid crystal, RBC suspension can be supplemented if hematocrit level is lower. For patients with acute massive blood loss, whole blood transfusion in appropriate amount should be performed for blood volume expansion. For the plain hemorrhagic shock, the indication for blood transfusion is the hematocrit level lower than 20% and when the hemoglobin is lower than 70 g/l after blood volume expansion. On the contrary, at the plateau, no consensus on the indication for blood transfusion has been achieved in the resuscitation of hemorrhagic shock, and further studies are required.

In order to improve the survival rate, fluid resuscitation is indispensable for patients with massive bleeding and shock but a rapid transfusion using large amount of liquid should be avoided. In fact, the previous approach of an immediate infusion of large fluid volume to resuscitate hemorrhagic shock is now discouraged and, instead, using limited amount of fluid has produced a better outcome. An

### Table 8 Renal function after resuscitation with different fluids in hemorrhagic shock rats

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>BUN (mmol/l)</th>
<th>Cr (µmol/l)</th>
<th>FENa (%)</th>
<th>RFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>18.92 ± 5.73a</td>
<td>128.93 ± 6.73c</td>
<td>3.57 ± 0.63c</td>
<td>4.76 ± 0.37c</td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>16.82 ± 6.16a</td>
<td>108.93 ± 6.05a</td>
<td>2.39 ± 0.45a</td>
<td>3.35 ± 0.57a</td>
</tr>
<tr>
<td>HS</td>
<td>10</td>
<td>9.41 ± 5.48</td>
<td>41.98 ± 5.89d</td>
<td>0.99 ± 0.21c</td>
<td>1.84 ± 0.31d</td>
</tr>
<tr>
<td>DX</td>
<td>10</td>
<td>9.82 ± 6.23</td>
<td>56.39 ± 6.36c</td>
<td>0.56 ± 0.13f</td>
<td>0.97 ± 0.33c</td>
</tr>
<tr>
<td>HH</td>
<td>10</td>
<td>12.32 ± 3.46b</td>
<td>31.75 ± 4.16b</td>
<td>0.13 ± 0.03</td>
<td>0.23 ± 0.04b</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>7.39 ± 6.42</td>
<td>23.45 ± 3.23</td>
<td>0.11 ± 0.04</td>
<td>0.13 ± 0.03</td>
</tr>
</tbody>
</table>

BUN blood urea nitrogen, Cr serum creatinine, FENa fractional excretion of filtrated sodium, RFI renal failure index

* P < 0.05 versus HS group, DX group, HH group, HO group
* P < 0.01 versus HS group
* P < 0.05 versus IS group, HS group, DX group, HH group, HO group
* P < 0.01 DX group, HH group, HO group
* P < 0.01 versus HO group, HH group
* P < 0.05 versus HO group
early resuscitation may increase the risk for bleeding. Besides, it may involve other complications such as hemodilution and decreased body temperature which may lead to inadequate oxygen delivery, coagulation dysfunction and hypothermia. It was suggested [26] that the decision of an early resuscitation of hemorrhagic shock should be based on the extent of blood loss and injuries. Overall, it is recommended to minimize the amount of resuscitation fluid in order to maintain a relatively low tissue perfusion called as permissive low blood pressure.

As mentioned before, rapid liquid transfusion in hemorrhagic shock patients leads to hemodilution, which causes inadequate oxygen supply and compromises the survival rate. This condition is more frequently encountered in the treatment of hemorrhagic shock at high altitude and may increase the mortality. In the present study, to avoid such complications, we performed intravenous transfusion of oxygenated solution or hypoxic liquid which refers to a solution rich in oxygen dissolved under high pressure. Oxygenated solution has unique advantages and is widely used in the treatment of ischemic/hypoxic disease, plain traumatic hemorrhagic shock and burn shock. Hypoxic liquid increased mean arterial pressure, heart rate and urine volume, improved metabolic acidosis, reduced lung-to-body weight ratio, reduced blood lactate level, and increased SaO2 and PaO2 [27, 28]. Hypoxic liquid expanded blood volume and improved oxygen supply without increasing blood viscosity and thus safely and effectively ameliorated the peripheral circulation and tissue perfusion during hemorrhagic shock [29]. In addition, hypoxic liquid improved morphology and function of mitochondria, increased expression of heat shock protein (Hsp) 90, and attenuated intestinal ischemia–reperfusion injury [30]. In severe hemorrhagic shock, hyperoxic solution improved survival and decreased severity of the lung injury in rabbits [31]; improved oxygen supply to the cardiac and intestinal microvessels in pigs [32]; and increased mean arterial pressure, tissue PO2 and oxygen delivery to skeletal muscles in rabbits [33]. In addition, high-oxygen ventilation via pure oxygen inhalation increased the blood and tissue oxygen, reduced the blood volume for transfusion as well as improved the O2-carrying capacity of hemoglobin which gave a better resuscitation effect in shock patients with low hemoglobin levels [34].

Overall, it implies that enhancing the oxygen concentration of resuscitation fluid can increase the oxygen supply to the microcirculation and improve the survival rate of hemorrhagic shock animals. Nonetheless, few studies have been so far conducted to determine the resuscitation effects of hyperoxic solution on hemorrhagic shock at high altitudes. We have shown previously in rabbit model [20] that hyperoxygenated hypertonic hypercolloid solution significantly increased the blood pressure, ventricular pressure, and ±dp/dt max in high-altitude hemorrhagic shock which resulted in improved heart, lung and kidney function, and increased the survival time and rate. It also decreased plasma ET and increased plasma NO levels, maintained ET/NO balance and improved lipid peroxidation injury. We also showed in a rat model study [35] that oxygenated fluid infusion lowered the pulmonary artery pressure and improved the right ventricle function in hemorrhagic shock resuscitation at high altitude. Importantly, the oxygenated fluid does not require lung oxygenation, and hence, the patients with acute lung injury can be benefited. Oxygenated fluid directly supplies tissues and cells with oxygen without relying on hemoglobin; therefore, it can have the similar resuscitation effect in shock patients with low hemoglobin levels as in patients with normal hemoglobin levels. These findings suggest that resuscitation with hyperoxic fluids provides increased oxygen supply for microcirculation and improves survival rate in experimental animals.

The present study shows that oxygenated fluid significantly increased blood pressure, ventricular pressure and ±dp/dt max, improved heart, lung and kidney function, and led to prolonged survival time and increased survival rate in hemorrhagic shock animals at high altitude. Our data also show that hyperoxic solution was superior to other non-oxygenated fluids with regard to resuscitative effects. Of note, resuscitation with oxygenated fluid significantly reduced levels of inflammatory cytokines and free oxygen radicals in animals with hemorrhagic shock at high altitude, which was consistent with previous studies [36–38].

Taken together, administration of hyperoxic fluid is an effective treatment strategy and, in this regard, hyperoxic, hypertonic, and hypercolloid solution is a novel and promising resuscitative agent which has potential benefits for liquid resuscitation of high-altitude hemorrhagic shock.

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