Original Article

Hepatopulmonary syndrome: the role of intra-abdominal hypertension and a novel mouse model

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Abstract: Objective: Hepatopulmonary syndrome (HPS) is considered as a triad of chronic liver disease, pulmonary vascular ectasia and severe hypoxemia. The study aims to investigate the pathological mechanism of intra-abdominal pressure (IAP) in HPS and establish a novel mouse model. Methods: Fifty male ICR mice were randomly divided into experimental and control group, receiving subcutaneous injection of carbon tetrachloride and water, respectively. Mice in experimental group were then divided into 4 sub-groups with the intraperitoneal injection of different volume of albumin to form different IAP (0, 5, 10 and 20 cmH2O). All the mice were then sacrificed 24 hours later and blood gas analysis was conducted. In addition, liver and lung histopathology was also examined. Results: Blood gas analysis in different IAP suggested the respiratory alkalosis. Arterial partial pressure of oxygen significantly decreased in the IAP=10 cmH2O (68.13 ± 3.56, P<0.01) and 20 cmH2O (66.00 ± 3.78, P<0.01). Alveolar-arterial oxygen pressure difference increased markedly in the IAP=10 cmH2O (54.60 ± 6.80, P<0.001) and 20 cmH2O (57.04 ± 5.60, P<0.001). According to lung histopathology, macrophages were found to accumulate in the alveolar spaces and the widened alveolar walls were detected. In addition, there was visible blood stasis in the alveolar walls and numerous red blood cells extravasated into air space in the IAP=10 and 20 cmH2O. Conclusions: Our study suggested that intra-abdominal hypertension was a significant pathological mechanism of HPS. Meanwhile, we have established a novel mouse model that will now be optimized with further investigation of the mechanism and therapeutic targets of HPS.

Keywords: Hepatopulmonary syndrome, intra-abdominal hypertension, abdominal compartment syndrome, animal model

Introduction

Hepatopulmonary syndrome (HPS) is considered as a triad of chronic liver disease, pulmonary vascular ectasia and severe hypoxemia. However, the certain pathogenesis of HPS remains unknown. The following mechanisms are commonly utilized to explain the reason of hypoxemia occurring in patients with HPS: 1. Enhanced mismatch of alveolar ventilation to pulmonary vascular perfusion; 2. Defect of diffusion-perfusion; 3. Deoxygenated blood directly into the pulmonary vein through abnormal dilated vessels, or bypass of the pulmonary-capillary alveolar surface; 4. Disorder of oxygen-hemoglobin affinity ability; 5. Mechanical effects, such as ascites [1-3]. All these changes may result from the expansion of pulmonary vessels due to the impaired liver function, imbalance between vasodilator and vasoconstrictor [4], intestinal bacterial translocation [5, 6], intestinal endotoxemia [7] and lung monocyte-macrophage system activation [3, 8].

Massive ascites, one of the major complications in decompensated cirrhosis, can significant increase intra-abdominal pressure (IAP) [9, 10]. Recent studies indicated that intra-abdominal hypertension (IAH) could also lead to elevation of diaphragm, translocation of bacterial, endotoxemia, activation of monocyte macrophage as well as declined hepatic clearance [11-13]. In addition, there are several drawbacks, such as short-term maintaining, in the
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Current animal models of HPS by intraperitoneal infusion of salt solution or continuous injection of CO₂ or nitrogen [9-11]. Therefore, this study aims to investigate the role of IAP in the development of HPS. Meanwhile, we aim to establish a novel mouse model of HPS.

Materials and methods

Animals and reagents

All animals received humane care during the study under a protocol in accordance with institutional guidelines for animal researches. This study was approved by the Animal Experimental Ethics Committee of Tongji Hospital, Tongji University. Male ICR mice, weighing 25-30 g (n=50), were housed under standard conditions at room temperature, humidity and regular 12h/12h light/dark cycles in the Animal Center of Tongji Hospital. The reagents used in the study included: Phenobarbital sodium (Beijing Double-Crane Pharmaceutical Co. Ltd.), carbon tetrachloride (Sinopharm Chemical Reagent Co., Ltd.) and albumin (Germany Jeter biomedical Asia Pacific Co., Ltd.).

Animal model of cirrhosis

Male ICR mice were randomly divided into two groups: experimental group (n=40), receiving subcutaneous injection of carbon tetrachloride (0.3 ml/100 g, 3 times/week); control group (n=10), receiving subcutaneous injection of water with the same concentration and frequency of carbon tetrachloride. After the sacrifice of mice in experimental group, liver histopathology was examined to confirm the cirrhosis.

Cirrhotic model with different IAP

Mice in experimental group were randomly divided into 4 sub-groups (10 mice per sub-group). Different volume of albumin (30 g/L) and saline were injected into the peritoneal cavity to form the different IAP (0, 5, 10 and 20 cmH₂O). Abdominal circumference and body weight were continuously monitored. As the circumference or weight decreased by 10%, albumin or normal saline were added to retain the certain level of IAP. After anesthesia with the intraperitoneal injection of Phenobarbital sodium, a percutaneous peripheral intravenous catheter was introduced into the peritoneal cavity of mice. The IAP was then recorded continuously by a pressure transducer of a monitor system (Petas, KMA 275, Ankara, Turkey).

Histopathology

After sacrifice of all the mice, specimens of liver and lung were surgically removed, fixed in 10% buffered formalin and processed with an automated tissue processing machine followed by paraffin wax embedding. The samples were then sectioned in the coronal plane (5 μm slice thickness) and stained with hematoxylin and eosin for routine light microscopy. Staining with Masson's Trichrome (Sigma, USA) was used as a marker of collagen fiber to assess the degree of liver fibrosis. Histopathological examination was performed by three experienced pathologists.

Blood gas analysis

Blood samples were collected for gas analysis under sterile condition as mice were sacrificed. The value of alveolar-arterial oxygen pressure difference (AaDO₂) was obtained via the modified alveolar gas equation.

Statistical analysis

Categorical variables were presented as frequencies and percentages, with continuous variables as mean ± SD. Data were evaluated through the analysis of variance and correlated by SPSS14.0 (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered as statistical significance.

Results

Cirrhosis in model group

Compared with the control group, the shape of liver in experimental group was rough and small nodules could be detected. According to liver histopathology, hepatocytes in control group showed a regular arrangement and structure (Figure 1A). However, hepatocytes in mice received subcutaneous injection of carbon tetrachloride arranged disorderly and regenerated nodules were observed (Figure 1B).

Lung histopathology

According to the lung histopathology of cirrhotic mice (hematoxylin-eosin stain, × 400), the nor-
mal alveolar spaces and walls were observed in the group of $IAP=0 \text{ cmH}_2\text{O}$ (Figure 2A). For the $IAP=5 \text{ cmH}_2\text{O}$, macrophages were found to accumulate in the alveolar spaces and walls (Figure 2B). In addition, the further accumulated macrophages and widened alveolar walls...
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**Table 1. Analysis of blood gas in mice with different IAP**

<table>
<thead>
<tr>
<th>IAP (cmH₂O)</th>
<th>PH</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>AaDO₂ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.42 ± 0.03</td>
<td>85.86 ± 2.03</td>
<td>35.63 ± 3.25</td>
<td>29.19 ± 4.87</td>
</tr>
<tr>
<td>5</td>
<td>7.43 ± 0.04</td>
<td>82.25 ± 4.62</td>
<td>35.00 ± 2.07</td>
<td>33.60 ± 5.75</td>
</tr>
<tr>
<td>10</td>
<td>7.52 ± 0.04</td>
<td>68.13 ± 3.56</td>
<td>29.50 ± 3.59</td>
<td>54.60 ± 6.80**</td>
</tr>
<tr>
<td>20</td>
<td>7.48 ± 0.05</td>
<td>66.00 ± 3.78*</td>
<td>29.25 ± 4.92</td>
<td>57.04 ± 5.60**</td>
</tr>
</tbody>
</table>

*Compared with PaO₂ in group of IAP=0 cmH₂O, P<0.01; **Compared with AaDO₂ in group of IAP=0 cmH₂O, P<0.001. AaDO₂=alveolar-arterial oxygen pressure difference; IAP=intra-abdominal pressure; PaO₂=arterial partial pressures of oxygen; PaCO₂=arterial partial pressures of carbon dioxide.

could be seen in the IAP=10 cmH₂O (Figure 2C). Moreover, there was significant blood stasis in the alveolar walls and numerous red blood cells extravasated into air space in the IAP=10 and 20 cmH₂O (Figure 2D).

**Analysis of blood gas**

Blood gas analysis in mice with different IAP suggested the respiratory alkalosis. In the group of IAP=0 and 5 cmH₂O, there was no statistical difference of PH, arterial partial pressure of oxygen (PaO₂) and carbon dioxide (PaCO₂), and AaDO₂ (Table 1). However, PaO₂ decreased gradually with the increasing IAP in the group of IAP=10 cmH₂O (68.13 ± 3.56, P<0.01) and 20 cmH₂O (66.00 ± 3.78, P<0.01). In addition, AaDO₂ increased markedly in the group of IAP=10 cmH₂O (54.60 ± 6.80, P<0.001) and 20 cmH₂O (57.04 ± 5.60, P<0.001) (Table 1).

**Discussion**

HPS is characterized by chronic liver disease, hypoxemia, increase of AaDO₂ and intrapulmonary vascular dilatation [14, 15]. For the diagnosis of HPS, an elevated AaDO₂ (>20 mmHg) and a dilatation of intrapulmonary vascular confirmed by contrast enhanced “bubble” echocardiography or radionuclide perfusion scanning is required [4, 16]. The manifestations closely related to HPS include cyanosis, digital clubbing and cutaneous spider nevi, etc. Generally, HPS is defined by a widened AaDO₂ on room air (>15 mmHg, or >20 mmHg and in patients >64 years old), with or without hypoxemia resulting from intrapulmonary vasodilatation in the presence of liver dysfunction or portal hypertension. From a practical vantage point, identifying patients with PaO₂ <70 mmHg is useful in recognizing those with clinically significant HPS. The presence of “clubbing” has the highest positive predictive value (75%) and “dyspnea” with the highest negative predictive value (100%) for patients with HPS [17].

Although several potential mechanisms were used to explain the occurrence and development of HPS, the certain pathogenesis remains unknown [1-3]. As the major complication of cirrhosis, ascites could obviously raise IAP. According to the consensus of World Society for Abdominal Compartment Syndrome, IAH was defined as sustained or repeated pathologic elevation of IAP≥12 mmHg. Sustained elevation of IAP>20 mmHg accompanying new organ dysfunction, such as cardiovascular, respiratory and renal systems, was treated as abdominal compartment syndrome. The adverse consequences of IAH can further lead in the diaphragm upward, bacterial translocation, intestinal endotoxemia, monocyte/macrophage activation and decreased hepatic clearance [11-13, 18].

Currently, the commonly used animal models of IAH are induced via intraperitoneal infusion of sterile salt solution or continuous injection of CO₂ or nitrogen [19]. Besides, chronic common bile duct ligation is widely used in the modeling of biliary cirrhosis and portal hypertension due to the hypoxemia and intrapulmonary vasodilator [20]. It is also considered as a classical model with the pathological characteristics of HPS. However, the drawbacks of these methods are as following: the balance of liquid is quickly absorbed, which could maintain IAP temporarily; the adoption of CO₂ or nitrogen or the ligation of common bile duct cannot simulate the effect of massive ascites in cirrhosis. Moreover, the biggest pitfall of the latter is an uncertain proportion of the bile duct recanalization rate. In this study, we introduced a novel model of HPS in cirrhotic mice, which could form different levels of IAP via injecting different volumes of albumin (30 g/L). Our results indicated that the novel model has effectively overcome the defects of modeling and preserve IAP for a long time with the simulation effect of ascites.

From the results of lung histopathology, the widened alveolar interval could be found in the
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group of IAP=10 and 20 cmH₂O (Figure 2C and 2D), which may lead to the time extension of pulmonary ventilation. In addition, the obvious blood stasis in the alveolar walls and red blood cells extracellularly extended into air space suggested the severe dilatation of alveolar capillaries and the increasing permeability of microcirculation (Figure 2C and 2D). Blood gas analysis showed that PaO₂ gradually decreased with the increased IAP. In the group of IAP=10 and 20 cmH₂O, PaO₂ are 68.13 ± 3.56 mmHg and 66.00 ± 3.78 mmHg, respectively (Table 1). Accordingly, HPS is definite as PaO₂<70 mmHg, which indicated the presence of hypoxia and the disturbance of acid-base as the result of the gas-exchange abnormality.

In conclusion, our study suggested that IAH was a significant pathological mechanism of HPS. Meanwhile, we have established a novel mouse model of HPS, demonstrating the hemodynamic deteriorations and organ dysfunctions similar to the patients with HPS. The model will now be optimized with further investigation of the mechanism and therapeutic targets of HPS.

Acknowledgements

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Disclosure of conflict of interest

None declared.

Abbreviations

AaDO₂, alveolar-arterial oxygen pressure difference; HPS, hepatopulmonary syndrome; IAH, intra-abdominal hypertension; IAP, intra-abdominal pressure; PaO₂, arterial partial pressure of oxygen.

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References

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