Effects of CO₂ pneumoperitoneum on blood flow volume of abdominal organs of rabbits with controlled hemorrhagic shock and liver impact injuries

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Objective: To investigate the effects of CO₂ pneumoperitoneum on blood flow volume of abdominal organs of rabbits with controlled hemorrhagic shock model and liver impact injuries.

Methods: After controlled hemorrhagic shock and liver impact injuries, the rabbit model was established. Eighteen rabbits subjected to hemorrhagic shock and liver impact injuries were divided into 3 groups randomly according to the volume of lost blood: light hemorrhagic shock (blood loss volume was 10%, 6 ml/kg), moderate hemorrhagic shock (20%, 12 ml/kg) and severe hemorrhagic shock (40%, 22 ml/kg). Intraabdominal pressures of CO₂ pneumoperitoneum was 10 mmHg. Color-labeled microspheres were used to measure the blood flow volume of the liver, kidney and stomach before pneumoperitoneum at 30 minutes and 2 hours after pneumoperitoneum and 30 minutes after deflation. And the mortality and hepatic traumatic condition of rabbits were recorded.

Results: Of the 18 rabbits, there were 9 with liver impact injuries at Grade I, 8 at Grade II and 1 at Grade III (according to AIS-2005). The mortality rate in light hemorrhagic shock group was 33.33%, and that in moderate or severe hemorrhagic shock group was 100% within 30 minutes and 2 hours after pneumoperitoneum, respectively. The blood flow volume in the organs detected decreased at 30 minutes under pneumoperitoneum in light and moderate hemorrhagic shock groups. At the same time, the blood flow volume of the liver in moderate hemorrhagic shock group decreased more significantly than that in light hemorrhagic shock group.

Conclusions: The blood flow volume of abdominal organs in rabbits is decreased obviously under CO₂ pneumoperitoneum, with fairly high mortality rate. It is believed that CO₂ pneumoperitoneum should cautiously be used in abdominal injury accompanied with hemorrhagic shock, especially under non-resuscitation conditions.

Key words: Abdominal injuries; Shock, hemorrhagic; Pneumoperitoneum, artificial; Regional blood flow

Th e diagnosis of abdominal injury sometimes remains difficult. Laparoscopic technique developed at the end of last century provides a new diagnostic method and facilitate definite management. But researches on the safety of CO₂ pneumoperitoneum established during these conditions of abdominal injury accompanied by hemorrhagic shock are rarely reported. In this study, rabbit models with different degrees of hemorrhagic shock and liver impact injuries were established to investigate the effects of CO₂ pneumoperitoneum on blood flow volume of abdominal organs.

METHODS

Animals and surgical preparation

Eighteen New Zealand rabbits of either sex weighing 2.0-3.7 kg (mean 2.42 kg ± 0.41kg) were used and randomly divided into 3 groups with 6 rabbits in each group according to the volume of blood loss: light hemorrhagic shock (blood loss volume was 10%, 6 ml/kg), moderate hemorrhagic shock (20%, 12 ml/kg) and severe hemorrhagic shock (40%, 22 ml/kg). The rabbits were given free access to water, but food was withdrawn for 12 hours before the experiment. Anesthesia was induced with 3% sonistan. A tracheotomy was performed and the rabbits were kept in a supine position throughout the experiment. Mechanical ventilation was not used but two catheters were inserted, ie, one catheter was inserted into the left cardiac ventricle (the position verified by typical pressure trace) for injection of color-labeled microspheres via the right carotid artery,
the other one into the right femoral artery to obtain reference blood samples and to draw blood according to the degree of hemorrhagic shock. During the course of shock no shed blood or fluid was infused into the body for resuscitation. Animals were impacted by the biology impact machine (BIM-IV) and had liver injured. A 10° syringe needle was inserted through the umbilicus into the abdomen for establishing CO₂ pneumoperitoneum and monitoring pressure of pneumoperitoneum.²⁻³ Body temperature was monitored and maintained at normal limits with a heating light. After the animals were killed, condition of the liver was investigated. Totally 18 rabbits were allowed to get into the experiment in which the traumatic condition of liver was at Grade I-III.

**Main reagents**

The reagents included Dye-Trak microspheres (Triton Technology Inc, USA), Triton X-100, Tween 80, dehydrated alcohol, potassium hydroxide, 37% hydrochloric acid and cellosolve acetate (Chemical Reagents Company of Chongqing, China).

**Main instruments**

The instruments included electrophysiograph (Power Lab/8SP, AD Instruments), centrifuger (type 80-2, Shanghai Hongshan Apparatus Factory, China), electrothermal airblast drying cabinet (Galaxy Machine Corporation of Chongqing, China), universal microplate spectrophotometer (Multiskan Spectrum, type 1500, Finland), self-made type BIM-IV bioimpactor device and self-made insufflator of CO₂.

**Regional blood flow**

Regional blood flow (RBF) of the pancreas was measured by the color-labeled microsphere technique using spheres of five different colors including blue, yellow, persimmon, orange and lemon (Dye-Trak Microspheres, Triton Technology Inc, USA). The spheres were suspended in saline with 0.05 Tween 80 and thimerosal. Ten seconds before injection, the reference sample was withdrawn from the femoral catheter which was set at a constant rate of 1 ml/min using a withdrawing pump. The right carotid catheter was flushed and blood lost was replaced with saline or blood via the femoral catheter throughout the experiment.

After the rabbits were killed, tissue samples were taken from the liver, kidney and stomach in a predetermined and standardized process. Tissue sample and reference sample were digested, centrifuged, washed and dried. Then, the color-labeled microspheres were recovered. Acidified cellosolve acetate was used to extract the dye of the microspheres. The photometric absorption of each dye solution was measured by the universal microplate spectrophotometer at the following wave lengths, ie, 670 nm, 545 nm, 495 nm, 450 nm and 390 nm. These data were input into the Excel files by the Triton Technology Inc. The RBF of the liver, kidney and stomach could be calculated according to following formula: RBF (ml/min/g) = the velocity of reference blood flow (ml/min) × the number of microsphere of tissue/the number of reference blood flow × tissue weight (g).

**Statistical analysis**

Data were expressed as mean ± standard deviation. A general linear model for repeated measures (SPSS version 13.0 software) was used. Post hoc tests were performed (Student-Newman-Keuls, Instat Statistic program) when justified by the preceding test. P<0.05 was considered statistically significant.

**RESULTS**

Nine rabbits had liver impact injury of Grade I, 8 Grade II and 1 Grade III. The volume of intra-abdominal hemorrhage was 1.85-2.56 ml. There were 8 cases accompanied by light pulmonary contusion. The mortality rate in light hemorrhagic shock group was 33.33%, and that in moderate or severe hemorrhagic shock group was 100% within 30 minutes and 2 hours after pneumoperitoneum.

RBF decreased at different degrees in every group under pneumoperitoneum and restored gradually after deflation. There was no remarkable difference in the RBF between Grade I and II groups (P>0.05). The difference of RBF was remarkable at the first phase and the difference was still remarkable at the second phase (P<0.05) in comparison with Grade II and III groups.

RBF in the organs decreased at 30 minutes after pneumoperitoneum in all groups. RBF in the liver decreased more significantly in moderate hemorrhagic shock group than that in light hemorrhagic shock group (Table 1). The rabbits with severe hemorrhagic shock were all died during 45 minutes after pneumoperitoneum.
DISCUSSION

Early diagnosis and definite management is the key to lowering mortality and complication rate in abdominal injury. Besides history of trauma and physical examination, the common auxiliary examinations include blood test, X-ray examination, abdominal paracentesis, diagnostic peritoneal lavage, type-B ultrasound, CT, and so on. But all these methods have some false-negative and false-positive rates, and therefore 15%-20% of exploratory operations are unnecessary. The diagnosis of abdominal injury sometimes remains difficult and laparoscopic technique is a new diagnostic method and can give definite management. Reduction of blood flow occurred in hemorrhagic shock which is often accompanied by abdominal injury, and the blood flow volume of the organs, such as the liver, kidneys and stomach, is decreased so as to increase the blood supply of more important organs (heart and brain). But there are no researches as we know on the safety of CO₂ pneumoperitoneum established during these conditions of abdominal injury accompanied by hemorrhagic shock. In this study, rabbit models with different degrees of hemorrhagic shock and liver impact injuries were established to investigate the effects of CO₂ pneumoperitoneum on blood flow volume of abdominal organs.

The whole blood volume of a rabbit is about 57.3 ml/kg. When the blood was drawn at the volume of 6 ml/kg, 12 ml/kg, 22 ml/kg, the hemorrhagic shock model would lose about 10%, 20%, 40% of its whole blood volume. The principle of color-labeled microspheres for detecting blood flow of organs is to infuse a few definite color-labeled microspheres into the heart, and the color-labeled microspheres reach pantosomatous tissues and organs following the blood flow. The organ blood flow is calculated through the microsphere number of tissues or organs gained at the end of experiment. The advantages of color-labeled microspheres are considered easy manipulation, simultaneous detection of multi-organs blood flow volume and non-radioactive pollution.

Under non-hemorrhagic shock condition, pneumoperitoneum, using carbon dioxide gas to provide intra-abdominal working space for laparoscopic surgery, would result in reduction of splanchnic blood flow. Schafer et al found that the RBF of the liver and kidneys decreased by about 29%-37.2% and 34.8%-40.6% in studies of splanchnic circulation alterations during CO₂ pneumoperitoneum in rats. The decrease of hepatic blood flow is mainly due to the reduction of portal venous flow. Yavuz et al found that splanchnic blood flow was susceptible to intra-abdominal pressures above the level of 15 mmHg. Similar to above researches, we found that when the pressure of CO₂ pneumoperitoneum was 10 mmHg, the blood flow volume of the kidney decreased at 30 minutes of pneumoperitoneum in moderate hemorrhagic shock group; while the blood flow volume of the liver decreased more significantly in moderate hemorrhagic shock group than that in light hemorrhagic shock group. It is believed that the influence of CO₂ pneumoperitoneum on the splanchnic circulation is accompanied by the aggravation of the severity of hemorrhagic shock, reasons for which may include direct oppression of CO₂ pneumoperitoneum on abdominal organs, decreased cardiac output owing to hypercapnia and returned blood volume reduction.

Table 1. RBF in the liver, kidney and stomach at different degrees of hemorrhagic shock (ml·min⁻¹·g⁻¹, n=6)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Groups</th>
<th>Pre-pneumoperitoneum</th>
<th>30 min after pneumoperitoneum</th>
<th>2 h after pneumoperitoneum</th>
<th>30 min after deflation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Light hemorrhagic shock</td>
<td>0.6590.247</td>
<td>0.4470.191*</td>
<td>0.3370.243</td>
<td>0.3910.177</td>
</tr>
<tr>
<td></td>
<td>moderate hemorrhagic shock</td>
<td>0.3300.095</td>
<td>0.2010.132*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Left kidney</td>
<td>Light hemorrhagic shock</td>
<td>1.9850.631</td>
<td>1.2600.349</td>
<td>0.8270.114</td>
<td>0.8820.137</td>
</tr>
<tr>
<td></td>
<td>moderate hemorrhagic shock</td>
<td>1.4480.228</td>
<td>1.0290.120*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Right kidney</td>
<td>Light hemorrhagic shock</td>
<td>2.0520.668</td>
<td>1.2930.303</td>
<td>0.9840.220</td>
<td>0.8220.137</td>
</tr>
<tr>
<td></td>
<td>moderate hemorrhagic shock</td>
<td>1.5210.293</td>
<td>1.1330.230*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stomach</td>
<td>Light hemorrhagic shock</td>
<td>0.3380.196</td>
<td>0.2350.083</td>
<td>0.2310.111</td>
<td></td>
</tr>
<tr>
<td></td>
<td>moderate hemorrhagic shock</td>
<td>0.2610.109</td>
<td>0.2450.132</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a: Compared with pre-pneumoperitoneum (P<0.05). b: Compared with light hemorrhagic shock (P<0.05). _ : The animals alive were so less that could not be analyzed statistically.
In this study, the mortality rate of light hemorrhagic shock group was 33.33%, and that in moderate and severe hemorrhagic shock groups was 100%. It would be hazardous to establish CO$_2$ pneumoperitoneum when abdominal injury is accompanied by hemorrhagic shock and insufficient resuscitation. But sufficient resuscitation may raise the mortality rate and complication rate when bleeding due to abdominal injury has not yet been controlled. Definite stop bleeding with open operation is believed to be the most important step for non-controlled hemorrhagic shock.

REFERENCES


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