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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Morphological changes in hepatic vascular endothelium after carbon dioxide pneumoperitoneum in a murine model

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Abstract
Background: Liver metastasis is an important prognostic factor in advanced colorectal cancer. Several studies have demonstrated that carbon dioxide (CO_2) pneumoperitonem enhances liver metastasis in an animal model. In the present study, we used scanning electron microscopy (SEM) to investigate morphological changes in hepatic vascular endothelium after CO_2 pneumoperitoneum in a murine model.
Methods: Thirty-three male BALB/c mice were randomized to undergo pneumoperitoneum (CO_2, air, or helium), open laparotomy, and anesthesia alone. After each procedure, the animals' livers were excised at days 0, 1, and 3 and examined by SEM.
Results: In the CO_2 pneumoperitoneum group, we observed rough surface and derangement of the hepatic vascular endothelial cells and intercellular clefts on day 1. In the other groups, no major morphologic changes were observed at any time.
Conclusions: Hepatic vascular endothelium changes after CO_2 pneumoperitoneum. Such characteristic changes may play an important role in establishing liver metastasis after CO_2 pneumoperitoneum.

Key words: Laparoscopic surgery — Pneumoperitoneum — Carbon dioxide — Murine model — Scanning electron microscopy — Colorectal cancer — Liver metastasis

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Laparoscopic surgery yields favorable short-term clinical outcomes in the management of colorectal malignancies. As a less invasive alternative to open surgery, it has been shown to be technically feasible and to improve patient comfort. There have been few reports, however, on long-term outcomes.

Liver metastasis is the most important prognostic factor in advanced colorectal cancer. Therefore, the effects of pneumoperitoneum on liver metastasis of colorectal cancer have been a matter of cancer. Several experimental studies have shown that carbon dioxide (CO_2) pneumoperitoneum enhances liver metastasis [4–7]. Carbon dioxide pneumoperitoneum causes a decrease in portal blood flow [13, 18, 19] and provides favorable conditions for the adherence of free tumor cells, which can then spread into the portal system, to the hepatic vascular endothelium. However, it is uncertain whether morphological changes occur in the hepatic vascular endothelium after surgical procedures such as the CO_2 pneumoperitoneum or open laparotomy.

In the present study, we used a murine pneumoperitoneum model to investigate morphological changes in hepatic vascular endothelium by scanning electron microscopy (SEM).

Materials and methods

Animals

Thirty-three male BALB/c mice weighting 20–24 g and aged 6–8 weeks were used in this study. All animals were kept under standard laboratory conditions (temperature, 20–24°C; relative humidity, 50–60%; 12 h light/dark cycles) and given a standard laboratory diet with free access to water and food. All experiments were performed under the guidelines for animal experimentation of the Oita University Faculty of Medicine.

Procedures

All surgical procedures were performed under general anesthesia induced by diethyl ether inhalation. Thirty-three BALB/c mice were divided into three operative groups; pneumoperitoneum, laparotomy, and anesthesia only (control).

In the pneumoperitoneum group, nine mice underwent CO_2 pneumoperitoneum at 8–10 mmHg for 60 min and were killed after either 0 h (n = 3), 24 h (n = 3), or 72 h (n = 3). In addition, six mice received an air or helium (n = 3 each) pneumoperitoneum at 8–10 mmHg for 60 min and were killed after 24 h. The procedure for

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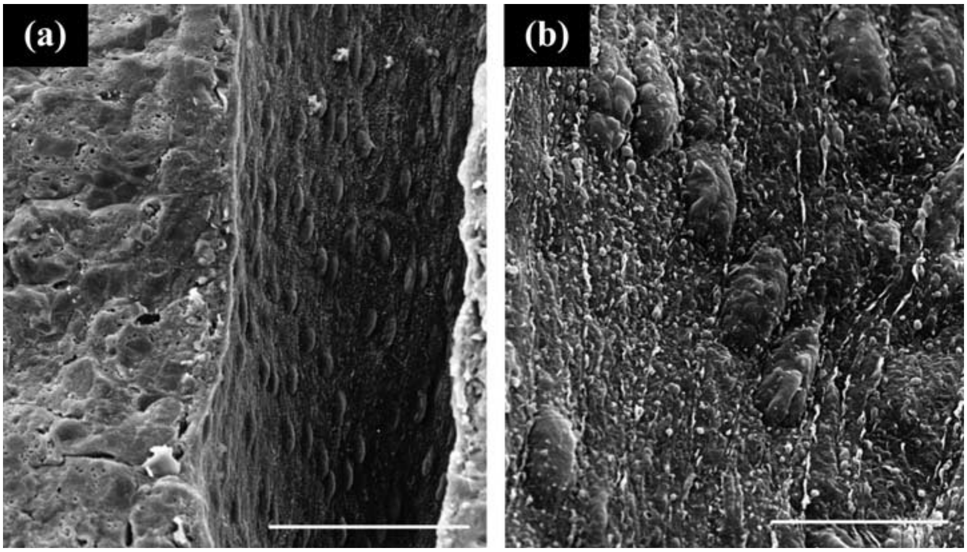

Fig. 1. Hepatic vascular endothelium of untreated mice. **a** Original magnification, ×500. The bar represents 60 μm. **b** Original magnification, ×2,000. The bar represents 15 μm.

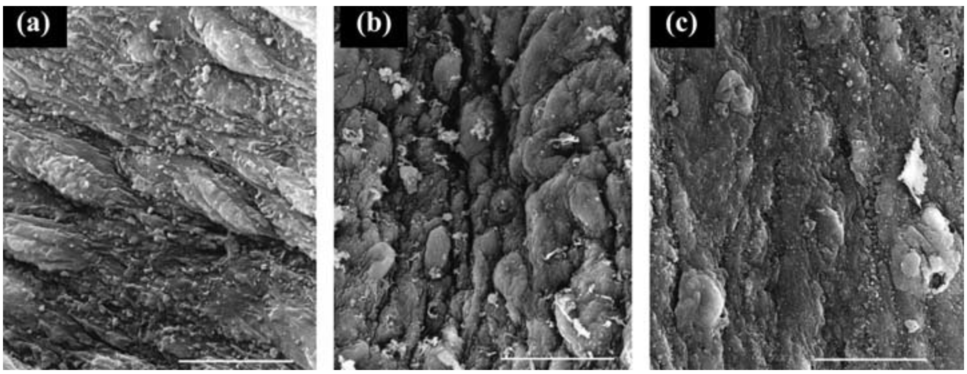

Fig. 2. In the CO_2 pneumoperitoneum group, there were marked changes in the hepatic vascular endothelium. Dilatation of intercellular clefts, irregular arrangement of endothelial cells, and a rough surface were observed. Original magnification, ×2,000. The bar represents 15 μm. **a** Immediately after CO_2 pneumoperitoneum. **b** Day 1. **c** Day 3.

establishing the pneumoperitoneum has been described previously [16]. A 22-gauge intravenous cannula was inserted into the left lower quadrant and used as an insufflation needle. A 20-gauge intravenous cannula was inserted into the right lower quadrant and used to measure intraperitoneal pressure. A disposable syringe was used to inject the gas. A syringe pump was used to maintain continuous insufflation, and intraperitoneal pressure was measured as the distance between the right and left water levels in the U-shaped tube.

In the laparotomy group ($n = 9$), a 3-cm abdominal midline incision was made, and the laparotomy conditions were maintained for 60 min. The mice were killled after either 0 h ($n = 3$), 24 h ($n = 3$), or 72 h ($n = 3$).

The control group ($n = 9$) underwent only diethyl ether anesthesia for 60 min. The mice were killed after either 0 h ($n = 3$), 24 h ($n = 3$), or 72 h ($n = 3$).

After each procedure, the left lobe of the murine liver was excised, and four samples from each liver were prepared for examination with an SEM. These samples were put into fixative solution composed of 2% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer solution. They were then placed into 1% osmium tetroxide for 60 min, dehydrated stepwise in alcohol, and dried by mens of a critical points apparatus. The dried specimens were mounted on aluminium stubs, spatter-coated with gold, and examined with an SEM (Hitachi S800, Ibaragi, Japan). Photographs of the five areas selected at random in each sample were analyzed.

None of the animals died at any time during this experiment.

Analysis

Following the same procedure used by Suematsu et al. [17], the photographs were evaluated by five independent observers (one histologist, one pathologist, and three surgeons) who were not informed of the procedures used to quantify the results. The following characteristics of the hepatic vascular endothelium were observed: (a) dilatation of intercellular clefts, (b) derangement of the endothelial cells, and (c) a rough surface. We then compared these characteristics with those of normal hepatic vascular endothelium of untreated mice and graded the changes as none or slight (−), moderate (+), or marked (+ +). If over half of the observers were in agreement, the results were adopted. In cases where the observers disagreed about the results, the lower grade was adopted.

Results

Figure 1 shows the normal hepatic vascular endothelium of untreated mice. Normal liver endothelium is characterized by a smooth surface, a regular arrangement of the endothelial cells that corresponds with the direction of the blood flow, and no intercellular clefts. In the CO_2 pneumoperitoneum group (Fig. 2), we observed both derangement of the hepatic vascular endothelial cells and intercellular clefts on day 1. These changes were recognized immediately after creation of the CO_2 pneumoperitoneum and persisted 3 days. However, in the air pneumoperitoneum group, the changes were not as marked on day 1 (Fig. 3a). Also, on day 1 after helium pneumoperitoneum, the changes were unremarkable (Fig. 3b). There were also no remarkable changes at any time in the control group or the laparotomy group

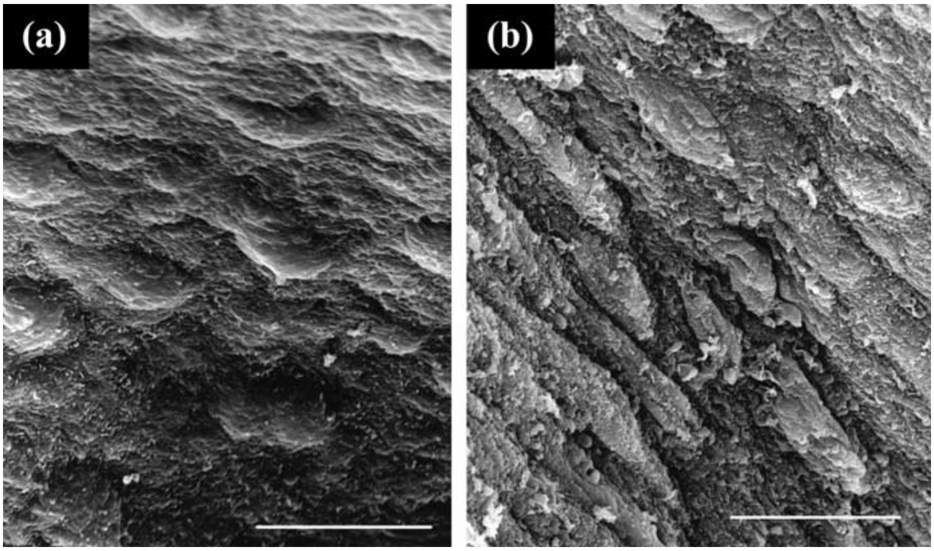

Fig. 3. In both the air (**a**) and helium (**b**) pneumoperitoneum groups, changes to the hepatic vascular endothelium were slight as compared with the CO_2 pneumoperitoneum group on day 1. Original magnification, ×2,000. The bar represents 15 μm.

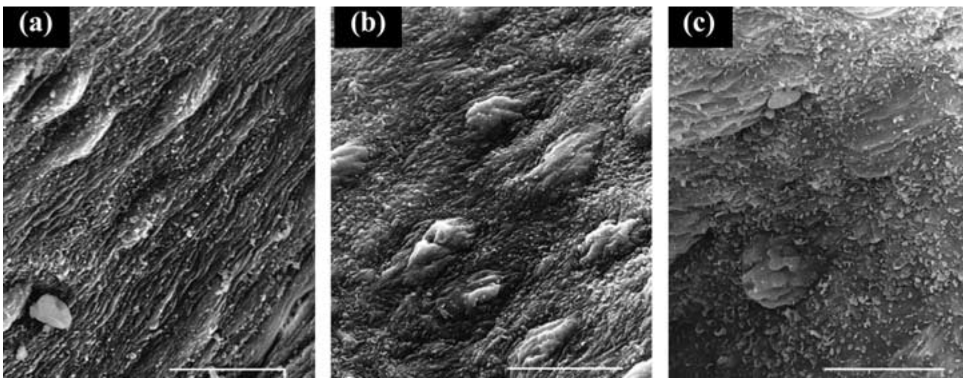

Fig. 4. In the control group, there were only minimal changes to the hepatic vascular endothelium. Original magnification, ×2,000. **a** Immediately after ether anesthesia. **b** Day 1. **c** Day 3. The bar represents 15 μm.

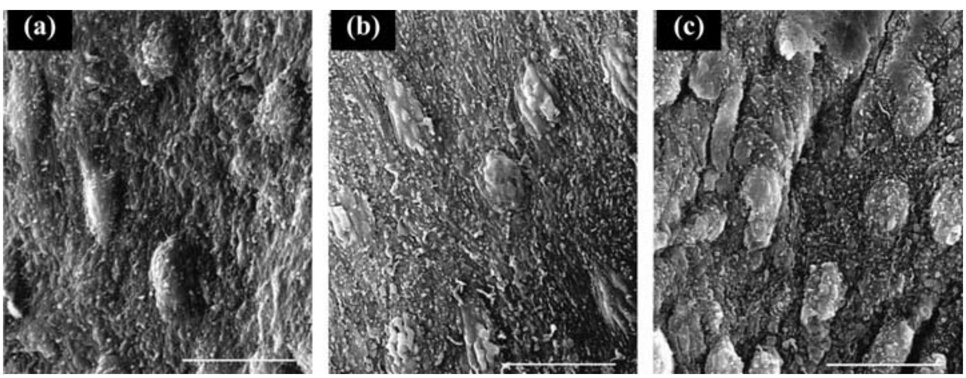

Fig. 5. In the laparotomy group, there were only minimal changes to the hepatic vascular endothelium. Original magnification, ×2,000. **a** Immediately after laparotomy. **b** Day 1. **c** Day 3. The bar represents 15 μm.

(Figs. 4 and 5). In the laparotomy group, only the rough surface of endothelium was observed on day 3, but this change was slight as compared with the CO_2 pneumoperitoneum group.

Table 1 summarizes the morphological changes in each group. There were no wide disparities among the five observers in grading the results.

Discussion

Laparoscopic surgery has been adopted for colorectal cancer because it is less invasive and yields a better cosmetic result. However, the effects of the pneumoperitoneum on liver metastasis, which is the most important prognostic factor for colorectal cancer, remain unclear. In the present study, we used SEM to investigated the morphologic changes to hepatic vascular endothelial cells that occur after CO_2 pneumoperitoneum. Other studies have used SEM to investigate morphological changes to the peritoneum after CO_2 pneumoperitoneum [17, 20], but this is the first study to clarify changes to the hepatic vascular endotheliem after CO_2 pneumoperitoneum.

The first step in liver metastasis is the adherence of tumor cells to the hepatic vascular endothelium. Thus,

Table 1. Summary of morphological changes of hepatic vascular endothelium after carbon dioxide (CO_2), helium, or air pneumoperitoneum, laparotomy, or anesthesia alone

	Dilatation of intercellular clefts	Irregular arrangement of endothelial cells	Rough surface
Immediately after each procedure			
CO_2	–	+	–
Laparotomy	–	–	–
Anesthesia	–	–	–
24 h after each procedure			
CO_2	+ +	+ +	+ +
Air	–	–	+
Helium	–	–	+
Laparotomy	–	–	–
Anesthesia	–	–	–
72 h after each procedure			
CO_2	+	+	+
Laparotomy	–	–	+
Anesthesia	–	–	–

–, none or slight; +, moderate; + +, marked

injury to the hepatic vascular endothelium may be associated with an increase in the incidence of liver metastasis. In hepatic ischemia-reperfusion injury, the damage to the ischemic lobe creates a favorable condition for liver metastasis or intrahepatic tumor growth [2], and the expression of adhesion molecules promotes the establishment of liver metastasis.

Several studies have previously shown that the intraabdominal insufflation of CO_2 causes a marked and rapid decrease (35% to 84%) in portal blood flow [8, 12, 14]. This reduction correlates with the degree of intraabdominal pressure, and may be caused by either mechanical compression of the thin-walled portal vein or hypercapnia-induced vasoconstriction; by contrast, the hepatic arterial flow appears to be less compromised. In this study, intraabdominal pressure was kept at 8–10 mmHg after insufflation in the pneumoperitoneum group. This high level of intraabdominal pressure must influence portal blood flow, and it may serve to induce hepatic ischemia after creation of the pneumoperitoneum.

In the CO_2 pneumoperitoneum group, we observed distinct morphological changes to the hepatic vascular endothelium, including (a) dilatation of intercellular clefts, (b) irregular arrangement of the endothelial cells, and (c) a rough surface. On day 1 after CO_2 pneumoperitoneum, these changes were remarkable. However, on day 1 after air and helium pneumoperitoneum, the changes to the hepatic vascular endothelium were comparatively slight. Our results suggest that these morphological changes are caused not only by the reduction in portal blood flow induced by intraabdominal high pressure but also by the CO_2 itself.

Shuto et al. [15] have shown that metabolic acidosis occurs after both helium and CO_2 pneumoperitoneum, but hypercapnia occurs only after CO_2 pneumoperitoneum. Furthermore, several studies have shown that whereas helium pneumoperitoneum does not cause either hypercapnia or acidic changes, CO_2 pneumoperitoneum induces both hypercapnia and acidosis[1, 3, 9–11]. Therefore, morphological changes to the hepatic vascular endothelium may be a result of a combination of CO_2 absorption and the hepatic ischemia induced by the CO_2 pneumoperitoneum.

In conclusion, morphological changes to the hepatic vascular endothelium occur after CO_2 pneumoperitoneum. Clinical studies are needed to investigate whether these changes are associated with the enhancement of liver metastasis after laparoscopic colorectal surgery.

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