Peritoneal tissue-oxygen tension during a carbon dioxide pneumoperitoneum in a mouse laparoscopic model with controlled respiratory support

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Introduction

Laparoscopic, or minimally invasive, surgery has revolutionized the surgical field. The technology has evolved dramatically over the past two decades and continues to advance. Laparoscopic surgery offers numerous patient benefits including less post-operative pain and impairment of lung function, better cosmetic results, shorter hospitalization and earlier recovery (Korolija et al., 2004; Schwenk et al., 2005). It is not, however, without limitations. Post-operative adhesion formation and peritoneal dissemination remain major clinical problems (Ray et al., 1998; Ellis et al., 1999; Canis et al., 2001; Koppe et al., 2006). Recent animal experiments suggest that the laparoscopic peritoneal environment is hypoxic (Molinas and Koninckx, 2000; Yesildaglar and Koninckx, 2000; Molinas et al., 2001; Mynbaev et al., 2002; Binda et al., 2003; Wildbrett et al., 2003; Elkelani et al., 2004; Molinas et al., 2004). Given that hypoxia has many adverse effects on biological systems (Crowther et al., 2001; Murdoch et al., 2004; Lewis and Murdoch, 2005), it is plausible that it may contribute to the formation of adhesions or to the growth of malignant cells (Molinas and Koninckx, 2000; Yesildaglar and Koninckx, 2000; Molinas et al., 2001, 2004; Mynbaev et al., 2002; Binda et al., 2003; Wildbrett et al., 2003; Elkelani et al., 2004).

In interpreting the results of animal studies, one must consider whether the findings obtained in the animal model are generalizable to the human clinical setting. A serious limitation of many of the laparoscopic experiments performed in rodent models is the lack of attention paid to confounding factors including the type of anaesthesia used, whether or not respiratory support was used and what insufflation pressures were used in the peritoneum (Gitzelmann et al., 2000; Shiromizu et al., 2000; Carter et al., 2003). A recent study
has clearly demonstrated that hypoxaemia and acidosis occur in anaesthetized, non-ventilated rats (Heijnen et al., 2002). Both hypoxaemia and acidosis can induce hypoxia. In another study in which laparoscopic surgeries were performed in mice anaesthetized with respiratory support, the animals developed a severe acidosis (Molinas et al., 2004). As is commonly practised in many laboratories (Gitzelmann et al., 2000; Shiromizu et al., 2000; Carter et al., 2003), these investigators applied the same absolute pneumoperitoneum pressure levels used in humans rather than a pressure level proportionate to the animals’ size (Molinas et al., 2001, 2004). It is likely that an excessively high intraperitoneal pressure (IPP) contributes to the development of acidosis. There is no study, however, that investigates the relationship between anaesthesia, ventilation and the laparoscopic peritoneal environment to the development of hypoxia, hypoxaemia and acidosis.

In this study, we developed mouse laparoscopic and laparotomy models that utilized controlled respiratory support (CRS). CRS was enabled by videoendoscopic endotracheal intubation and mechanical ventilation. We used this model to measure the peritoneal tissue-oxygen tension (PitO₂) in non-injured peritoneum under various conditions including anaesthesia alone, during a carbon dioxide (CO₂) pneumoperitoneum at both low and high IPP and laparotomy. We compared our findings in this model with those obtained when CRS was not used.

Materials and methods

Animals

Animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services, Public Health Service, 1985), and institutional review board approval was obtained for the current study. Studies were conducted in adult (8-week-old, 18–20 g) female C57BL6 mice (Ifla-Credo, Lyon, France). The mice were maintained in a light-and-temperature-controlled environment (14-h light, 10-h dark cycle, 22–25°C) and allowed a 2-week period of acclimation to the vivarium before any procedure was performed. After completion of the experiment, all mice were euthanized with an anaesthetic overdose.

Anaesthesia and videoendoscopy-assisted endotracheal intubation

All mice were anaesthetized in a container with 3–5% vaporized isoflurane with air. Each spontaneously breathing, anaesthetized animal was placed in a supine position on a surgical table. A 2 cm loop made from 4-0 suture was placed behind the front upper incisors and fixed to the surgical table and the mouse secured to the table with adhesive tape by the tail. A rigid 2 mm endoscope (Karl Storz Endoscopy & GmbH, Tuttlingen, Germany) was used to visualize the epiglottis, larynx and vocal cords with the image displayed on a monitor (Figure 1A and B). An endotracheal tube (24 gauge i.v. catheter, Insyte, Becton Dickinson, Le pont de claix, France), with a blunted and bent metal insertion needle as a stylet, was inserted parallel to the scope and threaded carefully and gently between the vocal cords under direct visualization.

A 24 gauge i.v. catheter (arrow) with a blunted and bent metal insertion needle as a stylet was inserted parallel to the scope and threaded carefully and gently between the vocal cords under direct visualization.

Figure 1. Videoendoscopy-assisted endotracheal intubation in mice. A rigid 2 mm endoscope (A) was used to visualize the epiglottis, larynx and vocal cords with the image displayed on a monitor (B). A 24 gauge i.v. catheter (arrow) with a blunted and bent metal insertion needle as a stylet was inserted parallel to the scope and threaded carefully and gently between the vocal cords under direct visualization.
Pneumoperitoneum at 2 mmHg of IPP with CRS were within the normal range (PaO₂, 105.3 ± 2.0; PaCO₂, 28.9 ± 1.0; pH, 7.367 ± 0.012, mean ± SEM, n = 3, our preliminary studies). Then, sufentanil (narcotic analgesic, 25 μg/kg, 0.05 ml) and vecuronium bromide (muscle relaxant, 0.4 mg/kg, 0.08 ml) were injected s.c. (right lateral upper quadrant). Vecuronium bromide was injected every 30 min. Anaesthesia was maintained by delivering 2% vaporized isoflurane with air. In the groups without CRS, following the injection of sufentanil (25 μg/kg) s.c. (right lateral upper quadrant), the spontaneously breathing mice received 2% vaporized isoflurane with air through a nose cone.

Surgical procedures
In the pneumoperitoneum group, a 24 gauge i.v. catheter was inserted into the middle of the abdomen and the peritoneal space insufflated with CO₂ or air to a pressure of 2 or 8 mmHg (flow rate: 2.1 min⁻¹ for both pressure) for 1 h using a Storz electronic endoflator (Karl Storz Endoscopy & GmbH). This electronic endoflator is suitable for both gas. An 18 gauge catheter was inserted i.p. (right lower quadrant) and connected to a water valve to permit the continuous escape of gas. The insufflation of gas was balanced with its exit to maintain a constant pressure.

In the laparotomy group, a 3 cm abdominal incision was made extending from the xiphoid to the pubis and two 5-0 sutures were applied to expose peritoneum to the air for 60 min.

Homeothermia
After the induction of anaesthesia, all procedures were performed on a thermostatically regulated, feedback-controlled heating pad (Homeothermic Blanket Control Unit, Harvard apparatus GmbH). After intubation, a temperature probe was inserted into the rectum. A rectal temperature of 37°C was maintained during the surgical procedure.

Peritoneal tissue-oxygen tension
A 16 gauge i.v. catheter was inserted into the retroperitoneal space (beneath the peritoneal membrane) from the left lateral upper quadrant to left lateral lower quadrant, just before surgical procedures. A polarographic oxygen electrode (Integra Neuroscience, Sophia Antipolis, France) was inserted through the catheter and placed in the retroperitoneal space just near the left lateral abdominal mammary gland to measure the PitO₂. The PitO₂ was continuously monitored on a LICOX CMP monitor (CC1.P1, oxygen sensitive part diameter, 0.65 mm; oxygen sensitive area, 18 mm²; Integra Neuroscience) during procedures. To obtain reliable PitO₂ readings, the tissue was allowed to stabilize following the microtrauma of implantation, and the intraoperative values were averaged across the last 30 min of the procedure in each mouse. The resulting values were then averaged among the mice in each group for Protocol 1 (see below). This is a well-established method for evaluating tissue-oxygen Pa (Akca et al., 1999; Greif et al., 2000).

Experimental design
Protocol 1
A total of 40 mice were randomized into the following eight groups of five animals each: (i) anaesthesia alone; (ii) anaesthesia with CRS provided by endotracheal intubation and mechanical ventilation; (iii) anaesthesia plus CO₂ pneumoperitoneum at 2 mmHg of IPP (the minimum required to perform the surgery according to our preliminary study); (iv) anaesthesia plus CO₂ pneumoperitoneum at 2 mmHg of IPP with CRS; (v) anaesthesia plus CO₂ pneumoperitoneum at 8 mmHg of IPP; (vi) anaesthesia plus CO₂ pneumoperitoneum at 8 mmHg of IPP with CRS; (vii) anaesthesia plus laparotomy and (viii) anaesthesia plus laparotomy with CRS.

Results
Arterial blood gas analysis
Results are shown in Table I. Animals in the CO₂ pneumoperitoneum groups without CRS (Groups 3 and 5) developed a hypercarbic acidosis. The PaCO₂ in these animals was considerably higher than the normal value of 25–35 mmHg (Schwarte et al., 2000) in mice. In addition, mice in Group 5 (CO₂ pneumoperitoneum, high IPP, no CRS) developed mild hypoxaemia. In contrast, mice in Group 6 (CO₂ pneumoperitoneum, high IPP, CRS) were mildly acidemic, but not hypercarbic. Blood gas parameters for all of the other groups (1, 2, 4 and 6–8) were within the normal range.

Peritoneal tissue-oxygen tension
Protocol 1
Results are shown in Figure 2A and B. When PitO₂ was measured during anaesthesia alone, CO₂ (Figure 2A) or air
Results of arterial blood gas analysis and peritoneal tissue-oxygen tension during a carbon dioxide pneumoperitoneum in a mouse laparoscopic model

Table I. Results of arterial blood gas analysis and peritoneal tissue-oxygen tension during a carbon dioxide pneumoperitoneum in a mouse laparoscopic model

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (anaesthesia alone)</th>
<th>CO2 pneumoperitoneum at a low IPP (2 mmHg)</th>
<th>CO2 pneumoperitoneum at a high IPP (8 mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>101.3 ± 5.2</td>
<td>106.8 ± 4.2</td>
<td>108.2 ± 4.2</td>
</tr>
<tr>
<td>Group 2</td>
<td>36.2 ± 3.9</td>
<td>26.8 ± 2.9</td>
<td>26.7 ± 2.9</td>
</tr>
<tr>
<td>Group 3</td>
<td>73.74 ± 0.04</td>
<td>7.269 ± 0.04</td>
<td>7.269 ± 0.04</td>
</tr>
<tr>
<td>Group 4</td>
<td>4.17 ± 0.03</td>
<td>4.17 ± 0.03</td>
<td>4.17 ± 0.03</td>
</tr>
<tr>
<td>Group 5</td>
<td>101.2 ± 3.2</td>
<td>104.2 ± 3.2</td>
<td>101.2 ± 3.2</td>
</tr>
<tr>
<td>Group 6</td>
<td>34.2 ± 3.6</td>
<td>32.2 ± 3.6</td>
<td>32.2 ± 3.6</td>
</tr>
<tr>
<td>Group 7</td>
<td>7.269 ± 0.04</td>
<td>7.269 ± 0.04</td>
<td>7.269 ± 0.04</td>
</tr>
<tr>
<td>Group 8</td>
<td>32.2 ± 3.6</td>
<td>32.2 ± 3.6</td>
<td>32.2 ± 3.6</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. CRS, controlled respiratory support; CO2, carbon dioxide; PitO2, peritoneal tissue-oxygen tension; IPP, intraperitoneal pressure. *P* values are obtained using the one-way analysis of variance with Fisher’s post hoc analysis.

Discussion

This study demonstrates that a CO2 pneumoperitoneum is in itself enough to cause acidosis in non-ventilated mice. In non-ventilated mice subjected to a high IPP, the acidosis was accompanied by hypercarbia and mild hypoxaemia. In ventilated mice, a mild metabolic acidosis was the only blood gas abnormality. As demonstrated in a recent haemodynamic study, an IPP of 6–8 mmHg in a rat (420–490 g) is comparable to an IPP of 15 mmHg in a human (Gutt and Schmandra, 2000). Thus, insufflation to 8 mmHg in a 20 g mouse is not equivalent to what is experienced by a human from a haemodynamic standpoint. Laparoscopic rodent experiments utilizing a disproportionately high IPP, particularly without CRS, are probably quantitatively and qualitatively different from the conditions in the human. Continued advancement in the field of laparoscopic surgery depends on the use of adequate animal models with sufficient similarity to human physiology as to enable valid comparisons. Their small size and relatively low maintenance cost make rodent models ideal for surgical studies, particularly those incorporating a time course. This study clearly demonstrates that both the use of an IPP in proportion to the animal’s size and mechanical ventilation are necessary in order to obtain near physiological conditions in the rodent, which are comparable to the conditions in the human.

Technical difficulties associated with endotracheal intubation are the main reason why only a limited number of laboratories use mechanical ventilation in rodent models. Several methods require a great deal of skill to perform (Molinas et al., 2001; Tarnavski et al., 2004). Additionally, the complication rates of these methods are high because even relatively mild iatrogenic laryngeal trauma could cause respiratory difficulties post-operatively. Consistent with studies in rats (Fuentes et al., 2004), the method of videoendoscopic endotracheal intubation used in this study is easy to learn and simple to perform accurately.

In this study, the highest PitO2 levels were seen in ventilated mice subjected to a CO2 pneumoperitoneum at a low IPP. Thus, in Protocol 2, we sought to investigate whether the PitO2 levels could also be elevated during an air pneumoperitoneum at a low IPP. Air insufflation at the same IPP, however, did not result in an increased PitO2. These findings suggest that it is the biological activity of CO2 gas that is contributing to the increase in peritoneal tissue-oxygen pressure levels during a CO2 pneumoperitoneum at a low IPP. PitO2 is based on the following factors: (i) delivery of oxygen from the lungs to the tissue (i.e. oxygenation of arterial blood, circulation); (ii) transport of oxygen from the blood to the tissue (i.e. PAO2 in blood, the diffusion distance); (iii)
oxygen consumption in the tissue (Gottrup, 2004). There are several possible explanations for the elevated PitO2 seen in the low IPP–CO2 pneumoperitoneum group. First, in rat laparoscopic models, the peritoneum becomes locally acido
tic during a CO2 pneumoperitoneum with CRS, even when the systemic pH is corrected (Hanly et al., 2005). The decreased pH and increased CO2 in peritoneal tissues during a CO2 pneumoperitoneum could increase the transport of oxygen from the blood to the tissue through the Bohr Effect. In the present study, we did not use humidified insufflation gas.

The hypothermic effect of laparoscopic insufflation is absorbed by the use of humidified gas (Bessell and Maddern, 1996) and thus, increased temperature might increase the transport of oxygen from the blood to the tissue through the Bohr effect. Further studies are necessary to investigate whether the use of humidified insufflation gas could increase the PitO2 levels. Second, in ventilated pigs, a CO2 pneumoperitoneum at 5–12 mmHg of IPP increases peritoneal blood flow (Brundell et al., 2002; Yavuz et al., 2003). It is likely that the increased peritoneal blood flow increases the delivery of oxygen to the tissue. In this study, the PitO2 during the CO2 pneumoperitoneum at the high IPP was significantly lower than that at the low IPP in ventilated mice. It is plausible that the high IPP would decrease peritoneal blood flow by a mechanical effect, resulting in a lower PitO2. Further studies are necessary to confirm whether the PitO2 during a CO2 pneumoperitoneum depends on IPP levels.

A recent study by Wildbrett et al. (2003) demonstrated a significant decrease in the tissue-oxygen tension during a CO2 pneumoperitoneum in a rat model without CRS. There are several possible explanations for the difference in findings. Primarily, their applied methodology was different from ours (i.p. injection of pentobarbital, absence of CRS and so on). Second, hypoxaemia might occur in their non-ventilated animals (Heijnen et al., 2002).

Normal level of tissue-oxygen tension is considered to be around 40 mmHg (Guyton and Hall, 2000) and thus, the present results suggested that non-injured peritoneal tissues in ventilated mice are not hypoxic during surgery. However, tissue hypoxia in wounds is common (Crowther et al., 2001) and thus, it is plausible that during surgical procedures in which the peritoneum is injured, the PitO2 level might be lower than the level in the non-injured peritoneum. Tissue oxygenation is one of the most important determinants in wound healing, adhesion formation and tumour growth (Crowther et al., 2001). Although it is necessary to investigate the PitO2 level in injured peritoneum under various surgical conditions, it is likely that the use of a low IPP during laparoscopic surgery may, through minimiz
ation of hypoxia, reduce the incidence of complications such as adhesions. Further studies are necessary to investigate the biological significance of an elevated PitO2 in normal peritoneum, the relationship between the IPP and PitO2 and complications such as adhesion formation or the peritoneal dissemination of tumours.

However, there are several methodological limitations to this study. First, we did not perform haemodynamics studies. Further studies are necessary to determine the most physiological IPP in mice, so that it mimics the situation in a clinical setting. Second, a polarographic oxygen electrode was placed in the retroperitoneal space to avoid any direct effects of CO2 gas. The present method measures overall average tissue oxygen tension, and thus, we might measure overall average tissue-oxygen tension for peritoneum and the surrounding tissues. Further studies are necessary to evaluate whether the peritoneal environment during a CO2 pneumoperitoneum is not hypoxic in each cellular level of

Figure 2. Peritoneal tissue-oxygen tension (PitO2, mmHg) during anaesthesia alone, CO2 (A) or air (B) pneumoperitoneum and laparotomy within the same mice. (A) After insufflation with CO2, the PitO2 was immediately elevated and maintained at higher levels than those seen with anaesthesia alone. Then, following laparotomy, PitO2 was immediately decreased in all three mice. (B) In contrast to the CO2 pneumoperitoneum, during an air insufflation, the PitO2 was maintained at levels comparable to those seen with anaesthesia alone and laparotomy. The PitO2 was significantly higher during CO2 pneumoperitoneum (A) than that during air pneumoperitoneum (B) (P < 0.05). Note: Observed peaks of PitO2 during transition phases between different surgical procedures might have occurred because of microtrauma of tissues as we described in Materials and methods section.
the peritoneal membrane, using a hypoxia marker (Haroon et al., 2000).

In conclusion, we used a mouse laparoscopic model to gain new insight into peri-operative peritoneal physiology. We demonstrated that an elevation in Pito2 occurs during a CO2 pneumoperitoneum at a low IPP with CRS. The present findings did not support the previous studies that the peritoneal environment during a CO2 pneumoperitoneum is hypoxic. The present method is well established for evaluating human tissue-oxygen partial pressure (Akca et al., 1999; Greif et al., 2000). Further studies should be performed to confirm our present findings in a clinical setting.

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References


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