FERTILITY AND STERILITY®
VOL. 81, NO. 3, MARCH 2004

Published by Elsevier Inc.
Printed on acid-free paper in U.S.A.

Role of CO_2 pneumoperitoneum-induced acidosis in CO_2 pneumoperitoneum-enhanced adhesion formation in mice

The effect of assisted ventilation and CO_2 pneumoperitoneum during laparoscopic surgery upon blood gases and adhesion formation were evaluated in mice. We confirmed that the CO_2 pneumoperitoneum induces acidosis and enhances adhesion formation, and an association between both effects was demonstrated, together with its modulation by the assisted ventilation. (Fertil Steril® 2004;81:708–11. ©2004 by American Society for Reproductive Medicine.)

Carbon dioxide is the most commonly used gas for pneumoperitoneum during laparoscopic surgery because of safety reasons, that is, its high solubility in water and its high exchange capacity in lungs. However, CO_2 pneumoperitoneum induces adverse systemic and local effects.

Systemically, CO_2 pneumoperitoneum can induce hypothermia (1), can impair venous return depending on the intraabdominal pressure (2), and causes CO_2 absorption from the abdominal cavity. If not compensated adequately by ventilation, this hypercarbia negatively affects cardiovascular and respiratory function (3).

Locally, CO_2 pneumoperitoneum induces desiccation (4), decreases the pH (5), and alters the peritoneal fluid (6), the microcirculation (7), and the morphology of the mesothelial cells in a time- and pressure-dependent manner (1). In addition, it is a cofactor in postoperative adhesion formation because adhesions increase with the duration of the pneumoperitoneum and with the insufflation pressure (8–11). These effects on adhesion formation are also observed with helium pneumoperitoneum and can be reduced by the addition of oxygen to the pneumoperitoneum (10, 11). This pneumoperitoneum-enhanced adhesion formation has been shown to be mediated, at least partially, by the up-regulation of plasminogen activator-1 (12), vascular endothelial growth factors (13), and hypoxia inducible factors (14), suggesting mesothelial hypoxia as the driving mechanism.

The relation between CO_2 pneumoperitoneum-induced systemic changes, such as acidosis and hypercarbia, and local changes, such as adhesion formation, has not yet been addressed, and therefore this prospective randomized study was performed in our laparoscopic mouse model.

The study was approved by the Institutional Review Animal Care Committee and was carried out in 250 female Naval Medical Research Institute mice (10–12 weeks old, 30–35 g). Anesthesia was induced with pentobarbital (IM, 0.07 mg/g), and endotracheal intubation was performed as described elsewhere (11). Mice were ventilated with room air with a tidal volume of 500 μL (Small Animal Ventilator, Harvard Apparatus Inc., Holliston, MA) or 250 μL (Mouse Ventilator MiniVent, Hugo Sachs Elektronik—Hardvard Apparatus GmbH, March-Hugstetten, Germany) at different ventilation rates, according to the experimental design.

Laparoscopy and induction of adhesions were performed as described elsewhere (11–14). A 2-mm endoscope with a 3.3-mm external sheath for insufflation was introduced into the abdominal cavity caudal to the xiphoids. Heated and humidified CO_2 was used for the pneumoperitoneum at 20 cm H_2O (~15 mm Hg) of insufflation pressure. Standardized lesions in uterine horns and pelvic sidewalls were performed with monopolar and bipolar coagulation. The pneumoperitoneum was maintained for the minimum time required to perform the lesions (standardized at 10 minutes) or for a longer period (60 minutes) to evaluate basal adhesions and pneumoperitoneum-enhanced adhesions, respectively (12). Adhesions were qualitatively (extent, type, tenacity, and total) and quantitatively (proportion) scored after 7 days during laparotomy, as described elsewhere (11–14).

Blood samples were obtained from the carotid artery as described elsewhere (15). A midline skin incision was performed in the ventral neck region, the thyroid lobes were separated, and the right carotid artery was dissected, avoiding any damage of the vagus nerve. Arterial blood samples (90 μL) were taken using heparinized glass capillaries (240 IU/mL) connected to a 23-gauge winged infusion set and stored at 4°C until analysis for pH, partial pressures of CO_2 (pCO_2) and oxygen (pO_2), and oxygen saturation (sO_2; IL Synthesis 15U, Instrumentation Laboratory, Belgium).

The effects of the CO_2 pneumoperitoneum and assisted ventilation with different ventilation patterns upon adhesion formation and upon blood gases were evaluated in two series of experiments using block randomization by days. The standard ventilation setting in series I was similar to that in all previous studies (11–14).

Received February 13, 2003; revised and accepted October 28, 2003.

Partially supported by Karl Storz Endoscopy (Tuttlingen, Germany) and by the Fonds Wetenschappelijk Onderzoek (Brussels, Belgium), grant number G.0324.01 and Onderzoeks Toelagen Katholieke Universiteit Leuven (Leuven, Belgium), grant number TBA/00/27.

Presented in part at the 10th Annual Meeting of the European Society for Gynecological Endoscopy, Lisbon, Portugal, November 21–24, 2001 and awarded the first prize of the Raul Palmer Award; also presented in part at the 6th International Symposium on Peritoneum, Amsterdam, The Netherlands, April 10–12, 2003.

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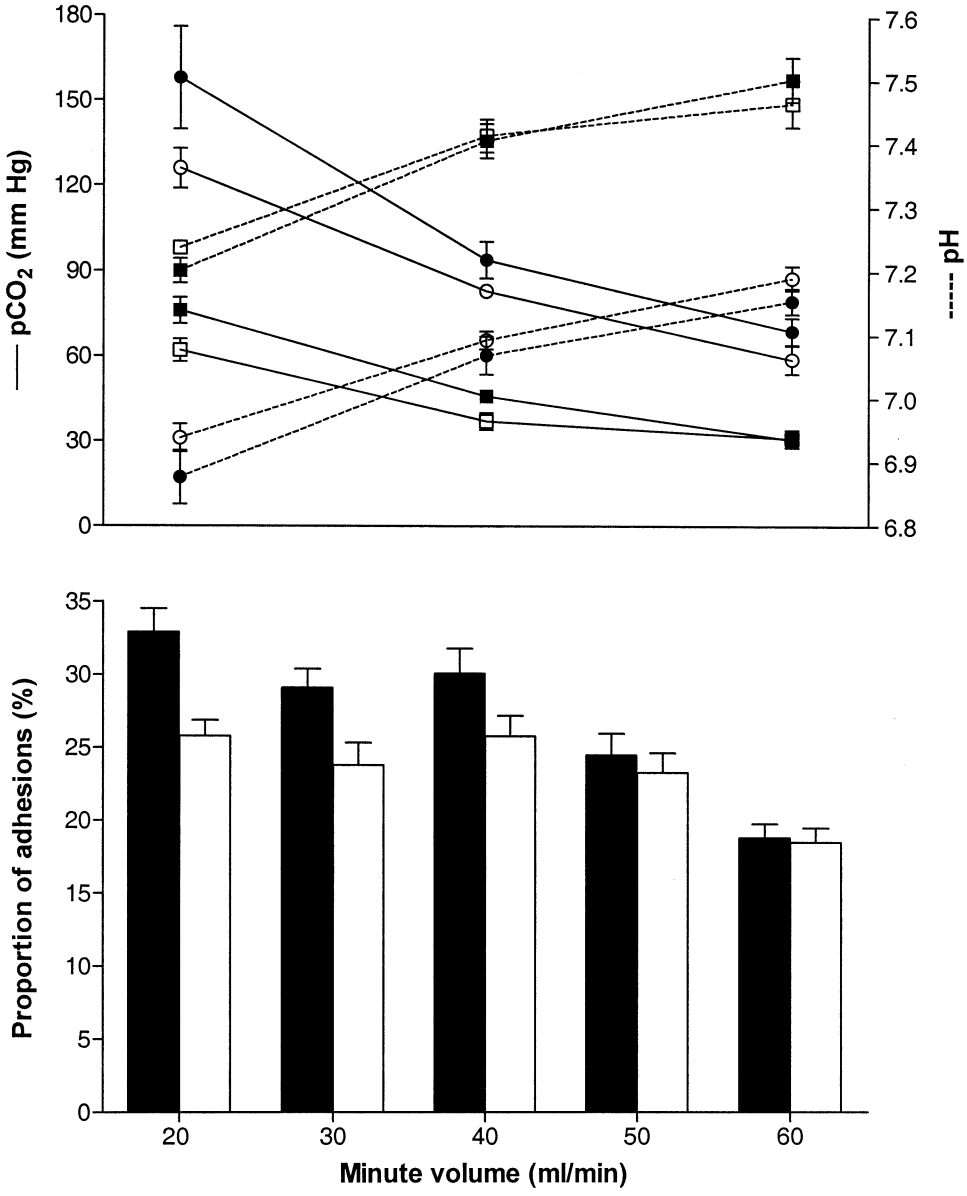
0015-0282/04/$30.00
doi:10.1016/j.fertnstert.2003.10.018

FIGURE 1

Effect of assisted ventilation upon CO_2 pneumoperitoneum-enhanced adhesion formation and arterial blood gases. Mice were ventilated with a minute volume of 20, 30, 40, 50, or 60 mL/min with a tidal volume of 500 μL at 40, 60, 80, 100, or 120 strokes per minute in series I (*filled symbols*) or of 250 μL at 80, 120, 160, 200, or 240 strokes per minute in series II (*open symbols*), respectively. pCO_2 (*solid line*) and pH (*dashed line*) were evaluated in mice with 60 minutes of ventilation with (*round symbols*) or without (*square symbols*) pneumoperitoneum.



Molinas. CO_2 pneumoperitoneum, acidosis, and adhesions. Fertil Steril 2004.

To ascertain the optimal work conditions for future studies, this experiment was duplicated in series II using a more recently available ventilator (Mouse Ventilator MiniVent). Differences between both series were disregarded for statistical analyses because they were performed separately. First, pneumoperitoneum-enhanced adhesions were evaluated in mice ventilated with a tidal volume of 500 μL at 40, 60, 80, 100, or 120 strokes per minute in series I (5 groups, 10 mice per group) and a tidal volume of 250 μL at 80, 120, 160, 200, or 240 strokes per minute in series II (5 groups, 10 mice per group), giving minute volumes of 20, 30, 40, 50, or 60 mL/min, respectively. Second, basal adhesions were evaluated in mice ventilated with minute volumes of 20, 40, and 60 mL/min in series I (3 groups, 10 mice per group) and II (3 groups, 10 mice per group). Finally, blood gases were evaluated in mice with and without 60

minutes of CO_2 pneumoperitoneum ventilated with minute volumes of 20, 40, or 60 mL/min and in mice with anesthesia only (breathing spontaneously without pneumoperitoneum) in series I (7 groups, 5 mice per group) and II (7 groups, 5 mice per group). Because carotid artery blood sampling is a terminal procedure, blood gases experiments were performed independently from adhesion formation experiments.

Statistical analyses were performed with the SAS system (SAS Institute, Cary, NC) using a factorial design. Logistic regression (proc logistic) and ANOVA were used for nonparametric (adhesion scores) and parametric (blood gases) variables, respectively. Unpaired *t*-test was used to compare blood gases between two groups. Absolute values of qualitative adhesion scores are not shown. All other data are presented as the mean ± SE.

Pneumoperitoneum-enhanced adhesions decreased (proc logistic) with higher ventilation rates. This was observed by analyzing all data together (proportion: $P=.01$; extent: $P=.04$; type: $P=.05$) and series I separately (proportion: $P=.03$; extent: $P=.04$; type: $P=.04$; total: $P=.03$), but the trend did not reach statistical significance for series II (Fig. 1).

Basal adhesions slightly decreased with higher ventilation rates but without reaching statistical significance. For mice ventilated with minute volumes of 20, 40, and 60 mL/min, the proportion of basal adhesions were 13 ± 4%, 13 ± 1%, and 10 ± 2% in series I, and 13 ± 3%, 11 ± 2%, and 10 ± 1% in series II, respectively. Similar trend was observed for extent, type, tenacity, and total adhesions scores (data not shown).

Anesthesia with pentobarbital, without pneumoperitoneum and without assisted ventilation, was associated with increased pCO_2(series I: 97.8 ± 7.1 mm Hg, series II: 97.5 ± 5.1 mm Hg) and decreased pH (series I: 7.17 ± 0.01, series II: 7.14 ± 0.02). These values returned within normal limits with assisted ventilation with ≥40 mL/min for tidal volumes of 500 μL or 250 μL. CO_2 pneumoperitoneum increased the pCO_2 and decreased the pH. This was observed (two-way ANOVA) after ventilation with a tidal volume of 500 μL (pCO_2: $P<.0001$; pH: $P<.0001$) and of 250 μL (pCO_2: $P<.0001$; pH: $P<.0001$). As could be anticipated, assisted ventilation decreased the pCO_2 and increased pH. This was observed (two-way ANOVA) after ventilation with a tidal volume of 500 μL (pCO_2: $P<.0001$; pH: $P<.0001$) and of 250 μL (pCO_2: $P<.0001$; pH: $P<.0001$) at higher minute volumes (Fig. 1). The highest minute volume, however, did not fully correct the pCO_2or the pH. The pO_2 and the sO_2 did not vary significantly in any series (data not shown).

All these data demonstrate that the CO_2 pneumoperitoneum increases the pCO_2 and decreases the pH in mice, as has been reported in pigs (16), dogs (17), rabbits (18), rats (19), and humans (3). They also demonstrate, as expected, that higher ventilation rates decrease the pCO_2 and increase the pH. However, the marked increase in pCO_2 and decrease in pH in animals with anesthesia only, that is, spontaneous breathing without pneumoperitoneum, was surprising, indicating insufficient spontaneous ventilation. Our data also demonstrate that, for the same minute volume, the combination of lower tidal volume and higher rate is more effective to correct the pCO_2 and pH than is the combination of higher tidal volume and lower rate. This is consistent with the physiologic ventilation pattern of mice (20). The even much higher ventilation rates required in mice with pneumoperitoneum, although it is not surprising, emphasize that ventilation should be well controlled in studies with pneumoperitoneum to avoid acidosis and hypercarbia.

A better assisted ventilation also slightly decreases adhesion formation, as was clearly shown for the overall study and specifically in mice that were ventilated with a tidal volume of 500 μL. This effect, however, did not reach statistical significance in mice ventilated with a tidal volume of 250 μL. This could be due to the overall less adhesion formation in this series or to the overall better ventilation with the Mouse Ventilator MiniVent, as demonstrated by the reduced acidosis and hypercarbia. To the best of our knowledge, this is the first demonstration of an association between CO_2 pneumoperitoneum-induced acidosis and hypercarbia and adhesion formation. The mechanism whereby this acidosis and hypercarbia becomes a cofactor in adhesion formation remains unclear. Carbon dioxide pneumoperitoneum induces respiratory acidosis that, if not corrected, leads to metabolic acidosis and metabolic hypoxia (18). This could be deleterious for the peritoneal cells and enhance the detrimental effect of the CO_2 pneumoperitoneum induced peritoneal ischemic hypoxia, which we suggested to be a driving mechanism in pneumoperitoneum-enhanced adhesion formation. Obviously, a direct effect of acidosis and hypercarbia upon cells and molecules involved in adhesion formation cannot be excluded. Indeed, acidosis affects lymphocyte and macrophage functions, altering cellular and humoral immune function (21), and up-regulates VEGF expression independently from hypoxia (22). The latter has been reported to be involved in adhesion formation (13, 23–25).

In conclusion, this study confirms that CO_2 pneumoperitoneum causes acidosis and hypercarbia and that it is a cofactor in adhesion formation and demonstrates an association between both effects, suggesting that CO_2 pneumoperitoneum-induced acidosis plays a role in the mechanism of CO_2 pneumoperitoneum-enhanced adhesion formation. It also indicates that in adhesion formation studies in animal models, the effect of anesthesia should not be underestimated and the need for assisted ventilation should be carefully evaluated. The clinical significance of these data remains unclear, but the data should be taken into account for patients in steep Trendelenburg position or with limited cardiovascular adaptation, such as obese and heavy-smoker patients, and for laparoscopic surgery of the retroperitoneum or of long duration.

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