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Effect of adding more than 3% oxygen to carbon dioxide pneumoperitoneum on adhesion formation in a laparoscopic mouse model

Osama Ali Elkelani, M.D., M.Sc.,[a,b] *Maria Mercedes Binda, Ph.D.,*[a] *Carlos Roger Molinas, M.D., Ph.D.,*[a,b] *and Philippe Robert Koninckx, M.D., Ph.D.*[a,b]

University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium

Objective: To investigate the effect of the addition of 3% or higher oxygen concentrations to the carbon dioxide (CO_2) pneumoperitoneum.

Design: Prospective, randomized trial.

Setting: Academic research center.

Animal(s): Female Naval Medical Research Institute mice (n = 100).

Intervention(s): Sixty minutes of CO_2 pneumoperitoneum with 0%, 3%, 6%, 9%, or 12% oxygen; induction of adhesions by the creation of standardized peritoneal lesions during laparoscopy.

Main Outcome Measure(s): Adhesions were quantitatively and qualitatively scored after 7 days during laparotomy to determine [1] the effect of 60 minutes of CO_2 pneumoperitoneum with 0%, 3%, 6%, 9%, or 12% oxygen on adhesion formation, and [2] the effect of duration of CO_2 pneumoperitoneum and insufflation pressure on adhesion formation with the addition of 0%, 3%, and 12% oxygen.

Result(s): Compared with a CO_2 pneumoperitoneum with 3% oxygen, adhesion formation is greater when either no oxygen or more than 3% oxygen is added to the CO_2 pneumoperitoneum. These effects persisted at higher insufflation pressures and longer duration of pneumoperitoneum, both known to increase adhesion formation with pure CO_2.

Conclusion(s): This study confirms that adhesion formation is decreased with the addition of 3% oxygen to the CO_2 pneumoperitoneum. The addition of higher oxygen concentrations, however, is deleterious. Adhesions always increase with time and duration of the pneumoperitoneum. (Fertil Steril® 2004;82:1616–22. ©2004 by American Society for Reproductive Medicine.)

Key Words: Adhesion formation, CO_2 pneumoperitoneum, laparoscopy, oxygen, mice

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Reprint requests: Maria Mercedes Binda, Ph.D., University Hospital Gasthuisberg, Department of Obstetrics and Gynecology, Herestraat 49, 3000 Leuven, Belgium (FAX: 32-16-34-42-05; E-mail: MariaMercedes.Binda@uz.kuleuven.ac.be).

[a] Department of Obstetrics and Gynecology.

[b] Center for Surgical Technologies.

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Intraperitoneal adhesions are clinically important. They are a major cause of intestinal obstruction (1), chronic pelvic pain (2, 3), female infertility (4), and difficulties at the time of reoperation. Adhesions thus have a huge economic impact on health care systems.

The overall mechanisms of adhesion formation are well known (5, 6). A peritoneal trauma causes an inflammatory reaction with fibrin deposition. If fibrin is not completely degraded because of an overload of fibrin, decreased fibrinolysis, or the presence of a prolonged inflammatory reaction, fibroblast proliferation will occur, leading to collagen deposition, angiogenesis, and ultimately to adhesion formation.

Laparoscopy, compared with laparotomy, has been claimed to be less adhesiogenic, but the data are not conclusive (7). Laparoscopy probably causes less direct surgical trauma because of the gentle tissue handling and the use of microsurgical instruments. During laparoscopy, a pneumoperitoneum is necessary, and for this CO_2 is generally used for safety reasons (i.e., high solubility in water and high exchange capacity in the lungs). Carbon dioxide pneumoperitoneum, however, also induces adverse effects, such as hypercarbia and acidosis (8) and hypothermia and desiccation (9). It also alters the peritoneal fluid (10) and the morphology of the mesothelial cells (11–13). Pure CO_2

pneumoperitoneum has been shown to increase reactive oxygen species (ROS) production and decrease ROS scavengers, which protect cells against ROS (14). Finally, CO_2 pneumoperitoneum increases adhesion formation, and this effect is time- (15–18) and pressure-dependent (18, 19).

It has been suggested that this pneumoperitoneum-enhanced adhesion is mediated by mesothelial hypoxia, because similar effects were observed with helium pneumoperitoneum, because the addition of 2%–4% oxygen to both CO_2 and helium pneumoperitoneum decreased adhesion formation (17, 18) and because this effect was absent in mice deficient for hypoxia inducible factor (HIF) (20), plasminogen activator 1 (PAI-1) (21), vascular endothelial growth factor (VEGF) and placental growth factor (P1GF) (22).

Supraphysiologic partial oxygen tension (pO_2) is known to be deleterious to cells (23), probably through increased ROS production (14). The pO_2 in mesothelial cells, as well as in all peripheral cells, is estimated to be some 23 mm Hg; thus these cells are exposed to supraphysiologic pO_2 during laparotomy because pO_2 in air is 159 mm Hg. This study was carried out to investigate in a mouse model for pneumoperitoneum-enhanced adhesion formation, the direct effect of physiologic and supraphysiologic pO_2 tension on adhesion formation.

MATERIALS AND METHODS

Animals

This study was carried out in 100 female, Naval Medical Research Institute, 10–14-week-old mice weighing 30–40 g. The animals were kept under standard laboratory conditions (temperature 20°–22°C, relative humidity 50%–60%, 14 hours light and 10 hours dark) at the animal facilities of the Katholieke Universiteit Leuven, Belgium. They were fed with a standard laboratory diet (Muracon.G; Carsil Quality, Turnhout, Belgium) with free access to food and water. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia

Animals were anesthetized with IM pentobarbital (0.07 mg/g) (Nembutal; Sanofi Sante Animale, Brussels, Belgium). The abdomen was shaved, and the animal was secured to the table in a supine position. Endotracheal intubation was performed with a ventilation cannula (blunt-edged 20-gauge needle; BD Microlance 3; Becton Dickinson, Fraga, Spain) introduced into the trachea as described previously (18, 20–22, 24). Ventilation was performed with a mechanical ventilator (Mouse Ventilator MiniVent, Type 845; Hugo Sachs Elektronik, Hardvard Apparatus, March-Hugstetten, Germany) with room air and a tidal volume of 250 μL at 160 strokes per minute.

Laparoscopic Surgery for Induction of Intraperitoneal Adhesions

All surgeries were performed by the same surgeon (O.A.E.), who was, for obvious technical reasons, not blinded to the group being operated on. As described previously (18, 20–22, 24), a 3.5-mm midline incision was made caudal to the xiphoid appendix, and a 2-mm endoscope with a 3.3-mm external sheath for insufflation (Karl Storz, Tüttlingen, Germany) was introduced into the abdominal cavity. The endoscope, connected to a video camera (Karl Storz) and light source (Karl Storz), was secured in a holder (Karl Storz). Because the mouse abdominal wall is very thin, variable gas leakage, and thus variable flow, occurred through the abdomen. To minimize variability, the incision was closed around the endoscope with a 6/0 polypropylene suture (Prolene; Ethicon, Johnson and Johnson, Brussels, Belgium). Because a gas-tight closure was difficult to achieve, a flow through the abdominal cavity of 23 mL/min was achieved in all mice by inserting a 26-gauge needle (BD Plastipak; Becton Dickinson, Madrid, Spain) through the abdominal wall. This continuous flow, moreover, ensured a constant gas concentration in the abdominal cavity because the gas was slowly but continuously replaced.

The pneumoperitoneum was created with the Thermoflator Plus (Karl Storz). The insufflation gas (pure CO_2 or CO_2 mixed with up to 12% oxygen) was heated (37°C; Optitherm, Karl Storz) and humidified (Aquapor; Dräger, Lübeck, Germany). To maintain accurately insufflation pressure with minimal fluctuation, a water valve with a free escape of gas and an elastic balloon were used. The water valve and the balloon were found to be necessary to adapt the flow rate to a mouse and to dampen pressure changes during insufflation. The insufflation gas and the insufflation pressure were determined according to the experimental design.

After the establishment of the pneumoperitoneum, two 14-gauge catheters (Insyte-W; Vialon Becton Dickinson, Madrid, Spain) were inserted under direct laparoscopic visualization in both right and left flanks for the working instruments. The uterus was grasped in the midline with a 1.5-mm grasper, and standardized 10 × 1.6-mm lesions were created in the antimesenteric border of both right and left uterine horns by monopolar (homemade probe with a ball-shape cautery surface of 1.6-mm diameter) or bipolar coagulation (cylindrical cautery surface of 6 × 1.6-mm Bicap [Circon, Santa Barbara, CA]) at 10 W (Autocon 350; Karl Storz). In addition, identical lesions were made in the right and left pelvic sidewalls. The type of lesion in each side was randomly determined.

The secondary ports were removed immediately, and the incisions were closed in a single layer with 6/0 polypropylene suture (Prolene). The procedure took, in general, 3 to 4 minutes, but the pneumoperitoneum was maintained a minimum standard time of 10 minutes for basal adhesions, or for longer periods to evaluate pneumoperitoneum-enhanced adhesions (18).

Scoring of Adhesions

A xiphopubic midline incision and a bilateral subcostal incision were performed, and the whole abdominal cavity

was explored during laparotomy 7 days after the induction of adhesions, as previously described (18, 20–22, 24). After the evaluation of port sites and viscera, the pelvic fat tissue was carefully removed, and adhesions were scored under a microscope, according to a qualitative and a quantitative scoring system. All scoring was done by the same surgeon, who was blind to the group being evaluated.

In the qualitative scoring system, modified from Leach et al. (25), extent (0 = no adhesions, 1 = 1%–25%, 2 = 26%–50%, 3 = 51%–75%, 4 = 76%–100% of the injured surface involved), type (0 = no adhesions, 1 = filmy, 2 = dense, 3 = capillaries present), and tenacity (0 = no adhesions, 1 = essentially fall apart, 2 = require traction, 3 = require sharp dissection) were assessed. The sum of extent, type, and tenacity is the total score. In addition, a quantitative scoring system was used, as previously described (26). This system has the advantage of being less dependent on a subjective interpretation. It measures the proportion of the lesions covered by adhesions, calculated by dividing the sum of the length of the individual attachments by the length of the initial lesion.

Adhesions were formed between fat tissue and lesions; no adhesions were observed in other parts of the peritoneal cavity. The results are presented as the average of the adhesions formed at the four individual sites (right and left visceral and parietal peritoneum, with lesions inflicted by monopolar or bipolar coagulation), which were scored individually.

Experimental Design

Experiments were designed to assess the effect of CO_2 pneumoperitoneum containing more than 3% oxygen (6%, 9%, and 12%), compared with 0% and 3% oxygen; 0% oxygen is a model for pneumoperitoneum-enhanced adhesions, and 3% oxygen is a model for reducing pneumoperitoneum-enhanced adhesions by the addition of small amounts of oxygen. The maximum amount of oxygen used was 12% because this is the highest concentration that could be obtained with the Thermoflator Plus. All experiments were performed according to block randomization by day, to avoid day-to-day variability. Thus, a block of animals, comprising one animal of each experimental group, was always operated on the same day.

In the first study, the effect of 60 minutes of CO_2 pneumoperitoneum with 0%, 3%, 6%, 9%, or 12% oxygen at 10 cm H_2O on adhesion formation was evaluated (five groups, five mice per group).

In the second study, the effect of the duration of CO_2 pneumoperitoneum (10 minutes, 30 minutes, and 60 minutes) with 0%, 3%, and 12% oxygen at 10 cm H_2O on adhesion formation was evaluated (nine groups, five mice per group).

In the third study, the effect of the insufflation pressure (5 cm H_2O and 20 cm H_2O) with CO_2 pneumoperitoneum with 0%, 3%, and 12% oxygen for 60 minutes on adhesion formation was evaluated (six groups, five mice per group).

Statistics

Statistical analyses were performed with a commercial software program (SAS System; SAS Institute, Cary, NC). The Wilcoxon test was used to compare individual groups. To evaluate simultaneously the variables of the experiments with a factorial design, analysis of variance for nonnormally distributed populations (General Linear Methods, PROC GLM) was used. As discussed previously (17), the advantage of the factorial design is the increase in statistical power for the same total number of animals. A two-by-two factorial design evaluating two effects (A and B) with n animals in each group achieves for a total number of 4n animals almost the same statistical power as would be achieved by doing a 4n experiment evaluating A and another 4n experiment evaluating B, thus requiring almost 50% fewer animals in total (27).

All data are presented as the mean and SE. To evaluate differences between experimental groups, only the combined scores of the adhesions after monopolar and bipolar lesions were used. This was done because in all previous studies (20–22) bipolar lesions induced systematically fewer adhesions than monopolar lesions and were therefore less sensitive to detect intergroup differences.

RESULTS

In the first experiment, the effect of adding different concentrations of oxygen to the CO_2 pneumoperitoneum on adhesion formation was evaluated (Wilcoxon; Fig. 1 and Table 1). It was confirmed that, compared with pure CO_2 pneumoperitoneum, adhesions decreased after the addition of 3% oxygen (proportion: $P=.02$; total: $P=.05$; extent: $P=.02$; type: $P=.04$). Compared with CO_2 pneumoperitoneum with 3% oxygen, adhesions increased with 6% (proportion: $P=.05$), 9% (proportion: $P=.01$; total: $P=.01$; extent: $P=.01$; type: $P=.01$; tenacity: $P=.03$), and 12% (proportion: $P=.02$) oxygen. No differences in adhesion formation were found between 0%, 6%, 9%, and 12% oxygen.

In the second experiment, the effect of 10 minutes, 30 minutes, or 60 minutes of CO_2 pneumoperitoneum with 0%, 3%, or 12% oxygen on adhesion formation was evaluated (Fig. 2 and Table 2). Mice with two different concentrations of oxygen were analyzed simultaneously (PROC GLM, six groups, two variables [time and oxygen]). In mice with CO_2 pneumoperitoneum with 0% and 3% oxygen, adhesions increased with time (proportion: $P<.0001$; total: $P<.0001$; extent: $P<.0001$; type: $P<.0001$; tenacity: $P<.0001$) and decreased with 3% oxygen (proportion: $P=.05$; total: $P=.02$; extent: $P=.03$; type: $P=.001$; tenacity: $P=.02$). In mice with CO_2 pneumoperitoneum with 3% and 12% oxygen, adhesions increased with time (proportion: $P<.0001$; total: $P=.0003$; extent: $P<.0001$; type: $P=.001$; tenacity:

FIGURE 1

Effect of the addition of different oxygen concentrations to the CO_2 pneumoperitoneum on adhesion formation in mice. Adhesions were induced during laparoscopy with 60-minute pneumoperitoneum at 10 cm H_2O and quantitatively scored after 7 days during laparotomy. Values are means ± SE. Significance (Wilcoxon): [a]$P<.05$ (vs. 3%).

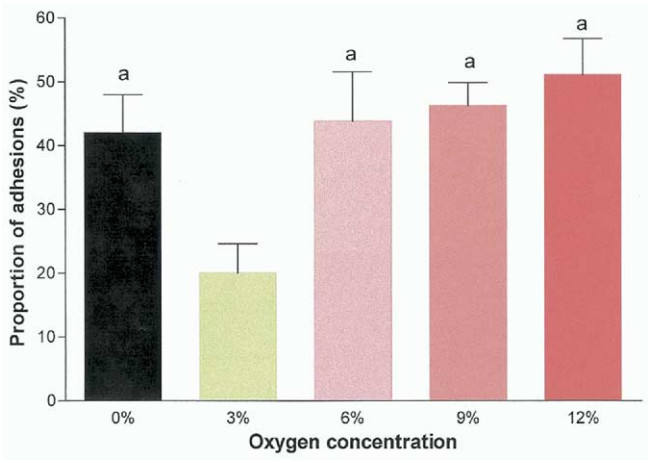

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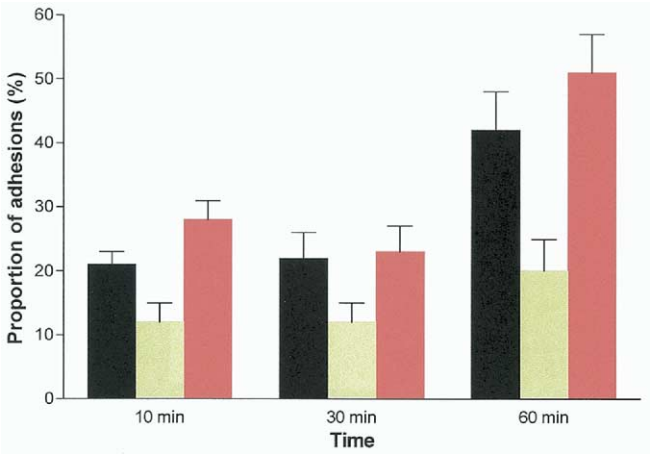
FIGURE 2

Effect of the duration of CO_2 pneumoperitoneum with 0% (*black bars*), 3% (*green bars*), or 12% (*red bars*) of oxygen on adhesion formation in mice. Adhesions were induced during laparoscopy with pneumoperitoneum at 10 cm H_2O and quantitatively scored after 7 days during laparotomy. Values are means ± SE. Significances (PROC GLM): 0%–3%: time: $P<.0001$, oxygen: $P=.05$; 3%–12%: time: $P=.0003$, oxygen: $P=.0005$; 0%–12%: time: $P<.0001$, oxygen: P=NS.



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$P=.0001$) and increased with 12% oxygen (proportion: $P=.0005$; total: $P=.001$; extent: $P=.001$; type: $P=.001$; tenacity: $P=.002$). In mice with CO_2 pneumoperitoneum with 0% and 12% oxygen, adhesions increased with time (proportion: $P<.0001$; total: $P<.0001$; extent: $P<.0001$; type: $P=.0002$; tenacity: $P<.0001$), whereas no differences were found between the two oxygen concentrations.

In the third experiment, the effect of 5 cm H_2O or 20 cm H_2O of CO_2 pneumoperitoneum with 0%, 3%, or 12% oxygen on adhesion formation was evaluated (Fig. 3, Table 3). Mice with two different concentrations of oxygen were analyzed simultaneously (PROC GLM, four groups, two variables [pressure and oxygen]). In mice with CO_2 pneumoperitoneum with 0% or 3% oxygen, adhesions increased

TABLE 1

Effect of the addition of different oxygen concentrations to CO_2 pneumoperitoneum on adhesion formation in mice.

Oxygen concentration (%)	Adhesion scores			
	Extent	Type	Tenacity	Total
0	2.2 ± 0.2[a]	1.6 ± 0.1[a]	1.7 ± 0.2	5.5 ± 0.5[a]
3	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	3.1 ± 0.8
6	2.0 ± 0.4	1.7 ± 0.2	1.6 ± 0.3	5.3 ± 0.8
9	2.2 ± 0.1[a]	2.0 ± 0.1[a]	2.2 ± 0.2[a]	6.4 ± 0.3[a]
12	2.1 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	5.7 ± 1.0

Note: Adhesions were induced during laparoscopy with 60-min pneumoperitoneum at 10 cm H_2O and qualitatively scored after 7 days during laparotomy. Values are means ± SE.

[a] Significance (Wilcoxon): $P<.05$ for group vs. 3% of oxygen.

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TABLE 2

Effect of the duration of the CO_2 pneumoperitoneum with 0%, 3%, or 12% of oxygen on adhesion formation in mice.

Time	Oxygen concentration (%)	Adhesion scores			
		Extent	Type	Tenacity	Total[a]
10 min	0	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	2.9 ± 0.3
	3	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	1.6 ± 0.4
	12	1.4 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	3.7 ± 0.4
30 min	0	1.0 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	2.8 ± 0.4
	3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	1.5 ± 0.2
	12	1.0 ± 0.2	1.0 ± 0.1	0.9 ± 0.2	2.9 ± 0.4
60 min	0	2.0 ± 0.1	1.6 ± 0.2	1.8 ± 0.2	5.4 ± 0.3
	3	1.0 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	2.7 ± 0.8
	12	2.2 ± 0.2	1.8 ± 0.3	1.9 ± 0.2	5.9 ± 0.7

Note: Adhesions were induced during laparoscopy with pneumoperitoneum at 10 cm H_2O and qualitatively scored after 7 days during laparotomy. Values are means ± SE.

[a] Significances for total (PROC GLM): 0%–3%: time: $P<.0001$, oxygen: $P=.02$; 3%–12%: time: $P=.0003$, oxygen: $P=.001$; 0%–12%: time: $P<.0001$, oxygen: P=NS. Significances for extent, type, and tenacity; see text.

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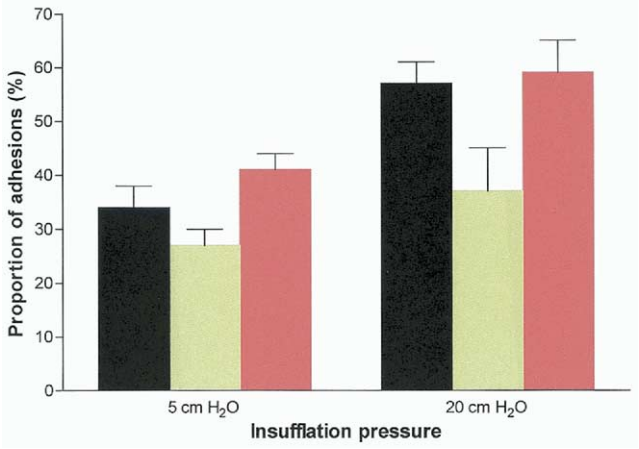
FIGURE 3

Effect of the insufflation pressure of CO_2 pneumoperitoneum with 0% (*black bars*), 3% (*green bars*), or 12% (*red bars*) of oxygen on adhesion formation in mice. Adhesions were induced during laparoscopy with pneumoperitoneum and quantitatively scored after 7 days during laparotomy. Values are means ± SE. Significances (PROC GLM): 0%–3%: pressure: P=.01, oxygen: P=.02; 3%–12%: pressure: P=.02, oxygen: P=.003; 0%–12%: pressure: P=.001, oxygen: P=NS.



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with pressure (proportion: P=.01; total: P=.0005; extent: P=.002; type: P=.002; tenacity: P=.003) and decreased with 3% oxygen (proportion: P=.02; total: P=.01; extent: P=.01; type: P=.02; tenacity: P=.03). In mice with CO_2 pneumoperitoneum with 3% or 12% oxygen, adhesions increased with pressure (proportion: P=.02; total: P=.02; extent: P=.01) and with 12% oxygen (proportion: P=.003; total: P=.005; extent: P=.001; tenacity: P=.04). In mice with CO_2 pneumoperitoneum with 0% or 12% oxygen, adhesions increased with pressure (proportion: P=.0001; total: P=.0002; extent: P=.0001; type: P=.04; tenacity: P=.005), whereas no differences were found between the two oxygen concentrations.

TABLE 3

Effect of the insufflation pressure of CO_2 pneumoperitoneum with 0%, 3%, or 12% of oxygen on adhesion formation in mice.

Pressure (cm H_2O)	Oxygen concentration, (%)	Adhesion scores			
		Extent	Type	Tenacity	Total[a]
5	0	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	4.1 ± 0.3
	3	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	3.1 ± 0.2
	12	2.0 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	5.0 ± 0.4
20	0	2.6 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	6.6 ± 0.3
	3	1.7 ± 0.4	1.5 ± 0.3	1.5 ± 0.3	4.7 ± 0.8
	12	2.7 ± 0.2	1.5 ± 0.1	1.8 ± 0.2	6.0 ± 0.4

Note: Adhesions were induced during laparoscopy with 60-min pneumoperitoneum and qualitatively scored after 7 days during laparotomy. Values are means ± SE.

[a] Significances for total (PROC GLM): 0%–3%: pressure: P=.0005, oxygen: P=.01; 3%–12%: pressure: P=.02, oxygen: P=.0005; 0%–12%: pressure: P=.0002, oxygen: P=NS. Significances for extent, type and tenacity: see text.

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DISCUSSION

This study confirms and extends our observations in mice that adhesion formation increases with the duration of pneumoperitoneum and with insufflation pressure and decreases with the addition of 3% oxygen to the CO_2 pneumoperitoneum (15–19). This beneficial effect of adding 3% oxygen persists over time, at least up to 60 minutes.

These data demonstrate that the decrease in adhesion formation achieved by adding 3% oxygen does not persist when higher oxygen concentrations are used. Clearly, 12% oxygen induces more adhesions than 3%. In addition, it is demonstrated that the increase of adhesion formation with the duration of the CO_2 pneumoperitoneum and with the insufflation pressure is not only valid for pure CO_2 pneumoperitoneum but also for CO_2 pneumoperitoneum with 3% or 12% oxygen.

The effect of CO_2 pneumoperitoneum on adhesion formation has been suggested to be mediated by mesothelial hypoxia, because adhesions increase with duration and with pressure and decrease with the addition of oxygen and because no differences were observed between CO_2 and helium pneumoperitoneum (18). This hypothesis of hypoxia is, moreover, consistent with the reported effects of CO_2 pneumoperitoneum in HIF-1α-, HIF-2α-, PAI-1-, and VEGF-deficient mice. Indeed, these factors are known to be upregulated by hypoxia (28, 29), and we have demonstrated that CO_2 pneumoperitoneum-enhanced adhesion formation was absent in mice deficient for HIF-1α and HIF-2α (20), deficient for PAI-1 (21), deficient for VEGF-B, and deficient for P1GF (22).

The beneficial effect of the addition of 3% oxygen could be explained by the fact that 3% oxygen at 770 mm Hg (atmospheric pressure of 760 mm Hg plus insufflation pressure of 10 mm Hg) results in a pO_2 of 23 mm Hg, which is remarkably similar to normal intracellular pO_2 (mesothelial normoxia) (30). The addition of 12% oxygen at 770 mm Hg results in a pO_2 of 92 mm Hg, which is higher than the normal intracellular pO_2 and thus should be called mesothelial hyperoxia.

We would like to stress the confusion resulting from the indiscriminate use of the words "hypoxia," "normoxia," and "hyperoxia" in the literature. These words are generally used to indicate a lower, similar, or higher pO_2, respectively, than

observed in air at normal atmospheric pressure, in which a concentration of 20.9% results in a pO_2 of almost 160 mm Hg. "Normoxia," however, is also used to indicate the physiologic pO_2 in peripheral cells of living organisms. It is important to realize that, according to the oxygen cascade model of mammals, the pO_2 decreases progressively from 159 mm Hg in air to 95 mm Hg in the arterial end of capillaries, 40 mm Hg in the interstitial fluid, and some 23 mm Hg in the peripheral cells (30). This intracellular pO_2 varies from 5–40 mm Hg, depending on the type of cells and on the distance to the capillaries (30–35). Taking these concepts into account, it is clear that intracellular pO_2 lower or higher than 5–40 mm Hg should be considered "cellular hypoxia" or "cellular hyperoxia," respectively. This definition of "cellular normoxia" is moreover consistent with several in vitro studies reporting a better cellular growth at pO_2 around 5–40 mm Hg. This was demonstrated for human lung fibroblasts (36), human melanocytes (37), human skin fibroblast cultures derived from fetal and postnatal tissue donors (38), and human hematopoietic stem cell (39).

Because a pneumoperitoneum with 12% oxygen induces mesothelial hyperoxia, the increase in adhesion formation might be caused by ROS (14). Indeed, hyperoxia generates ROS (e.g., superoxide anion, hydrogen peroxide, and nitric oxide), which have deleterious effects in cells. Cells protect themselves from the deleterious effects of ROS by producing ROS scavengers. The balance between ROS and ROS scavengers will determinate ROS availability and toxicity. Reactive oxygen species are suggested to be involved in tissue destruction and fibrosis in patients with endometriosis (40) and in adhesion formation (41, 42). Furthermore, this latter effect was shown to be reduced by ROS scavengers, such as catalase and superoxide dismutase (43, 44), vitamin E (45), methylene blue (46), and melatonin (47).

In addition, ROS might be involved in the increased adhesion formation after pure CO_2 pneumoperitoneum because ROS can be generated after the reperfusion of an ischemic tissue (48) (i.e., after mesothelial hypoxia during CO_2 pneumoperitoneum). Furthermore, the generation of ROS after open and laparoscopic surgery is well reported (49, 50). Laparoscopic surgery increases ROS availability by increasing ROS production (50) or by decreasing ROS scavengers (41, 42). Therefore, we hypothesize that this increased ROS availability plays a role in adhesion formation (14). This is fully consistent with the similar adhesion formation observed with CO_2 pneumoperitoneum with 0% or 12% oxygen and with the reduction of adhesion formation with 3% oxygen. Indeed, with 12% oxygen, mesothelial cells are in a hyperoxic environment that could lead to increased ROS production or decreased ROS scavenger production, whereas pneumoperitoneum with both 0% and 12% oxygen causes an ischemia/reperfusion process, especially at high insufflation pressure, that could be an additional source of ROS. Although pneumoperitoneum with 3% oxygen also alters microcirculation, cells do not become hypoxic because they receive oxygen from the more physiologic pneumoperitoneum environment (pO_2 around 23 mm Hg).

In conclusion, our data confirm that pure CO_2 pneumoperitoneum increases adhesion, that this effect is reduced by adding 3% oxygen, and that higher oxygen concentrations also increases adhesion formation. Our data also demonstrate that all these effects are present at low and high insufflation pressure and with short and long duration of the pneumoperitoneum. The observed effects with 12% oxygen (mesothelial hyperoxia model) could be similar to those during open surgery, which is performed in air that also has a relatively high oxygen concentration. The available data moreover suggest that the mechanisms involved in adhesion formation after laparoscopy and laparotomy could be partially similar (ROS availability) and partially different (HIF, PAI-1, and VEGF induction by pure CO_2 pneumoperitoneum), indicating that observations on adhesion prevention in one approach cannot simply be extrapolated to the other.

The extrapolation of these data to adhesion formation in the human cannot be made until appropriated trials are performed. These are planned as soon as the mechanisms involved are adequately understood. It is appealing, however, to consider the role of such fundamental mechanisms as cellular hypoxia and hyperoxia and of ROS in adhesion formation. These observations suggest at least that the mechanism involved in adhesion formation, and thus also in adhesion prevention, might be different between laparoscopy and laparotomy.

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