Reduction of CO₂-pneumoperitoneum-induced metabolic hypoxaemia by the addition of small amounts of O₂ to the CO₂ in a rabbit ventilated model. A preliminary study

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BACKGROUND: CO₂-pneumoperitoneum used in endoscopic surgery induces system effects by CO₂ absorption. This study investigated the effect of the addition of O₂ to CO₂-pneumoperitoneum, upon CO₂ absorption.

METHODS: The effect of a pneumoperitoneum using 100% CO₂ or 94% CO₂/6% O₂ upon arterial blood gases, acid base and O₂ homeostasis was evaluated. In series A suboptimal ventilation and a pneumoperitoneum pressure (PP) of 10 mmHg was used. In series B adequate ventilation and PP of 6 mmHg was used. RESULTS: CO₂-pneumoperitoneum profoundly affected blood gases and acid base homeostasis i.e. increasing pCO₂, HCO₃⁻ (P < 0.001) and lactate concentrations (P < 0.05) and decreasing pH, actual base excess and standard bicarbonate (P < 0.001), resulting in metabolic hypoxaemia with desaturation, lower pO₂ (P < 0.001) and O₂Hb (P < 0.05). These effects were more pronounced with higher PP and suboptimal ventilation. CONCLUSION: CO₂-pneumoperitoneum profoundly affected blood gases and acid base homeostasis resulting in metabolic hypoxaemia. The addition of 6% of O₂ to the CO₂-pneumoperitoneum prevented these effects to a large extent. If these preliminary data are confirmed in the human, the addition of a few percent of O₂ to CO₂ could become important for endoscopic surgery of long duration, especially in obese patients with limited cardiorespiratory adaptation and steep Trendelenburg.

Key words: acidosis/carboxaemia/CO₂-pneumoperitoneum/metabolic hypoxaemia/oxygen

Introduction

Endoscopic surgery is associated with less postoperative pain, lower morbidity, shorter hospitalization, better cosmetic results and a faster return to normal activities. CO₂ is generally used for the pneumoperitoneum for safety reasons because of its high solubility in water and its high exchange capacity in the lungs. The concentration of CO₂ can moreover easily be monitored by capnography and controlled by ventilation (Wright et al., 1995; Gebhardt et al., 1997).

CO₂-pneumoperitoneum induces systemic effects by CO₂ absorption, and by the intraperitoneal pressure which affects venous return (Kotzampassi et al., 1993). Firstly, CO₂ absorption increases the end tidal CO₂, arterial pCO₂ and mixed venous pCO₂ (Kotzampassi et al., 1993; Gandara et al., 1997). This carboxaemia induces a respiratory and metabolic acidosis, decreasing both arterial and mixed venous pH and arterial pO₂ (Liem et al., 1996; Gandara et al., 1997; Gebhardt et al., 1997; Knolmayer et al., 1998). CO₂ absorption negatively affects respiratory function (Junghans et al., 1997) an effect not observed by inert gases such as helium and argon. Minute ventilation, peak inspiratory pressure, pulmonary vascular resistance, alveolar CO₂ concentration, calculated physiological shunt, central venous pressure, systolic and diastolic arterial pressure and systemic vascular resistance and the cardiac output are increased (Kotzampassi et al., 1993; Gebhardt et al., 1997; Knolmayer et al., 1998). These effects of CO₂ absorption are more pronounced in those patients with limited pulmonary or cardiovascular adaptation (Gebhardt et al., 1997) with liver or blood disease (Cunningham and Schlanger, 1992; Haydon et al., 1996) and also with long duration of endoscopic surgery and steep Trendelenburg (Stone et al., 1998). Higher intraperitoneal pressures are associated with a reduction of visceral blood flow and urinary output (Caldwell and Ricotta, 1987; Kotzampassi et al., 1993). In rats the portal blood flow linearly decreases with intraperitoneal pressures of 2–12 mmHg affecting hepatic function and cellular immunity (Gutt et al., 1984).
addition of small amounts of O₂ to the CO₂-pneumoperitoneum, 2001). This increase in adhesions can be prevented by the administration of 30 mg/kg Ketamine 1000 (Sano 
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In rabbits and mice adhesions increase with duration and pressure of the CO₂-pneumoperitoneum (Yesildaglar et al., 1999, 2000; Molinas and Konincx, 2000; Molinas et al., 2001). This increase in adhesions can be prevented by the addition of small amounts of O₂ to the CO₂-pneumoperitoneum, suggesting local mesothelial hypoxia as a mechanism (Konincx, 2000; Molinas et al., 2001). Since these local effects of the addition of small amounts of O₂ were so pronounced, the systemic effects of adding small amounts of O₂ to the CO₂-pneumoperitoneum were investigated in a rabbit model.

Materials and methods

Animals

Adult female New Zealand white rabbits (n = 20) weighing between 2.7 and 3.0 kg were used. They were kept under standard laboratory conditions at a temperature of 20–25°C, and a relative humidity of 40–70%. They had a day cycle of 14 h light and 10 h dark, a standard laboratory diet (Hope Farms, Woerden, The Netherlands) and free access to food and water. The animals were housed at the Centre for Laboratory Animal Care of the Catholic University of Leuven (Animalium, St Rafael Hospital. K.U.Leuven, Belgium) and the experiments were approved by The Instituitional Review Animal Care Committee.

The animals were premedicated with an i.m. injection of 30 mg/kg Ketamine 1000 (Sanofi®; Sante Animale Benelux, Brussels, Belgium) and 6 mg/kg of 2% xylazine hydrochloridum solution (VMD, Arendonk, Belgium). After intubation with a 3.5 mm endotracheal tube (Sheridan Catheter Corp., New York, NY, USA) inhalation anaesthesia was performed with 2.5% halothane (Fluothane®; Zeneca, Destelbergen, Belgium) mixed with O₂ and room air with concentrations of O₂ in inspired gas fractional inspired O₂ concentration (FiO₂) 0.7, using a vaporizer (Drägerwerk, Lubeck, Germany) connected to a small animal ventilator (Model 683; Harvard Apparatus Inc., Holliston, MA, USA). During anaesthesia the haemodynamic and respiratory parameters were monitored continuously, i.e. pulse rate and O₂ saturation (SpO₂, in %) in the peripheral blood (ear vessels), end tidal CO₂ (PetCO₂) and respiratory pressure, using an electrocardiogram, a blood pressure meter (Hewlett Packard, Boeblingen, Germany), a pulse oximeter (Nellcor, Hayward, CA, USA), a capnograph (Capnomac; Datex, Finland) and a manometer respectively.

Surgical protocol

The animals were placed in the supine position and the abdomen was shaved and disinfected with polyvidone iodine (Iso-Betadine; Asta Medica, Brussels, Belgium). The surgical procedures included a pneumoperitoneum created with a 10 mm trocar (Apple®; Medical Corporation, USA) placed caudally to the sternum. For the pneumoperitoneum the Thermo 
fl (Karl Storz, Tüttlingen, Germany) was used with a humidifier (Aquapor, Drägerwerk) and with a heating device (Opti Therm; Karl Storz) keeping the insufflation temperature between 35–37°C. In addition a water valve was used to dampen changes in the insufflation pressure. Taking into account the high exchange capacity of the peritoneum and to maintain a 100% concentration of CO₂, a continuous flow rate through the abdominal cavity of some 80 ml/min was used to constantly remove any O₂ which might have diffused from the circulation. To achieve this a 22 gauge catheter (Insys-W®; Vialon®; Becton Dickinson, Madrid, Spain) was inserted through the abdominal wall. This flow rate with heated and humidified CO₂ caused hardly any desiccation (Yesildaglar et al., 2000). Insufflation was carried out through the 10 mm trocar inserted superficially.

Experimental design

In superficially and adequately ventilated rabbits a control group without pneumoperitoneum (n = 4 and 3 respectively) was compared with animals using either 100% O₂ (n = 4 and 3), or 6% of O₂ + 94% CO₂ (n = 3 and 3). In the superficially ventilated (tidal volume of 6.7 ml/kg and a respiratory rate of 27–29 per min) animals (series A) intraperitoneal pressure was 10 mmHg and in the adequately ventilated (tidal volume of 11.3 ml/kg and a respiratory rate of 18–21 per min) animals (series B) intraperitoneal pressure was 6 mmHg. Ventilation (superficially or adequately) and intraperitoneal pressures were chosen as described (Mynbaev et al., 2002). From these experiments the groups with the most and least pronounced effects of CO₂ pneumoperitoneum were chosen to investigate the effect of the addition of 6% of O₂. A concentration of 6% O₂ was chosen since in adhesion prevention studies optimal effects between 2–10% of O₂ were observed (Molinas et al., 2001). For both series of experiments, animals were block randomized by day. In series A, one animal died in the group with 94% CO₂ + 6% O₂.

Assays

The ear artery was catheterized with a 20 gauge catheter (Insys-W®; Vialon®; Becton Dickinson). The syringes and catheters were rinsed with 0.3 ml of saline with 5 IU heparin/l (Rhône-Poulenc Rorer, Brussels, Belgium). The first sample was taken before starting pneumoperitoneum and the following samples were taken every 30 min for 210 min in series A and every 15 min for 120 min in series B. Syringes with blood samples were put on ice immediately and analysed in duplicate in the blood gas analyser (Ablhim System 625/620; Radiometer, Copenhagen, Denmark). At the end of the experiment the animals were killed with an i.v. injection of 0.3 ml/kg T61 (Intervet, Mecheden, Belgium).

The following values were measured: arterial blood gas parameters such as pH, partial pressures of O₂ (pO₂) and CO₂ (pCO₂); acid base parameters such as concentrations of hydrogen carbonate (HCO₃⁻), standard bicarbonate (SBC), actual base excess (ABE), standard base excess (SBE) and the concentration of total carbon dioxide (tCO₂); blood oximetry parameters such as O₂ saturation (SO₂), oxihaeemoglobin (O₂HB) and reduced haemoglobin (RHb); O₂ status parameters such as total O₂ concentration (tO₂) and O₂ tension at half saturation assessing the haemoglobin O₂ affinity (p50). Finally the lactate concentration was measured.

Data analysis and statistical methods

Data were analysed using Graph Pad Prism (Graph Pad Software Inc., San Diego, CA, USA). Differences between the three experimental groups in each series were evaluated by repeated measurement ANOVA. Subsequently differences between groups one and two, between groups one and three, and between groups two and three were evaluated by Turkey’s multiple comparison tests. Mean ± SEM is given unless stated otherwise.
CO₂-pneumoperitoneum-induced hypoxamia reduced by O₂ addition

Figure 1. Arterial blood gases (pCO₂ and pH) in rabbits without pneumoperitoneum (group one — and — in series A and B respectively), during pneumoperitoneum with 100% CO₂ (group two — and — in series A and B respectively) and 6% O₂ + 94% CO₂ (group three — and — in series A and B respectively). X: time, min and Y: means ± SD are given.

Results

In both control groups anaesthesia and ventilation alone did not cause major changes in the concentrations of arterial pCO₂ (Figure 1), tCO₂ and PĒCO₂. A slight decrease in pH, ABE and SBC (Figures 1 and 2), and SBE was seen in series A at the end of the experiment. The pO₂, however, increased as estimated by pulse oxymetry and as measured in blood. In both series 70% FiO₂ caused an increase of pO₂ from 95–100 mmHg to 350 mmHg (Figure 3). In both control groups O₂ parameters tO₂, sO₂, p50, RHb and O₂Hb, as well as the lactate and HCO₃⁻ concentrations remained unchanged.

In superficially ventilated animals (series A) the CO₂-pneumoperitoneum (group two) caused a pronounced and progressively increasing carboxaemia, as evidenced by the elevated pCO₂ (Figure 1, P < 0.001), tCO₂ (not shown, P < 0.05) and PĒCO₂ (not shown, P < 0.01) in comparison with the control group. This CO₂ accumulation caused acidaemia, which was initially a respiratory acidosis and subsequently a metabolic acidosis as shown by the progressively decreasing pH (P < 0.001) and the increased concentrations of lactate (P < 0.05) and HCO₃⁻ after 90 min (P < 0.001) (Figure 2). The carboxaemia also affected the acid base balance as manifested by a progressively increasing deficiency of ABE (P < 0.001), SBE (not shown, P < 0.001) and SBC (P < 0.001). Simultaneously sO₂ (P < 0.01) and the O₂Hb (P < 0.05), concentration decreased, whereas the p50 (P < 0.001) and the concentration of RHb (P < 0.001), increased (Figure 2). The pO₂ (P < 0.001) and tO₂ (not shown, P < 0.01) decreased at the end of the experiment.

In superficially ventilated animals (series A) adding 6% of O₂ to the CO₂-pneumoperitoneum (group three) dramatically changed (Figures 1 and 2) the effects of pure CO₂ (group two). In comparison with pure CO₂ the carboxaemia (pCO₂) and acidosis (pH) were not only less pronounced (P < 0.001 for both values), but after 60 min a plateau was observed, whereas with pure CO₂ both effects increased progressively at least until 150–180 min. Metabolic acidosis was much less pronounced, and the lactate concentrations showed a small increase only, at the end of the experiment. In comparison with the pure CO₂-pneumoperitoneum group, the p50 (group two versus group three; P < 0.001) increased less whereas the values of ABE (group two versus group three; P < 0.01), SBC (group two versus group three; P < 0.01) and SBE (not shown, group two versus group three; P < 0.01) increased less whereas the values of ABE (group two versus group three; P < 0.01), SBC (group two versus group three; P < 0.01) and SBE (not shown, group two versus group three; P < 0.01) remained within background levels.

In adequately ventilated animals (series B) the effects of pure CO₂-pneumoperitoneum (group two) were similar but much less pronounced than in superficially ventilated animals (series A versus B: all values P < 0.001), i.e. slight carboxaemia with moderately increased arterial pCO₂ (group one versus
two; \( P < 0.001 \), \( tCO_2 \) (not shown, group one versus two; \( P < 0.001 \)) and \( P_{ETCO_2} \) (not shown, group one versus two; \( P < 0.001 \)) and a slight respiratory acidosis (pH, group one versus two; \( P < 0.001 \)) (Figure 1) without metabolic acidosis. In series B the effects of adding 6% of \( O_2 \) were similar to those in series A, i.e. less carboxaemia, almost no acidosis, and no changes for acid base and \( O_2 \) parameters.

**Discussion**

CO\(_2\) used for the pneumoperitoneum during laparoscopy is absorbed in humans (Shuto et al., 1995; Berg et al., 1997) and in large (Leighton et al., 1993; Liem et al., 1996) and small animals (Kuntz et al., 2000). The resulting increase of arterial pCO\(_2\) and decrease of pH can be stabilized within 15–40 min by adequate ventilation (Kotzampassi et al., 1993; Leighton et al., 1993). Inadequate ventilation can lead to respiratory and metabolic acidosis and changes in acid base balance (Shuto et al., 1995; Liem et al., 1996; Berg et al., 1997; Gebhardt et al., 1997; Taura et al., 1998) These effects are known to increase with pneumoperitoneum pressure, because of increased absorption and impaired CO\(_2\) excretion and venous return (Shuto et al., 1995; Liem et al., 1996; Bazin et al., 1997).

These observations are confirmed in our experiments—in animals with superficial ventilation and higher insufflation pressure (10 mmHg)—changes in both arterial pCO\(_2\) and pH are more pronounced without reaching equilibrium within the first hours. In animals with adequate ventilation and lower insufflation pressure (6 mmHg) a slight increase of arterial pCO\(_2\) and a slight decrease of pH, which stabilizes after 15–40 min, are seen. These results are also consistent with the recently reported arterial pCO\(_2\) and pH changes in rabbits (Portilla et al., 1998).

The reported data on acid base balance and \( O_2 \) values in blood during endoscopic surgery are not consistent. The HCO\(_3^-\) concentration in arterial blood is reported to increase (Liem et al., 1996), to decrease (Shuto et al., 1995; Gandara et al., 1997) or to remain constant (Leighton et al., 1993; Wright et al., 1995). The concentration of SBC has been reported to remain unchanged (Leighton et al., 1993). The hydrogen ion concentration (H\(^+\)) increases (Wright et al., 1995; Taura et al., 1998) whereas the base excess (BE) decreases (Shuto et al., 1995; Fernandez-Cruz et al., 1998; Taura et al., 1998) or remains stable (Horzic et al., 1998). The arterial blood concentrations of lactate can increase (Berg et al., 1997; Taura et al., 1998) or decrease (Knolmayer et al., 1998). The pO\(_2\) is proportional to the FiO\(_2\), e.g. a FiO\(_2\) increase from 20–100% causes a pO\(_2\) increase from 95–100 to 500 mmHg respectively. During endoscopic surgery with adequate ventilation pO\(_2\) and sO\(_2\) do not change (Leighton et al., 1993). With higher intraperitoneal pressures, however, a slight decrease of pO\(_2\) and sO\(_2\) in arterial and mixed venous blood was described i.e. with pressures >12 mmHg in humans (Wright et al., 1995;
CO₂-pneumoperitoneum-induced hypoxemia reduced by O₂ addition

Figure 3. Arterial blood partial pressures of O₂ (pO₂), oximetry (sO₂, O₂Hb and RHb) and O₂ status (p50 or O₂ tension at half saturation assessing the haemoglobin O₂ affinity) parameters in rabbits without pneumoperitoneum (group one —— and —— in series A and B respectively), during pneumoperitoneum with 100% CO₂ (group two —— and —— in series A and B respectively) and 6% O₂ + 94% CO₂ (group three —— and —— in series A and B respectively). X: time, min and Y: means ± SD are given (pO₂).

Berg et al., 1997; Gebhardt et al., 1997), >14 mmHg in dogs (Kotzampassi et al., 1993), and >10 mmHg in pigs (Liem et al., 1996).

Our data give a comprehensive picture of changes caused by CO₂ absorption and confirm previous results (Mynbaev et al., 2002). The key event is the progressive accumulation of CO₂, causing increases in carbonic acids and a base deficit. This leads initially to respiratory and later to metabolic acidosis. Excess of acids, base deficits and a lower pH reduce haemoglobin O₂ affinity, as evidenced by the increased O₂ tension at half saturation (p50), the increased concentration of reduced haemoglobins (RHb) and the decreased pO₂, sO₂, O₂Hb, known as the Bohr effect (Siggaard-Andersen et al., 1990). This results in tissue ischaemia and increased lactate concentrations (Berg et al., 1997; Taura et al., 1998).

The addition of 6% of O₂ to the CO₂ pneumoperitoneum has important and unexpected effects. The increase of pCO₂ and decrease of pH during CO₂-pneumoperitoneum could be interpreted as an accumulation of resorbed CO₂. This hypothesis, however, does not explain the dramatic effect of adding 6% O₂ to the CO₂-pneumoperitoneum, since this only changes the CO₂ concentration from 100 to 94%. We therefore suggest another mechanism for the progressive rising CO₂ and declining pH during CO₂-pneumoperitoneum: namely that these effects do not accumulate, but reflect a progressively increasing absorption of CO₂ secondary to mesothelial damage by hypoxia. The addition of small amounts of O₂ to the CO₂ pneumoperitoneum prevents mesothelial damage. Hence, pCO₂ and pH changes occur for some 30 min only, i.e. the time to reach an equilibrium between absorption and evacuation by ventilation. This hypothesis of CO₂-pneumoperitoneum induced mesothelial damage through hypoxia and its prevention by adding small amounts of O₂ has been described previously (Yesildaglar et al., 1999, 2000; Molinas and Koninckx, 2000; Molinas et al., 2001) based on data in rabbits (Molinas and Koninckx, 2000; Yesildaglar et al., 2000) and mice (Yesildaglar et al., 1999; Molinas et al., 2001) showing that adhesion formation increases with the duration of CO₂- or helium-pneumoperitoneum and with insufflation pressure, and decreases with the addition of small amounts of O₂ (Yesildaglar et al., 1999, 2000; Molinas and Koninckx, 2000; Molinas et al., 2001). To explain why the
addition of 6% of O2 to the CO2-pneumoperitoneum does not change HCO₃⁻ and iCO₂ whereas pCO₂, pH, ABE, SBE, SBC, pO₂, sO₂, O₂Hb, RHb p50 and lactate are affected is more difficult. Factors that should be taken into consideration include: that CO₂-pneumoperitoneum finally leads to metabolic hypoxia (Mynbaev et al., 2002) through the Bohr effect and that CO₂-pneumoperitoneum not only induces mesothelial hypoxia but also causes some hypoxia in the organs of the abdominal cavity. In order to understand the effect of the addition of O2 to CO₂ on blood gases, acid base and O₂ homeostasis, it could also be compared with the treatment of hypoxia with O₂. Acute asphyxia increases the arterial pCO₂ and lactate concentration. Exercise hypoxia causes lactacidemia with increases in arterial lactate and decreases in pH, pCO₂, HCO₃⁻, base excess, sO₂ and haemoglobin O₂ affinity (Wasserman, 1986; Yoshida et al., 1989). Treatment with O2 normalizes the acid base balance and blood gases with a decrease of acid excess, compensation of base deficit, increases in saturation and in haemoglobin O₂ affinity (Adams and Welch, 1980; Yoshida et al., 1989). Similarly, the addition of 6% of O2 to the CO₂ could prevent the mesothelial hypoxemia and the metabolic changes in the peritoneum and in the organs of the abdominal cavity with subsequent stabilization of the acid base and blood gases homeostasis.

The concept that the addition of small amounts of O2 to the CO₂ prevents hypoxic damage to the mesothelial and splanchic organs, could explain the clinical observation that the absorption of CO₂ is more important during retroperitoneal surgery in humans. The effects of pneumoperitoneum observed in non-animal studies obviously cannot be extrapolated to human surgery. Indeed, in the human, increased ventilation is performed during surgery in order to keep pCO₂ within acceptable limits. In our experiments, we intentionally have chosen not to increase ventilation in order to show the effects clearly. Moreover, a model with superficial ventilation was used to enhance changes in order to better understand the underlying mechanism. This could be important in the human where similar hyperventilation experiments obviously cannot be performed, for ethical reasons.

In conclusion, the addition of 6% of O₂ to CO₂ used for the pneumoperitoneum dramatically affects the known increase in arterial pCO₂ and decrease in pH, with, in addition, a prevention of the subsequent metabolic changes. The prevention of local hypoxia in the peritoneum and in the organs of the abdominal cavity is suggested as a mechanism. If these preliminary data are confirmed in the human, the addition of a few percent of O₂ to CO₂ could become important during endoscopic surgery of longer duration, especially in patients with limited cardio-respiratory adaptation and steep Trendelenburg.

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References