Hypoxaemia induced by CO₂ or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits

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A prospective randomized trial in a rabbit model was performed to test the hypothesis that the increase in adhesion formation following prolonged pneumoperitoneum is mediated by peritoneal hypoxaemia. Laparoscopic standardized opposing lesions were performed in uterine horns and pelvic sidewalls by bipolar coagulation and CO₂ laser in six groups of eight animals. Pure CO₂ or helium pneumoperitoneum was used for 10 (groups I and IV) or 45 min (groups II and V) to confirm the effect of duration of pneumoperitoneum and 96% of CO₂ or helium with 4% of oxygen (group III and VI) for 45 min to assess the effect of the addition of oxygen. After 7 days, adhesion formation was scored by laparoscopy. By two-way analysis of variance, total, extent, type and tenacity of adhesion scores increased (P = 0.0003, P = 0.0004, P = 0.0004 and P = 0.0004) with increasing duration of pneumoperitoneum and decreased (P = 0.02, P = 0.03, P = 0.01 and P = 0.05) with the addition of oxygen. No differences were found between CO₂ and helium. In conclusion these data confirm the effect of pneumoperitoneum upon adhesions and demonstrate its reduction by oxygen, strongly suggesting that the main cause of adhesion formation is the relatively superficial hypoxaemia produced by the pneumoperitoneum.

Key words: adhesions/helium/hypoxaemia/laparoscopy/pneumoperitoneum

Introduction

Intraperitoneal adhesions are clinically important because of the associated complaints and complications. They are a major cause of intestinal obstruction, chronic pelvic pain and infertility (Rapkin, 1986; Ellis, 1997). Adhesions may be classified as congenital or acquired which can be post-inflammatory or post-operative. Some 10% of patients without previous surgery have adhesions, 9% post-inflammatory and 1% congenital. In contrast, adhesions are found in 94% of patients with at least one experience of previous surgery, 92% post-operative, 1% post-inflammatory and 1% congenital (Ellis, 1997). The peritoneal injury during either a laparotomy or a laparoscopy activates the cascade which leads to adhesion formation (Chegini, 1997; diZerega, 1997; Holmdahl, 1997). In humans, laparoscopy was claimed to be less adhesiogenic than laparotomy (Lundorff et al., 1991). More and large clinical studies have not been performed because of the obvious difficulties and ethical concerns for follow-up and scoring of adhesions. In animals, some studies failed to show differences between laparoscopy and laparotomy (Filmar et al., 1987; Marana et al., 1994; Jorgensen et al., 1995) whereas others did show significantly less adhesions after laparoscopy in rats (Schäfer et al., 1998), dogs (Schippers et al., 1998), pigs (Fowler et al., 1994) and rabbits (Luciano et al., 1989).

CO₂ pneumoperitoneum was recently shown to play a role in peritoneal adhesion formation in rabbits (Ordoñez et al., 1997) and mice (Yesildaglar et al., 1999). This could be important since CO₂ is generally used for pneumoperitoneum for safety reasons because of its high solubility in water and exchange rate in the lungs. CO₂ induces local changes such as intraperitoneal acidosis (Volz et al., 1996, 1997; West et al., 1997). In the absence of moistening, desiccation of mesothelial layers will occur (Ryan et al., 1973), whereas the intraperitoneal pressure will induce adverse effects upon peritoneal microcirculation (Taskin et al., 1998, 1999) possibly inducing hypoxaemia. The hypothesis of hypoxaemia in the peritoneal superficial layers was moreover suggested by the observations that adhesions decrease when oxygen is added to the CO₂ during pneumoperitoneum (P.R. Koninckx et al., unpublished).

In order to confirm that superficial mesothelial hypoxaemia is a key adhesiogenic factor, this prospective, randomized trial in a rabbit model was designed using helium instead of CO₂. This experiment should confirm the effect of duration of pneumoperitoneum and evaluate the relative importance of acidosis and hypoxaemia upon adhesion formation.

Materials and methods

Subjects and surgical procedures

Laparoscopies were performed in 48 adult, female, New Zealand white rabbits weighing between 2.5 and 3 kg. The study was approved by the Institutional Review Animal Care Committee and the animals were kept under standard laboratory conditions at the Centre for Laboratory Animal Care of the Catholic University of Leuven.

The anaesthesia was induced with i.m. ketamine (50 mg/kg, Ketalin®, Aphamco, Duiven, The Netherlands) and xylazin (6 mg/kg, XYL-M 2%, VMD, Arendonk, Belgium) and maintained with inhalational halothane (2%, Fluothane®; Zeneca, Destelbergen, Belgium) and oxygen (1.5 l/min).

The surgery was performed under strict aseptic condition and no antibiotics were administered. The rabbits were placed in supine
position and the abdomen was shaved and disinfected with polyvidone iodine (iso-Betadine®; Asta Medica, Brussels, Belgium). A 12 mm trocar (Apple®; Medical Corporation, Bolton, MA, USA) was introduced by open laparoscopy through a 1 cm incision caudal to the sternum. The pneumoperitoneum was created using 100% of CO₂ or helium or a mixture of 96% of CO₂ or helium with 4% of oxygen. This was achieved using two insufflators (Thermofoam®; Karl Storz, Tuttingen, Germany), one for CO₂ or helium and one for oxygen. To obtain a homogeneous mixture, the output of both insufflators was mixed in a mixing chamber which was connected to a water valve to limit the insufflation pressure at 10 cm of water (Koninckx and Vandermeersch, 1991). Therefore a slightly higher insufflation pressure was used for both insufflators (8 mm Hg), whereas 4% of oxygen was achieved using 24 l/min of CO₂ or helium and 1 l/min of oxygen. For reasons of standardization 25 l/min was used for pure CO₂ or helium, knowing that all excess gas would escape from the water valve. A 12 mm 0° endoscope (Karl Storz) connected to a single chip video camera (Karl Storz) and light source (Karl Storz), was used. After the establishment of the pneumoperitoneum a 5 mm trocar (Apple®; Medical Corporation) was introduced, under direct laparoscopic vision, at the level of the umbilicus to allow the introduction of the necessary instruments. Taking into account the high exchange capacity of the peritoneum and to maintain the extent (L-extent), laser type (L-type) and laser tenacity (L-tenacity) an 18 gauge catheter (Insyte-W®; Vialon®; Becton Dickinson, Madrid, Spain) was inserted in between the first and second trocars.

The rabbits were placed in 45° Trendelenburg position. Standardized opposing lesions of 2 cm² were performed randomly on the uterine horns and in the pelvic side walls by bipolar coagulation in one side, thus was operated on during the same day. All surgery was performed by the same surgeon during 8 consecutive days for the first and the second look respectively.

All second look laparoscopies were video-recorded and subsequently adhesion formation was scored blindly by two independent observers taking into account extent (0: no adhesions, 1: 1–25%, 2: 25–50%, 3: 51–75%, 4: 76–100%), type (0: no adhesions, 1: filmy avascular, 2: dense avascular, 3: dense with capillaries, 4: dense with larger vessels) and tenacity (0: no adhesions, 1: essentially fall apart, 2: required traction, 3: required sharp dissection).

Since the lesions inflicted in either the right or the left side were performed in the same way (laser or bipolar), scoring was done separately for right and left side, thus obtaining separate laser (L) and bipolar (B) lesions adhesion scores.

Laser total (L-total) adhesion score was obtained adding laser extent (L-extent), laser type (L-type) and laser tenacity (L-tenacity) adhesion scores. Bipolar total (B-total) adhesion score was obtained adding bipolar extent (B-extent), bipolar type (B-type) and bipolar tenacity (B-tenacity) adhesion scores. Extent, type, tenacity and total adhesion scores were defined as the sum of L-extent and B-extent, L-type and B-type, L-tenacity and B-tenacity and L-total and B-total, respectively.

Statistics

Statistical analysis was performed with the SAS system (SAS Release 6.12, 1998) using Wilcoxon analysis and two-way analysis of variance. Since data were not normally distributed because of 0 scores, the general linear model (Proc GLM) was used instead of analysis of variance. All data are presented as mean ± SEM. The advantage of the 2×2 factorial design was that to achieve the same statistical precision as with a one at a time approach, twice as many observations would have been needed. The power of the observed effect of duration of CO₂ and helium, and the effect of adding oxygen thus is comparable to experiments with 16 animals in each group. This increase in power of the factorial design was only valid when the effects of the two factors were additive, i.e. when no interaction between the two factors was present. The possibility of detecting an interaction, i.e. a different effect of one factor at different levels of the other factor, could however also be considered an advantage of the factorial design, since this effect could otherwise easily be missed. When the number of observations is low, one should be aware that a positive interaction (with subsequent reduction of power to demonstrate the effect of the two factors) could be missed, especially when the between subject variability is high (Armitage and Berry, 1987).

Results

By increasing the duration of pure CO₂ or helium pneumoperitoneum from 10 to 45 min the total adhesion score increased (Wilcoxon) from 4.6 ± 1.3 to 10.5 ± 1.8 for CO₂ (P = 0.04) and from 3.1 ± 1.2 to 9.7 ± 1.8 for helium (P = 0.02) confirming the effect of duration of the pneumoperitoneum. For CO₂, extent increased from 1.7 ± 0.5 to 4.5 ± 0.9 (P = 0.03), type from 1.3 ± 0.3 to 3.1 ± 0.5 (P = 0.01) and tenacity from 1.6 ± 0.4 to 2.9 ± 0.6 (not significant, NS).
For helium, extent increased from 1.1 ± 0.4 to 3.6 ± 0.6 (P = 0.01), type from 1 ± 0.4 to 3.1 ± 0.6 (P = 0.02) and tenacity from 1 ± 0.4 to 3 ± 0.6 (P = 0.03). By two-way analysis of variance, the effect of duration of pneumoperitoneum was highly significant for total (P = 0.0003), extent (P = 0.0004) and tenacity (P = 0.004) scores, whereas no differences between CO₂ and helium and no interaction between duration of pneumoperitoneum and the type of gas used were found (Figure 1).

Adding 4% of oxygen to CO₂ or helium pneumoperitoneum during 45 min, total adhesion score decreased (Wilcoxon) to 6 ± 1.8 for CO₂ (NS) and to 6.1 ± 0.9 for helium (NS). For CO₂, extent decreased to 2.4 ± 0.8 (NS), type to 1.8 ± 0.5 (NS) and tenacity to 1.8 ± 0.4 (NS). For helium, extent decreased to 2.1 ± 0.3 (NS), type to 1.9 ± 0.2 (NS) and tenacity to 2.1 ± 0.3 (NS). By two-way analysis of variance, the addition of oxygen was significant for total (P = 0.02), extent (P = 0.01) and tenacity (P = 0.05) scores, whereas no differences between CO₂ and helium and no interaction between the addition of oxygen and the type of gas used were found (Figure 1).

Laser and bipolar lesions were evaluated separately. By two-way analysis of variance, increasing duration of the pneumoperitoneum increased L-total (P = 0.0001), L-extent (P = 0.0001), L-type (P = 0.0001) and L-tenacity (P = 0.003) adhesion scores, as well as B-total (P = 0.02), B-extent (P = 0.04), B-type (P = 0.01) and B-tenacity (P = 0.05) adhesion scores (Table I).

Laser lesions induced more adhesions than bipolar lesions as evidenced by higher total scores (P = 0.0001), and higher scores for extent (P = 0.0001), type (P = 0.0001) and tenacity (P = 0.0001).

Discussion

It appears that this is the first study of post-laparoscopic adhesion formation using helium pneumoperitoneum. Helium was chosen as an alternative for CO₂ pneumoperitoneum because it is chemically, physiologically and pharmacologically inert, non-explosive and since it does not produce hypercarbia and acidosis and the related haemodynamic and cardiopulmonary effects observed with CO₂ (Kotzampassi et al., 1993; Leighton et al., 1993; McMahon et al., 1994; Rademaker et al., 1995; Neuberger et al., 1996; Declan et al., 1997; Volz et al., 1997). Its lower solubility in water, however, carries a higher risk of embolization and an increased lethal effect of gas embolism (Wolf et al., 1994; Rudston-Brown et al., 1997).

Helium pneumoperitoneum was used in this experiment to evaluate the hypothesis that the hypoxaemia, induced by compression of the capillary flow in the superficial peritoneal layers during prolonged pneumoperitoneum, is a cause of adhesion formation. It is logical to assume that increasing duration of pneumoperitoneum and higher intra-abdominal

Figure 1. Effect of CO₂ (■) and helium (□) pneumoperitoneum (10 or 45 min) and of the addition of 4% of oxygen upon adhesion formation following a laser and bipolar opposing lesions. Total, extent, type and tenacity mean scores ± SEM are indicated together with P values (two-way analysis of variance).
pressures limit the peritoneal capillary flow, increasing hypoxaemia, and that the addition of oxygen could decrease hypoxaemia. This assumption is consistent with previous findings that duration of CO₂ pneumoperitoneum (Ordoñez et al., 1997; Yesildaglar et al., 1999) and that higher intraperitoneal pressures (Yesildaglar and Koninckx, 2000) increase adhesions whereas the addition of oxygen decreases adhesion formation (P.R. Koninckx et al., unpublished). This experiment confirmed the observations using CO₂ pneumoperitoneum and demonstrated the same effect using helium pneumoperitoneum whereas no differences were found between CO₂ and helium. All these data together confirm the hypothesis that hypoxaemia is more important than acidosis in adhesion formation following prolonged pneumoperitoneum.

The effect of a low oxygen concentration in blood (hypoxaemia) or in tissues (hypoxia) differs between tissues, which could be explained by different microvascular flow reserves, oxidative capacity and metabolic need (Scannell, 1996). Hypoxia alters the metabolism of endothelial cells as shown by vascular damage and increased vascular permeability (Scannell, 1996), increased expression of adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 and intracellular adhesion molecule (ICAM)-1 (Setty and Stuart, 1996) and increased polymorphonuclear (PMN) adhesion to the vascular endothelium (Kalra et al., 1996). At the same time, hypoxia decreases T-lymphocyte production of interleukin (IL)-2, a key cytokine responsible for B-cell proliferation and immunoglobulin secretion, and increases the release of tumour necrosis factor (TNF)-α, IL-1 and IL-8 by human macrophages (Scannell, 1996).

Hypoxia could modulate directly or indirectly, during pneumoperitoneum, the production of cytokines and growth factors by peritoneal mesothelial cells, macrophages and fibroblasts. Macrophages secrete cytokines and growth factors, such as IL-1, IL-4, IL-6, IL-10, TNF and transforming growth factor (TGF), which are involved in peritoneal wound healing and modulate the process that leads to adhesion formation (Chegini, 1997). TGF-β1, TNF-α and IL-1β up-regulate plasminogen activator inhibitor-1 (PAI-1) and down-regulate tissue-type plasminogen activator (t-PA), decreasing plasmin and thus inhibiting the lysis of fibrin (Tietze et al., 1998). TGF-β decreases the expression of matrix metalloproteinases (MMP) and increases the expression of tissue inhibitors of metalloproteinases (TIMP), thus decreasing matrix degradation and increasing fibrous adhesions (Chegini, 1997). The effect of hypoxaemia, however, has not been investigated as was done for mesothelial cells. Recently it was shown that human peritoneal mesothelial cells cultured under hypoxic conditions (2% oxygen) increase amounts of TGF-β1 mRNA and collagen III mRNA after 6 h (Saed et al., 1999a), amounts of TGF-β1 and TGF-β2 mRNA after 24 h (Saed et al., 1999b) and amounts of TIMP-1 mRNA, possibly via a TGF-β1 dependent mechanism (Saed et al., 1999c). To interpret these data it should be realized that in some experiments hypoxaemia varies from 0–5% of oxygen. This could be important since it should be compared to the partial oxygen pressure in the abdominal cavity.

The effect of mesothelial hypoxaemia, induced by the pneumoperitoneum during a laparoscopic surgery, upon vascular endothelial growth factor (VEGF) expression should be considered to explain the increase in adhesion formation. Indeed, hypoxia, together with other growth factors and cytokines, stimulates the production of VEGF by a variety of normal and transformed cell types (Neufeld et al., 1999). Hypoxia up-regulates the production of VEGF by non-activated and by interferon-β (IFNβ) and/or lipopolysaccharide (LPS) activated murine peritoneal macrophages (Xiong et al., 1998). Increased concentrations of VEGF were detected, generally under hypoxic conditions, in ovarian hyperstimulation syndrome (Chen et al., 1999), ovarian neoplasm (Yamamoto et al., 1997), endometriosis (McLaren et al., 1996) and ascites tumours (Luo et al., 1998) and during the normal cyclic changes in the female reproductive system (Shweiki et al., 1993). Furthermore, VEGF was found in peritoneal adhesions of women by immunohistochemistry (Wiczynski et al., 1998) and of men and women by enzyme-linked immunosorbent assay (Diamond et al., 1999) and it was shown that a polyclonal rabbit antibody to VEGF limits adhesion formation after laparotomy in a murine model (Saltzman et al., 1996).
data indicate that VEGF has a role in the development of post-operative adhesions.

It is unclear at present why laser lesions induce more adhesions than bipolar lesions. It can only be speculated that the larger denuded area might be important, but it should be investigated what is the exact role of depth and amount of tissue necrosis and whether this effect might be specific for hypoxia induced adhesions.

In conclusion, this experiment confirms the key role of mesothelial hypoxaemia in adhesion formation. Recent publications indicate that this could be mediated by a growth factor such as TGF-β or VEGF and more specific studies are currently being performed in order to clarify this point.

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Hypoxaemia induced by pneumoperitoneum increases adhesions


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