Adhesion formation in intubated rabbits increases with high insufflation pressure during endoscopic surgery

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The aim of the study was to test the hypothesis that the increase in adhesion formation by CO2 pneumoperitoneum is caused by mesothelial hypoxaemia. Therefore the effect of the intra-abdominal pressure together with the flow rate upon adhesion formation was evaluated in rabbits following laser and bipolar lesions during endoscopic surgery using humidified CO2 at 35 ± 1°C. The intra-abdominal pressure and flow rate were 5 mmHg and 1 l/min in group 1 (n = 5), 5 mmHg and 10 l/min in group 2 (n = 4), 20 mmHg and 1 l/min in group 3 (n = 5) and 20 mmHg and 10 l/min in group 4 (n = 4) respectively. A rapid and reliable intubation method for rabbits was developed to permit high insufflation pressure. By two-way analysis of variance, total adhesion scores following a laser lesion increased with flow rate (P = 0.0003) and insufflation pressure (P = 0.002). Total adhesion scores of bipolar lesions increased with pressure (P = 0.02) but not with flow rate (P = 0.1). The total adhesion scores of laser and bipolar lesions together increased with flow rate (P = 0.005) and with insufflation pressure (P = 0.004). There was no statistical interaction between flow rate and insufflation pressure. In conclusion, the insufflation pressure in endoscopic surgery with CO2 pneumoperitoneum is a co-factor in adhesion formation, together with desiccation.

Keywords: adhesions/desiccation/insufflation pressure/intubation

Introduction

Formation of peritoneal adhesions is a peritoneal response to injury and results in major clinical problems relating to peritoneal repair. The aetiology of pelvic adhesions is multifactorial. The most common cause of peritoneal adhesions is prior surgery. After multiple laparotomies, 93% of patients have intra-abdominal adhesions. Postoperative adhesions were found in 10% of patients after one laparotomy (Menzies and Ellis, 1990). Possible complications produced by intraperitoneal adhesions are intestinal obstruction, pelvic pain and infertility. The most serious complication of intraperitoneal adhesions is intestinal obstruction. It was estimated that one-third of all intestinal obstructions are caused by adhesions (Ellis, 1982).

Adhesions have been implicated as a cause of pelvic pain by their restriction in movement and distensibility of the pelvic organs (Kresch et al., 1984; Bahary and Gorodeski, 1987; Peters, et al., 1992). Adhesions are a major cause of infertility (Drake and Grunert, 1980; DeCherney and Mezer, 1984; Trimbos-Kemper et al., 1985). These can be treated by surgery but recurrence remains problematic (Diamond et al., 1987). Moreover, adhesive disease can produce a significant economic charge to community (Ray et al., 1993).

Laparoscopic surgery has been claimed to be less adhesiogenic than laparotomy (Luciano et al., 1989). This, however, has never been demonstrated conclusively, at least partially because of ethical constraints in man, and because most efforts were devoted to show benefits of laparoscopic surgery, such as lower postoperative morbidity and pain. Animal models allow a better control of variables. The most commonly used animal models are rats (Filmar et al., 1987; Evrard et al., 1994) and rabbits (Doody et al., 1989; Marana et al., 1994). Rabbits are a good model and have been widely used for the study of induction and prevention of adhesion formation. The rabbit model moreover permits the performance of endoscopic procedures with conventional instruments. Using this model, it was shown (Ordonez et al., 1997) that duration of pneumoperitoneum during endoscopic surgery was a co-factor in adhesion formation. This observation was confirmed in an endoscopic mouse model (Yesildaglar et al., 1999), suggesting that either changes in pH or anoxaemia of the superficial mesothelial layers was the aetiological factor.

Since hypoxaemia of the superficial mesothelial layers of the peritoneum must increase with the pneumoperitoneum pressure, the effect of 5 and 20 mmHg insufflation pressure upon adhesion formation was evaluated to test this hypothesis.

Materials and methods

Animals

Adult female New Zealand white rabbits weighing 2.7–3.2 kg were used. Animals were kept under standard laboratory conditions at a temperature of 20–25°C, a relative humidity of 40–70%, a day cycle of 14 h light and 10 h dark, a standard laboratory diet (Hope Farms, Woerden, The Netherlands) and had free access to food and water before and after the surgical procedures. The animals were housed at the centre for laboratory animal care (Animalium, St Rafael Hospital, Catholic University of Leuven, Belgium).

Experiments

To evaluate the effect of intra-abdominal pressure during pneumoperitoneum upon adhesion formation, 5 and 20 mmHg insufflation pressure were used, being respectively the lowest pressure for adequate peritoneal distension, and the highest pressure which could reliably...
be used in rabbits without anaesthetic problems. Since this experiment was designed to test the hypothesis of mesothelial anoxaemia, and knowing that CO₂ is readily absorbed and that the peritoneum has a high exchange capacity, a continuous flow of CO₂ through the abdominal cavity was used to maintain a nearly 100% concentration of CO₂ by removing continuously all oxygen which could have diffused from the circulation. Since some desiccation, a known co-factor of adhesion formation, could not be excluded, a 2 × 2 factorial design (Armitage and Berry, 1987) was used to evaluate the effect of insufflation pressure (5 and 20 mmHg) at two different flow rates (1 and 10 l/min) together with the effect of flow rate. The effect of pressure was investigated at low and high flow rates since it was not possible to predict at which flow rate the effect of pressure would become most significant, and since this could be done without increasing the number of experiments simply by using a 2 × 2 factorial design. The four groups thus had an intra-abdominal pressure and a flow rate of 5 mmHg and 1 l/min (group 1, n = 5), of 5 mmHg and 10 l/min (group 2, n = 4), 20 mmHg and 1 l/min (group 3, n = 5) and 20 mmHg and 10 l/min (group 4, n = 4) respectively. The CO₂ was warmed to 35 ± 1°C and humidified using the Thermoflator (Karl Storz, Brussels, Belgium) and a humidifier (Dräger®, Wemmel, Belgium).

**Anaesthesia and intubation under endoscopic view**

After premedication with 50 mg/kg i.m. ketamine (Ketalin; Aphanro, Arnhem, The Netherlands) and 0.3 mg/kg i.m. xylazin hydrochloride (Rompun, 2%; Bayer, Brussels, Belgium), anaesthesia was maintained with inhalation of 2–3% halothane (Fluothane®, Zeneca, Brussels, Belgium) using 1 l/min of oxygen. Pulse rate and oxygen saturation was monitored continuously using a pulse oximeter (Nellcor, Leuven, Belgium).

Because of the anatomy of the oral fissure and pharynx, orotracheal intubation of rabbits is difficult, with a high failure rate. Since intubation was necessary for these experiments using 20 mmHg of insufflation pressure, a new technique for endotracheal intubation of rabbits was developed. Approximately 5 min after the beginning of halothane anaesthesia, the animals were secured in a supine position on the operation table. A flexible salpingoscope (2.7 mm diameter; Karl Storz) in a straight, 20-cm-long metal tube with an external diameter of 3.2 mm, to obtain rigidity, was inserted in the endotracheal desiccation during insufflation. A 200 ml Falcon dish was designed to test the hypothesis of mesothelial anoxaemia, and since some desiccation, a known co-factor of adhesion formation, could not be excluded, a 2 × 2 factorial design (Armitage and Berry, 1987) was used to evaluate the effect of insufflation pressure (5 and 20 mmHg) at two different flow rates (1 and 10 l/mm) together with the effect of flow rate. The effect of pressure was investigated at low and high flow rates since it was not possible to predict at which flow rate the effect of pressure would become most significant, and since this could be done without increasing the number of experiments simply by using a 2 × 2 factorial design. The four groups thus had an intra-abdominal pressure and a flow rate of 5 mmHg and 1 l/min (group 1, n = 5), of 5 mmHg and 10 l/min (group 2, n = 4), 20 mmHg and 1 l/min (group 3, n = 5) and 20 mmHg and 10 l/min (group 4, n = 4) respectively. The CO₂ was warmed to 35 ± 1°C and humidified using the Thermoflator (Karl Storz, Brussels, Belgium) and a humidifier (Dräger®, Wemmel, Belgium).

In all animals, two opposing lesions were made on each side using either a CO₂ laser (Sharplan, 1060, Brussels, Belgium) at 10 W in a continuous superpulse mode or a 5 mm endoscopic bipolar forceps (Ethicon Endosurgery) at 10 W. Randomly, 2 cm² of the oviduct and of the ipsilateral pelvic side wall were superficially vaporized whereas on the other side, the same surface was coagulated using a bipolar forceps. Following this procedure, taking 5–6 min, the CO₂ pneumoperitoneum was maintained for 30 min total time. Animals were assigned, on a daily basis using randomization tables with blocks of four animals. Each block of four animals was operated on during the same day. Thus only one animal in each treatment group was operated on in one day. These experiments were performed by one investigator (N.Y.) over a 10 day period.

**Second-look laparoscopy and scoring system for assessment of adhesions**

Seven days after the initial procedures, adhesions were scored during a second look laparoscopy under general anaesthesia. Adhesions were scored (Fiedler et al., 1996) as published before (Ordonez et al., 1997). This scoring system took into account type (0 = no adhesions; 1 = filmy adhesions; 2 = firm adhesions; 3 = dense adhesions, require sharp dissection to be separated), tenacity (1 = easily fall apart; 2 = require traction; 3 = require sharp dissection) and extent of adhesions (1 to 4 points: 1–25%, 26–50%, 51–75%, 76–100%) respectively. Total adhesion scores were the sum of type, tenacity and extent scores of lesions. All the procedures and second-look laparoscopies were videotaped, and subsequently scored blindly by two independent investigators in order to minimize inter-observer variability in adhesion scoring (Corson et al., 1995).

The number of the animals was planned to be five in each group, but two rabbits (one rabbit from the second group and one rabbit from the fourth group) were excluded because of intraperitoneal infection observed during the second-look operations.

**In-vitro experiments**

Desiccation during high flow insufflation was evaluated in vitro as described previously (Yesildaglar et al., 1999). A 200 ml Falcon dish with two holes of 7 mm on each side, containing 20 ml of water, i.e. covering the entire bottom surface, was used to evaluate water loss/desiccation during insufflation at continuous flow rates of 1, 5, 10, 15 and 20 l/min with and without a humidifier (Dräger). The insufflation was carried out with a Thermoflator, which kept the insufflated CO₂ at 35 ± 1°C. The dish was kept at exactly 36.5 ± 0.5°C using two heaters (Maquet Rastatt, Maquet, St-Pieters-Leeuw, Belgium; Ameda, Zug, Switzerland). During the experiments, the temperature of the dish was monitored continuously using a temperature probe (Hewlett Packard, Brussels, Belgium). In all experiments, observations were made in triplicate.

**Statistics**

Data were analysed by two-way analysis of variance (ANOVA; general linear models, GLM procedure) using the SAS system (SAS Release 6.12, 1998). All data are presented as means ± SD unless indicated otherwise. The advantage of a 2 × 2 factorial design, with five animals in each cell, was that to achieve the same statistical precision with a one-factor-at-a-time approach, twice as many observations would have been needed. The factorial design moreover had the advantage of permitting the detection of an effect of one factor at different levels of the other, i.e. it permitted the detection of an interaction between the two factors (Armitage and Berry, 1987). In these experiments, a difference in total adhesion score of ≥3 had a power of 89% given an SD of 2, and 8 and 10 animals in each group. The SAS GLM test also compensated for the unbalanced design.
Adhesion formation increases with high insufflation pressure

Table I. Type, tenacity, extent and total adhesion scores following a superficial laser or a deeper bipolar lesion according to the pneumoperitoneum insufflation pressure and the flow rate

<table>
<thead>
<tr>
<th>Insufflation pressure (mmHg)</th>
<th>Flow rate</th>
<th>Laser lesion</th>
<th>Bipolar lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(1 l/min)</td>
<td>(10 l/min)</td>
<td>(1 l/min)</td>
</tr>
<tr>
<td>Type</td>
<td>1.0 ± 0</td>
<td>1.0 ± 0</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Tenacity</td>
<td>1.0 ± 0</td>
<td>1.3 ± 0.5</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Extent</td>
<td>3.0 ± 0</td>
<td>5.0 ± 0.8</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>Total</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>2.6 ± 0.5</td>
</tr>
</tbody>
</table>

**Values are means and SD.**

Results

Intubation under endoscopic vision was a safe and quick procedure taking <1 min, without a single injury, mortality or failure in this series.

Adhesion scores obtained in the four treatment groups are shown in Table I. By two-way ANOVA, total adhesion scores of laser lesions increased with flow rate ($P = 0.0003$) and insufflation pressure ($P = 0.002$). Total adhesion scores following a bipolar lesion increased with insufflation pressure ($P = 0.02$) but not with flow rate ($P = 0.1$). Sum of total adhesion scores of laser lesions and total adhesion scores of bipolar lesions increased with flow rate ($P = 0.005$) and with insufflation pressure ($P = 0.004$) (Figure 1).

Following a laser lesion, the individual adhesion scores for type, tenacity and extent increased with flow rate ($P = 0.003$, $P = 0.0005$ and $P = 0.004$ respectively) and with insufflation pressure ($P = 0.02$, $P = 0.001$ and 0.001 respectively). Following a bipolar lesion, the individual adhesion scores for extent increased with pressure ($P = 0.02$) but not with flow. Interaction between flow rate and insufflation pressure was not observed for total scores nor for type, tenacity or extent of adhesions.

In vitro, desiccation was linear with flow rate (Figure 2) being 1.61, 3.24, 4.83 and 6.53 g of water loss after insufflation for 30 min at 36.5 ± 0.5°C without humidification and at flow rates of 5, 10, 15 and 20 l/min respectively (Yesildaglar et al., 1999). However, when the CO₂ gas was humidified, water loss was much less and desiccation was exponential, with flow rates being 0.160 ± 0.018, 0.358 ± 0.015, 0.675 ± 0.021, 1.266 ± 0.027 and 2.153 ± 0.025 g of water after insufflation for 30 min at 1, 5, 10, 15 and 20 l/min flow rates for 30 min respectively (Figure 2).

Discussion

Endotracheal blind intubation of rabbits is difficult. It can take a long time to complete the procedure and there is a considerable failure rate (Corleta et al., 1994). Nevertheless, under endoscopic vision it is very safe, precise and practical time-wise to use the endotracheal intubation technique. To the best of our knowledge, this is the first report of this intubation technique.
The increase of adhesion formation with the duration of CO₂ pneumoperitoneum (Ordonez et al., 1997; Yesildaglar et al., 1999) could be caused by changes in pH or by anoxaemia of the superficial mesothelial layers or both. These experiments were designed to investigate the effect of anoxaemia, assuming that higher insufflation pressures would increase the depth and extent of anoxaemia by mechanical compression of the capillary bed, together with passive diffusion of CO₂. In order to maintain a near 100% concentration of CO₂ in the peritoneal cavity, taking into account the high exchange capacity of the peritoneum, a continuous flow of CO₂ was used, in order to remove any oxygen which could have diffused passively from the circulation. The result was that adhesions, following both a laser and a bipolar lesion, increased with the insufflation pressure, confirming the hypothesis that anoxaemia is a co-factor in adhesion formation, although an additional deleterious effect of changes in pH cannot be excluded.

The mechanism of adhesion formation as a consequence of anoxaemia remains unclear. Although it is logical to assume that the depth of anoxaemia increases with the insufflation pressure, the exact depth and extent of anoxaemia together with the consequences of the effects of CO₂ pneumoperitoneum upon the peritoneal microcirculation (Taskin et al., 1998) can only be speculated. Little is known about the possible effects of anoxaemia in the mesothelium to induce angiogenic factors such as vascular endothelial growth factor (VEGF) (Wiczyk et al., 1998) and upon the immune system, either directly upon the macrophages in the peritoneal fluid (Kopernik et al., 1998) or by attraction of monocytes from the circulation (Zeyneloglu, 1998).

Both factors, angiogenesis and the immune system, are candidates to mediate the observed effects upon adhesion formation. Desiccation is a known co-factor of adhesion formation. Since it could not be ruled out that a longer duration of CO₂ pneumoperitoneum would not cause some desiccation, the effects of anoxaemia and of desiccation were impossible to separate completely. Also the use of a humidifier could not guarantee the absence of desiccation. Therefore the effect of desiccation (i.e. flow rate) was introduced and evaluated in the model as a second factor using a 2×2 factorial design, permitting the simultaneous evaluation of the effects of pressure and of flow rate, together with the interaction between both factors. Since a higher flow rate probably resulted in more desiccation, the results suggest a direct effect of desiccation upon adhesion formation. In addition these experiments show that this effect cannot be prevented completely by using a humidifier, and that the effect increases with flow rate. From the in-vitro experiments, it can be postulated that in-vivo desiccation also must increase exponentially with flow rate since the efficacy of humidification decreases with increasing flow rate.

The relevance of desiccation at higher flow rates for surgery in the human is obvious, especially when using a high flow insufflator to remove smoke during CO₂ laser surgery. Also the effect of insufflation pressure and superficial mesothelial anoxaemia could be important since operations of much longer duration than those in this study are performed. Moreover, the mechanisms involved in adhesion formation by CO₂ pneumoperitoneum might be equally important for implantation and growth of malignant cells (Koster et al., 1996). Although the extrapolation of data from an animal model to the human should be done with great caution, the rabbit seems to be an appropriate model since reduction of adhesions by Interceed should be done with great caution, the rabbit seems to be an appropriate model since reduction of adhesions by Interceed (Marana et al., 1997) or by low dose aspirin (Muzii et al., 1998) and the effect of thrombin application (Yarali et al., 1998) are comparable with the effects observed in the human.

In conclusion, our data demonstrate that insufflation pressure is a co-factor in adhesion formation. These observations are consistent with the hypothesis that the increase in adhesion formation by CO₂ pneumoperitoneum is caused by hypoxaemia of the superficial cell layers of peritoneum. Moreover, this study shows the importance of adequate humidification of CO₂, especially at high flow rates.

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