Effect of temperature upon adhesion formation in a laparoscopic mouse model

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BACKGROUND: Pneumoperitoneum can be a cofactor in adhesion formation. Pneumoperitoneum with non-humidified gas causes desiccation in the peritoneal cavity which decreases temperature. The effect of desiccation upon adhesion formation is widely accepted. The specific effect of the associated cooling upon adhesion formation remains unexplored, and was addressed specifically in our laparoscopic mouse model. METHODS: Adhesions were induced during laparoscopy and scored after 7 days during laparotomy. Pneumoperitoneum was performed using CO2 or CO2 with oxygen with or without humidification. Animals were placed at different environmental temperatures to modulate body and intraperitoneal temperature. RESULTS: Anaesthesia, environment with a lower temperature and pneumoperitoneum all independently decrease body temperature. A decrease in body temperature decreases adhesion formation (P = 0.004). Therefore, at 37°C, pneumoperitoneum-enhanced adhesion formation is more important than at room temperature (P = 0.04). As was observed at room temperature, adhesion formation at 37°C increases with the duration (P = 0.01) of pneumoperitoneum and decreases with the addition of 3% of oxygen (P = 0.03). CONCLUSIONS: Hypothermia reduces pneumoperitoneum-enhanced adhesion formation, which supports hypoxia as a driving mechanism, since hypothermia decreases the toxic effects of hypoxia and of the ischaemia–reperfusion process. These data could open up new possibilities for adhesion prevention in laparoscopic surgery.

Key words: body temperature/desiccation/hypoxia/intraperitoneal adhesion formation/laparoscopy/pneumoperitoneum

Introduction

CO2 pneumoperitoneum during laparoscopy can decrease body temperature, especially when cold and dry CO2 gas at high flow rates is used (Bessell et al., 1999). As can be expected from thermodynamics, this cooling effect is caused less by the gas temperature but mainly by the energy necessary to evaporate body water in order to humidify the dry CO2 (Bessell et al., 1995). Indeed, cooling cannot be prevented with warm and dry gas (Bessell et al., 1995; Hazebroek et al., 2002), whereas cooling can be prevented to a large extent by cold and humidified gas (Hazebroek et al., 2002). Cooling can be fully prevented using warm and humidified gas, as shown in rats (Hazebroek et al., 2002) and pigs (Bessell et al., 1995, 1999; Mouton et al., 1999).

Pneumoperitoneum with dry and cold CO2 alters the morphology of the mesothelium, i.e. destroys the hexagonal pattern, reduces the microvilli (Hazebroek et al., 2002) and bulges up the cells (Volz et al., 1999; Suematsu et al., 2001). Whether this can be prevented with warm and humidified gas is controversial (Mouton et al., 1999; Hazebroek et al., 2002). Anyway, since the introduction of high flow insufflators for endoscopic surgery in the human (Kokinckx and Vandermeersch, 1991), the CO2 used became progressively warmed and humidified. The use of warm and humidified gas was claimed to reduce post-operative pain and duration of hospitalization (Demco, 2001) and to reduce intraperitoneal cytokine response (Puttick et al., 1999) and tumour growth (Nduka et al., 2002).

Over the last years, CO2 pneumoperitoneum became known as a cofactor in post-operative adhesion formation (Ordonez et al., 1997), and several mechanisms seem to be involved. First, peritoneal hypoxia was suggested as a mechanism since the effect increased with duration of pneumoperitoneum and with insufflation pressure, since similar effects were observed with helium pneumoperitoneum and since the addition of 2–4% of oxygen to both CO2 and helium pneumoperitoneum decreased adhesion formation (Molinas and Koninckx, 2000; Molinas et al., 2001). This was supported recently by the observations that the partial pressure of oxygen in the abdominal wall was reduced during a pneumoperitoneum with CO2 and with helium, whereas insufflation with a non-hypoxic gas mixture (80% CO2 and 20% O2) induced no changes (Wildbrett et al., 2003). This pneumoperitoneum-induced hypoxia was also supported by the fact that this effect was absent in mice deficient for hypoxia-inducible
factor (HIF) (Molinas et al., 2003b), plasminogen activator 1 (PAI-1) (Molinas et al., 2003a), vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) (Molinas et al., 2003c). Secondly, a role for reactive oxygen species (ROS) in adhesion formation has been suggested (Binda et al., 2003) since ROS activity increases during both laparotomy and laparoscopy, since they are produced during the ischaemia–reperfusion process and since the administration of ROS scavengers decreases adhesion formation in several animal models. Thirdly, other mechanisms could be involved such as cooling and desiccation.

During pneumoperitoneum, desiccation and cooling are intimately linked. First, desiccation is a key factor in cooling since at 37°C (body temperature), 577 cal are needed to vaporize 1 g of water, in contrast to only 1 cal needed to cool 1 g of water by 1°C and 0.2 cal to cool 1 g of CO2 by 1°C. Secondly, desiccation will be more important at higher gas temperature since absolute humidity increases with temperature, e.g. relative humidity of 100% corresponds to 25 mg of water/l of gas at 25°C and to 44 mg of water/l of gas at 37°C. Therefore, in desiccation experiments to study adhesion formation, some cooling will always be evident unless temperature is strictly controlled.

Since the exact roles of desiccation and cooling in peritoneal damage and in adhesion formation have not yet been studied in detail, we therefore planned to evaluate in our laparoscopic mouse model the specific effect of cooling during CO2 pneumoperitoneum upon adhesion formation.

Material and methods
The experimental set-up, i.e. animals, anaesthesia and ventilation, laparoscopic surgery, induction of intraperitoneal adhesions and scoring of adhesions, has been described in detail previously (Molinas et al., 2001, 2003a,b,c; Elkelani et al., 2002).

Animals
The study was performed in 86 female, Naval Medical Research Institute (NMRI), 9- to 10-week-old mice weighing 30–40 g. The animals were kept under standard laboratory conditions and they were fed with a standard laboratory diet with free access to food and water at any time. The study was approved by the Institutional Review Animal Care Committee.

Anaesthesia and ventilation
Animals were anaesthetized with i.p. pentobarbital (Nembutal, Sanofi Sante Animale, Brussels, Belgium) with a dose of 0.08 mg/g. The abdomen was shaved and the animal was secured to the table in the supine position. Animals were intubated with a 20-gauge catheter and ventilated with a mechanical ventilator (Mouse Ventilator MiniVent, Type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) using non-humidified or humidified room air (according to the experiment) with a tidal volume of 250 μl at 160 strokes/min.

Laparoscopic surgery
A midline incision was performed caudal to the xiphoides appendix, a 2 mm endoscope with a 3.3 mm external sheath for insufflation (Karl Storz, Tüttlingen, Germany) was introduced into the abdominal cavity and the incision was closed gas tight around the endoscope in order to avoid leakage.

The pneumoperitoneum was created using the Thermodrator Plus (Karl Storz, Tüttlingen, Germany), which permits addition of a variable concentration of O2 to the CO2. Insufflation gas, humidification and temperature varied with the experimental design. For humidification, the Storz Humidifier 204320 33 (Karl Storz, Tüttlingen, Germany) was used.

Induction of intraperitoneal adhesions
After the establishment of the pneumoperitoneum, two 14-gauge catheters (Insyte-W, Vialon, Becton Dickinson, Madrid, Spain) were inserted under laparoscopic vision. Standardized 10 mm × 1.6 mm lesions were performed in the antimesenteric border of both right and left uterine horns and in both the right and left pelvic side walls with monopolar coagulation (10 W, standard coagulation mode, Autocon 350, Karl Storz, Tüttlingen, Germany).

The pneumoperitoneum was maintained for the minimum time needed to perform the surgical lesions, standardized at 10 min or for 60 min (basal and pneumoperitoneum-enhanced adhesion, respectively).

Scoring of adhesions
During laparotomy 7 days after the induction of adhesions, the adhesions were scored blind (of the group being evaluated) under microscopic vision using a qualitative and a quantitative scoring system. The qualitative scoring system assessed: extent (0, no adhesions; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100% of the injured surface involved, respectively), type (0, no adhesions; 1, filmy; 2, dense; 3, capillaries present), tenacity (0, no adhesions; 1, easily fall apart; 2, require traction; 3, require sharp dissection) and total (extent + type + tenacity). The quantitative scoring system assessed the proportion of the lesions covered by adhesions using the following formula: (sum of the length of the individual attachments/length of the lesion) × 100. The results are presented as the average of the adhesions formed at the four individual sites (right and left visceral and parietal peritoneum), which were scored individually.

Environmental and animal temperatures
To control temperature, animals and equipment, i.e. insufflator, humidifier, water valve, ventilator and tubing, were placed either at 23–25°C (room temperature) or in a closed chamber maintained at 37°C (heated air, WarmTouch, Patient Warming System, model 5700, Mallinckrodt Medical, Hazelwood, MO). The insufflation gas temperature was determined by the environmental temperature, i.e. either at room temperature or at 37°C. Indeed, previous experiments showed equilibration of the gas temperature with environmental temperature after some 50 cm of tubing with 7 mm inner diameter and a flow rate of 2.5 l/min.

The temperature of the environment was measured with Testo 645 (Testo N.V./S.A., Lenzkirch, Germany), whereas the temperature of the animal was measured via the rectum with the Hewlett Packard 78353A device (Hewlett Packard, Böblingen, Germany) and recorded every 10–20 min.

Experimental design
The time of anaesthesia injection was considered time 0 (T0). The animal preparation and ventilation started after 10 min (T10). The pneumoperitoneum started at 20 min (T20) and was maintained for 10 min (T20–T30) or 60 min (T20–T80). Ventilation always finished at T80.
In experiment I (n = 32), basal and pneumoperitoneum-enhanced adhesion formation, together with body temperature, were evaluated in mice placed either at room temperature or at 37°C. Non-humidified CO₂ was used for the pneumoperitoneum and special care was taken to have a gas tight seal around the trocar in order to avoid any flow through the abdominal cavity and thus minimize desiccation. Ventilation was performed with non-humidified air (four groups, n = 8 per group).

In experiment II (n = 6), the effect of ventilation with or without humidified air upon body temperature was evaluated in mice placed at 37°C during 60 min of humidified CO₂ pneumoperitoneum (two groups, n = 3 per group).

In experiment III (n = 48), the effect of body temperature during humidified pneumoperitoneum upon adhesion formation was evaluated. To achieve a body temperature with minimal cooling, i.e. ~37°C, mice were placed at 37°C and ventilated with humidified air. To achieve a slightly lower body temperature, i.e. ~36°C, mice were placed at 37°C and ventilated with non-humidified air. To achieve a body temperature of some 32°C, mice were placed alternately at room temperature (T₀–T₂₀, T₃₀–T₄₀, T₅₀–T₆₀ and T₇₀–T₈₀) and at 37°C (T₂₀–T₃₀, T₄₀–T₅₀ and T₆₀–T₇₀) and ventilated with humidified air. These settings were determined based on previous experiments. Pneumoperitoneum-enhanced adhesion formation was evaluated using pure CO₂ in mice at 37°C (group I), 36°C (group II) and 32°C (group III). Pneumoperitoneum-enhanced adhesion formation at 37°C was also evaluated using CO₂ with 3% oxygen (group IV) and 12% oxygen (group V). Simultaneously, basal adhesion formation was evaluated using pure CO₂ (group VI). A flow of 23 ml/min through the abdominal cavity was used in all the groups (six groups, n = 8 per group).

Statistics
Statistical analyses were performed with GraphPad Prism version 4 (GraphPad Software Inc., San Diego CA). The Mann–Whitney test was used to compare adhesion formation between individual groups. Intergroup differences in body temperature were evaluated with two-way ANOVA. Linear regression and Pearson correlation were used to analyse adhesions and body temperature data. All data are presented as the mean ± SEM.

Results
In experiment I (Figure 1, Table I), during anaesthesia and ventilation only (T₀–T₂₀), body temperature decreased from some 36.5 to 31°C and from 37.5 to 35°C at room temperature and 37°C, respectively. At room temperature, body temperature further decreased to 28.5 and to 26.5°C at T₅₀ in mice with 10 and 60 min of pneumoperitoneum, respectively. At 37°C, body temperature remained constant at some 35.5 and 34.5°C up to T₈₀ for 10 and 60 min of pneumoperitoneum, respectively. Overall, body temperatures were always lower after 60 min of pneumoperitoneum than after 10 min, i.e. both at room temperature (P < 0.0001) and at 37°C (P = NS); body temperatures also were always lower at room temperature than at 37°C, i.e. after both 10 min (P < 0.0001) and 60 min (P < 0.0001) of pneumoperitoneum (two-way ANOVA). At room temperature, adhesion formation increased with the duration of pneumoperitoneum (proportion, P < 0.05). At 37°C, this effect of duration of pneumoperitoneum was more pronounced (proportion, P = 0.01; total, P = 0.04; extent, P = 0.02; type, P = 0.03). In addition, at 37°C, adhesion formation was higher than at room temperature, clearly for pneumoperitoneum-enhanced adhesions (proportion, P = 0.04; total, P < 0.05; extent, P = 0.03) and slightly for basal adhesions (P = NS) (Mann–Whitney test).

In experiment II, body temperatures were some 1°C higher when humidified air was used for ventilation (P = 0.003, two-way ANOVA), being 38.1 ± 0.1 (T₀), 36.4 ± 0.1 (T₁₀), 35.9 ± 0.3 (T₂₀), 36.2 ± 0.5 (T₃₀), 36.5 ± 0.6 (T₄₀), 36.5 ± 0.6 (T₅₀), 36.8 ± 0.5 (T₆₀), 37.0 ± 0.5 (T₇₀) and 37.1 ± 0.5 (T₈₀)°C for humidified ventilation, and 37.8 ± 0.4 °(T₀), 36.1 ± 0.1 (T₁₀), 35.0 ± 0.4 (T₂₀), 35.3 ± 0.5 (T₃₀), 35.4 ± 0.5 (T₄₀), 35.8 ± 0.7 (T₅₀), 35.7 ± 0.6 (T₆₀), 35.6 ± 0.5 (T₇₀) and 36.1 ± 0.5 (T₈₀)°C for non-humidified ventilation.

In experiment III (Figure 2), during anaesthesia and ventilation only, body temperature decreased to 35.5°C for groups I and II and to 31°C for group III at T₂₀. Afterwards, body temperature remained constant till T₅₀ at some 37°C for
group I, 36°C for group II and 32.5°C for group III (group I versus III, \( P < 0.0001 \); II versus III, \( P < 0.0001 \); and I versus II, \( P = 0.02 \)). Body temperatures of groups IV, V and VI were similar to those of group I (\( P = \text{NS} \), data not shown, two-way ANOVA). Adhesion formation after 60 min of pure CO\(_2\) pneumoperitoneum (groups I, II and III) decreases with body temperature (proportion, \( P = 0.02 \) Pearson correlation, Figure 2, Table II). In mice with body temperature of 37°C (Figure 3, Table II), adhesion formation increased with the duration of pneumoperitoneum (proportion, \( P = 0.04 \); total, \( P = 0.02 \); extent, \( P = 0.04 \); type, \( P = \text{NS} \); tenacity, \( P = 0.04 \)). In comparison with pure CO\(_2\) (group I), the addition of 3% oxygen to the pneumoperitoneum (group IV) decreased adhesion formation (proportion, \( P = 0.03 \); total, \( P = 0.04 \); extent, \( P < 0.05 \); type, \( P = \text{NS} \); tenacity, \( P = \text{NS} \)), whereas no differences were observed with the addition of 12% oxygen (group V).

To evaluate the effect of body temperature upon adhesion formation, data of experiments I and III were combined (Figure 4). Taking all data together, pneumoperitoneum-enhanced adhesion formation decreased with lower body temperatures (proportion, \( P = 0.004 \); total, \( P = 0.02 \), linear regression and Pearson correlation).

Discussion
This study confirmed and extended previous data concerning the effects of anaesthesia, ventilation and pneumoperitoneum upon body temperature.

We confirmed that anaesthesia decreases the body temperature of mice, as demonstrated previously in rats (Torbati et al., 2000), mice (Gardner et al., 1995) and humans (Buhre and Rossaint, 2003). As expected, this cooling effect is more pronounced at room temperature than at 37°C. Patients remain normothermic after anaesthesia when they are kept in a warmer operating room, whereas they become hypothermic in a colder operating theatre (Morris and Wilkey, 1970; Morris, 1971a,b). This pure anaesthetic side effect is caused by cutaneous vasodilatation, which abolishes heat conservation. Consequently, anaesthetized subjects become poikilothermic and their body temperature varies with environmental temperatures (Morris, 1971b).

This study demonstrated in mice that non-humidified ventilation can decrease body temperature, confirming previous data in humans (Dery, 1973; Fonkalsrud et al., 1980; Bissonnette and Sessler, 1989). Since unsaturated air will absorb water by evaporation from a wet surface (Williams et al., 1996), water loss from the respiratory airways is the most plausible explanation.

We confirmed that pneumoperitoneum causes cooling. Since non-humidified gas at higher flow rates causes important cooling due to desiccation, we took great care to prevent desiccation as much as possible by avoiding any flow through the abdomen (experiment I) and by humidifying the gas (experiments II and III). It is difficult, however, to rule out completely at least some desiccation by the pneumoperitoneum since gas-tight seals of the trocar insertions are

![Figures and tables](attachment://figures.png)

Table I. Effect of environmental temperature upon adhesion formation in mice

<table>
<thead>
<tr>
<th>Environmental temperature</th>
<th>Pneumoperitoneum Oxygen</th>
<th>Duration</th>
<th>Adhesion scores (mean ± SE)</th>
<th>Extent</th>
<th>Type</th>
<th>Tenacity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>0%</td>
<td>10 min</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>60 min</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>2.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td>0%</td>
<td>10 min</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>60 min</td>
<td>1.8 ± 0.2 a,b</td>
<td>1.5 ± 0.2 a</td>
<td>1.3 ± 0.2</td>
<td>4.5 ± 0.6 a,b</td>
<td></td>
</tr>
</tbody>
</table>

Adhesions were induced during laparoscopy at 20 cm H\(_2\)O insufflation pressure.

a 10 versus 60 min at room temperature (25°C) or 37°C, \( P < 0.05 \).

b Room temperature versus 37°C, 10 or 60 min \( P < 0.05 \), Mann–Whitney test.
between 37 and 60°C decreases with temperature. Hypothermia decreases the pneumoperitoneum-induced hypoxia, since oxygen consumption by cells directly protect tissues and cells from the pneumoperitoneum as the driving mechanism. First, hypothermia could et al. indicated.

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peristalsis, thus decreasing adhesion formation (Gataullin et al., 1988). These observations support our hypoxia hypothesis as the driving mechanism. First, hypothermia could directly protect tissues and cells from the pneumoperitoneum-induced hypoxia, since oxygen consumption by cells decreases with temperature. Hypothermia decreases the energetic parameters during reperfusion (Erecinska et al., 2003), endothelium (Zar and Lancaster, 2000) and muscle (Yoshioka et al., 2003), heart (Prasad et al., 1992), gut (Attuwaybi et al., 2003), endothelium (Zar and Lancaster, 2000) and muscle (Yoshioka et al., 1992). Hypothermia improves recovery of energetic parameters during reperfusion (Erecinska et al., 2003). Hypothermia also suppresses the inflammatory response after hepatic ischaemia–reperfusion, decreasing the infiltration of polymorphonuclear cells (Patel et al., 2000), and the production of tumour necrosis factor-α, interleukin-1β and macrophage inflammatory protein-2 also decreases (Patel et al., 2000; Kato et al., 2002).

The relationship between desiccation and cooling is complex, since desiccation causes cooling whereas desiccation is more important at higher gas temperature. This might explain why the effect upon adhesion formation can be variable if both desiccation and cooling are not strictly controlled. Therefore, experiments evaluating the effect of desiccation
might underestimate the effect since desiccation decreases temperature which itself reduces desiccation.

We confirmed and extended to 37°C our previous observations at room temperature which showed that the addition of 3% oxygen to the pneumoperitoneum decreased pneumoperitoneum-enhanced adhesion formation, and that the addition of 12% oxygen in comparison with 3% oxygen increases adhesion formation to a similar level as with pure CO₂ (Molinas and Koninckx, 2000; Molinas et al., 2001).

In summary, this study confirms that environmental temperature, anaesthesia, ventilation and pneumoperitoneum all influence the body temperature of mice. The most important observation is that hypothermia decreases pneumoperitoneum-enhanced adhesion formation. This could be due to prevention of the toxic effects caused by hypoxia and/or the ischaemia–reperfusion process. Other effects, i.e. reduction of the inflammatory response and an increase of intestinal peristaltic movements, cannot be excluded. Whether basal adhesions, i.e. without a pneumoperitoneum, are also decreased by lower temperatures is still uncertain since, in our model, the group of 'basal adhesions' still had 10 min of pneumoperitoneum-enhanced adhesion formation. This could be due to prevention of the toxic effects caused by hypoxia and/or the ischaemia–reperfusion process. Whether basal adhesions, i.e. without a pneumoperitoneum, are also decreased by lower temperatures is still uncertain since, in our model, the group of 'basal adhesions' still had 10 min of pneumoperitoneum-enhanced adhesion formation. This could be due to prevention of the toxic effects caused by hypoxia and/or the ischaemia–reperfusion process.

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