Effect of desiccation and temperature during laparoscopy on adhesion formation in mice

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Objective: To investigate the effects of desiccation (without cooling) and of oversaturation of the pneumoperitoneum on adhesion formation.
Design: Prospective randomized trial.
Setting: Academic research center.
Animal(s): BALB/c and NMRI female mice.
Intervention(s): The effect of desiccation using nonhumidified CO_2 on adhesion formation was evaluated in a laparoscopic mouse model. Body temperature (BT) was maintained at 37°C using a homeothermic blanket. In addition to controls without desiccation, the effect of both hypothermia and desiccation on adhesion formation was evaluated. Subsequently the effect of oversaturating the pneumoperitoneum using a high energy gas to avoid any desiccation was studied.
Main Outcome Measure(s): During surgery BT, pneumoperitoneum temperature, and relative humidity were monitored. Adhesions were scored after 7 days.
Result(s): Adhesions increased with increasing levels of desiccation when BT was kept at 37°C. This was prevented with humidified gas. If BT decreased, adhesions were fewer. Oversaturating the pneumoperitoneum increased adhesions due to high energy gas causing an increase in both BT and pneumoperitoneum temperature.
Conclusion(s): Adhesions increase with desiccation and decrease when BT is reduced. Adhesions are minimized when humidified gas is used. Since desiccation is associated with cooling, its effect is generally underestimated because of the counterbalance with cooling. The concept of combining controlled intraperitoneal cooling with a rigorous prevention of desiccation might be important for clinical adhesion prevention. (Fertil Steril® 2006;86: 166–75. ©2006 by American Society for Reproductive Medicine.)

Key Words: Body temperature, desiccation, hypothermia, hypoxia, intraperitoneal adhesion formation, laparoscopy, pneumoperitoneum, humidification

The CO_2 pneumoperitoneum has become known as a cofactor in postoperative adhesion formation (1) and several mechanisms seem to be involved. First, peritoneal hypoxia was suggested as a mechanism, as adhesion formation increased with insufflation pressure and with duration of pneumoperitoneum, as similar effects were observed with CO_2 and helium pneumoperitoneum, and as the addition of 2%–4% of oxygen to both CO_2 and helium pneumoperitoneum decreased adhesion formation (2–4). This hypothesis was supported by the observation that the partial pressure of oxygen in the abdominal wall is reduced during CO_2 or helium pneumoperitoneum (5). In addition, pneumoperitoneum-enhanced adhesion formation was absent in mice deficient for genes encoding for factors upregulated by hypoxia, such as hypoxia inducible factors (6), vascular endothelial growth factor and placental growth factor (7), and plasminogen activator 1 (8).

Second, the pneumoperitoneum induces ischemia at the time of insufflation and reperfusion at the time of deflation. Pneumoperitoneum-enhanced adhesion formation thus could be the consequence of an ischemia–reperfusion process with a role of reactive oxygen species (ROS) (9). This ischemia–reperfusion hypothesis is supported by a reduced adhesion formation after the administration of ROS scavengers in several animal models (10–15).

A third mechanism is peritoneal temperature. We recently demonstrated that adhesion formation is less when body temperature is lower (16). This indirectly supports the previous hypotheses—hypothermia decreases the toxic effects of hypoxia and of the ischemia–reperfusion process, suppressing the inflammatory response (17–25).

Finally, desiccation has been claimed to enhance adhesion formation, although clear experimental evidence is lacking. Dry and cold gas for the pneumoperitoneum not only induces desiccation (26), but also is deleterious for the peritoneum,

Received January 18, 2005; revised and accepted November 29, 2005.
Supported by Onderzoeks Toelagen Katholieke Universiteit Leuven (Leuven, Belgium grant TBA/00/27 and in part by Karl Storz Endoscopy (Tuttlingen, Germany).
Part of these results were presented at the 13th Annual Congress of the European Society for Gynecological Endoscopy, which was held in Cagliari, Sardinia, Italy, on October 14–17, 2004, and was awarded with the "R. Palmer Prize" for the best oral presentation.
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0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2005.11.079

altering the morphology of the mesothelium, destroying the microvilli, and bulging up the cells with exposure of the basal lamina (27–30).

Desiccation in the abdominal cavity will inevitably occur whenever the gas entering the peritoneal cavity is not fully saturated at the intraperitoneal temperature, normally 37°C. The peritoneum has a large surface with a thin serous fluid layer facilitating humidification of the pneumoperitoneum gas. Desiccation can be locally aggravated by a jet stream of CO_2 forcing tissue surfaces apart and exposing directly the tissue surfaces to this stream of gas (26).

Desiccation requires high amounts of energy and thus is associated with cooling. Quantitatively, 577 cal is needed to vaporize 1 mL of water at 37°C, whereas only 0.00003 cal is needed to heat 1 mL of CO_2 by 1°C (31). The caloric equivalent of heating cold dry gas is thus very small in comparison with the effect of vaporization. This cooling effect of desiccation in the airways during ventilation (16, 32–34) and in the abdomen during both open (35) and laparoscopic (36) surgery has been well documented. As expected, the cooling observed during laparoscopy with cold and dry gas can be fully prevented using warm and humidified gas (27, 28, 36) but not warm and dry gas (27, 37). Desiccation quantitatively depends on the volume of gas to be humidified, and thus increases tremendously when a continual supply of gas through the abdominal cavity occurs (e.g., due to leaks).

The loss of water content from the serous fluid, moreover, increases the osmolarity of the fluid, causing an osmotic imbalance between the intracellular and the extracellular space of the mesothelial cells. This then causes fluid of the intracellular space to diffuse through the cell membrane to equalize the osmotic imbalance. This mechanism then dehydrates the cell, leading to desiccation and trauma of the cell (27–30), resulting in a peak inflammatory response (38, 39).

Desiccation and cooling, two intimately linked processes, have opposite effects on adhesion formation, the former increasing (widely accepted but not proven) and the latter decreasing (16) adhesions. Therefore, the aim of this study was, first, to confirm that desiccation increases adhesion formation and to quantify this effect when the associated cooling was prevented. Second, the effect of avoiding completely desiccation by insufflating oversaturated gas turned out to be predominantly an experiment of increasing the intra-abdominal temperature due to the condensation.

MATERIALS AND METHODS

The Laparoscopic Mouse Model for Adhesion Formation

Experimental setup, that is, animals, anesthesia and ventilation, laparoscopic surgery, induction, and scoring of intraperitoneal adhesions (Fig. 1), has been described in detail previously (3, 4, 6–8, 16, 40).

Animals In the oversaturation experiment, 10-week-old female Naval Medical Research Institute (NMRI) mice weighing 25–35 g were used as in previous experiments. In the desiccation experiment, 10-week-old female BALB/c mice weighing 19–21 g were used. After it had become clear that the interanimal variability was much less in this inbred strain, whereas the adhesion formation was similar than in NMRI mice (41), we decided to use this strain for further experiments.

Animals were kept under standard laboratory conditions and they were fed with a standard laboratory diet with free access to food and water. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia and Ventilation Mice were anesthetized with intraperitoneal (IP) 0.08 mg/g pentobarbital, intubated with a 20-gauge catheter and mechanically ventilated (Mouse Ventilator MiniVent, type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) using humidified room air with a tidal volume of 250 μL at 160 strokes/min. Humidified air for ventilation was used to prevent cooling, as occurs during ventilation with nonhumidified air (16).

Laparoscopic Surgery A midline incision was performed caudal to the xyphoides, 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 to avoid leakage.

The pneumoperitoneum was created with the Thermoflator Plus (Karl Storz) using humidified or nonhumidified insufflation gas.

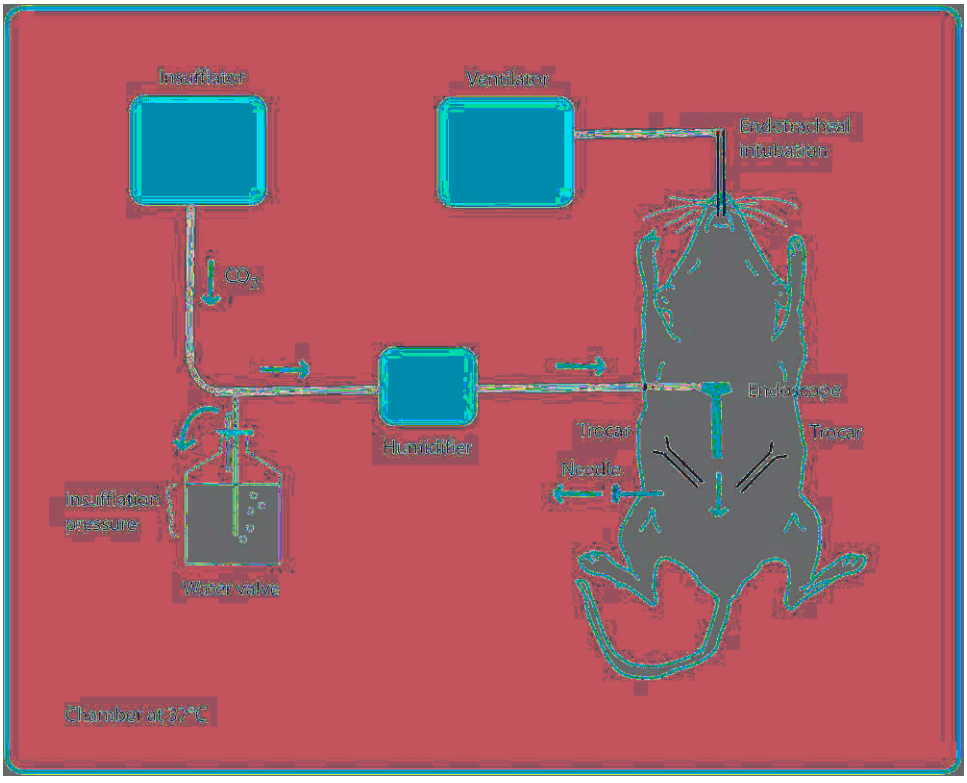
Induction of Intraperitoneal Adhesions After the establishment of the pneumoperitoneum, two 14-gauge catheters were inserted under laparoscopic vision. Standardized 10- by 1.6-mm lesions were performed in the antimesenteric border of both right and left uterine horns and pelvic sidewalls with bipolar coagulation (BICAP, bipolar hemostasis probe, BP-5200A, 5 Fr, 200 cm; IMMED Benelux, Linkebeek, Belgium) at 20 W (standard coagulation mode, Autocon 200, Karl Storz).

Because previous data indicate that adhesion formation increases with the duration of the pneumoperitoneum (3), pneumoperitoneum-enhanced adhesion formation was evaluated by maintaining the pneumoperitoneum for 60 minutes.

Scoring of Adhesions Adhesions were qualitatively and quantitatively scored, blindly (the investigator was not informed of the group being evaluated) under microscopic vision during laparotomy 7 days after their induction. The qualitative scoring system assessed as follows: 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), 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: adhesion (%) =

FIGURE 1

Laparoscopic mouse model.



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(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 sites (right and left visceral and parietal peritoneum), which were individually scored.

Setup and Design of the Experiments

Environmental Temperature To control animal and gas temperature, animals and equipment (i.e., insufflator, humidifier, water valve, ventilator, and tubing) were placed in a closed chamber maintained at 37°C with heated air (WarmTouch, Patient Warming System, model 5700, Mallinckrodt Medical, Hazelwood, MO).

Body and Pneumoperitoneum Temperature and Pneumoperitoneum Relative Humidity Animal body temperature was continuously monitored in the rectum (Hewlett Packard 78353A, Hewlett Packard, Böblingen, Germany) and registered every 10 minutes. Pneumoperitoneum temperature and relative humidity (RH) were measured with the Testo 645 device and a 4-mm probe (Testo N.V./S.A., Lenzkirch, Germany) introduced in the abdomen. Due to the size of this probe, measurements were not done systematically in the same experiments performed to induce adhesions.

Because desiccation or vaporization requires 577 cal/mL of water and thus produces cooling, the mice could not maintain their body temperature at 37°C during desiccation experiments, notwithstanding the box heated to 37°C. Therefore, to evaluate the pure effect of desiccation without cooling, keeping mouse body temperature at 37°C, an additional heating system had to be used (i.e., the homeothermic Blanket System; Harvard Apparatus LTD, Edenbridge, UK). This system includes a small rectal probe for continuous temperature monitoring and a heating blanket to provide sufficient heat for accurate control of mouse body temperature, both connected to a control unit. The control unit varies the current flowing through the heating blanket in an inversely proportional manner to the temperature monitored by the temperature probe.

Desiccation and Humidification of Pneumoperitoneum To induce desiccation, a controlled flow of nonhumidified CO_2 was obtained using 26- and 22-gauge needles, which at 15 mm Hg insufflation pressure induced a 23- or 100-mL/min flow of CO_2 gas through the abdominal cavity, respectively. Without a needle, in the absence of any leak, no flow through the abdominal cavity occurred.

To humidify the insufflated gas two types of humidifiers were used. For the desiccation experiment, the Storz Humidifier (204320 33, Karl Storz) and the 37°C chamber were used, in which CO_2 at 37°C and nearly 100% RH can be

obtained (this was measured in pilot studies). For the oversaturation experiment, the insufflation humidifier MR860 (Fisher & Paykel Healthcare Ltd, Auckland, New Zealand) was used to avoid any desiccation by oversaturation. This newly developed humidifier permits "oversaturation" of the CO_2, with some condensation in the peritoneal cavity. By varying the temperature in the humidification chamber, discrete levels of absolute humidity can be obtained (42). To prevent condensation between the humidifier and the animal or trocar, the tubing heats the CO_2 gas temperature above the dew point of the gas, using an internal heating wire. With entrance into the peritoneal cavity, the CO_2 will cool to 37°C, and if the absolute humidity is above 44 mg/L condensation will occur. In the oversaturation experiment, the humidifier was used at discrete levels of humidification, which, expressed relative to body temperature saturated (BTS) conditions (37°C, 100% RH, i.e., 44 mg water/L CO_2), corresponded to 0%, 75% (33 mg water/L), 100% (44 mg water/L), and 125% (55 mg water/L) BTS. For the dry group or 0% BTS, the same humidifier was used but the humidification chamber was not filled with water.

Experimental Design Because anesthesia and ventilation can influence body temperature, the timing was strictly controlled. The time of the anesthesia injection was considered time 0 (T_0). The animal preparation and ventilation started after exactly 10 minutes (T_{10}). The pneumoperitoneum started at 20 minutes (T_{20}) and was maintained for 60 minutes until T_{80}.

Two sets of experiments were performed. Historically, the oversaturation experiment was done first and later the desiccation experiment. Because it is easier and more logical to present the desiccation experiment first and subsequently the oversaturation experiment, we deliberately chose to describe throughout the article, first, the effect of desiccation without cooling and subsequently the effect of oversaturating the insufflation gas. In each experiment the measurement of temperature and humidification and the evaluation of adhesion formation were done in different mice to avoid any influence of the temperature and humidification measurements on adhesion formation.

In the desiccation experiment, desiccation was induced using nonhumidified CO_2 for the pneumoperitoneum at flows of 23 mL/min (group II) and 100 mL/min (group III) through the abdominal cavity. Two control groups with minimal desiccation were used: the first with no flow of nonhumidified gas (group I) and the second with a flow of 100 mL/min of humidified gas (group IV). Because desiccation decreases body temperature, a homeothermic blanket was used to keep body temperature strictly at 37°C. As a control for the effect of the homeothermic blanket on temperature and adhesion formation, a group of animals was treated with a flow of 100 mL/min of nonhumidified gas and without the homeothermic blanket (group V).

In the desiccation experiment, first body temperature, pneumoperitoneum temperature, and RH were measured, and the difference between peritoneum and body temperatures (δT = peritoneum − body temperature) was calculated (5 groups, n = 3/group). Subsequently, the effect of desiccation, without the associated decrease in body temperature, was evaluated on adhesion formation (5 groups, n = 56). A total of nine animals per group was planned. In group I, however, intended to have no flow through the abdominal cavity, an important leakage around the port sites occurred in four animals and this resulted in a dry abdominal wall and hypothermia, despite of the use of the homeothermic blanket. Because the degree of desiccation could not be estimated, these mice were immediately replaced during the experiments without changing the randomization order to have the required number of animals with temperature at 37°C. Also in groups II and III, a leakage occurred in two and five mice, respectively, and these mice could not maintain their body temperature at 37°C notwithstanding the homeostatic blanket. These mice also were replaced during the experiment without changing the randomization order, as the aim of this study was to maintain body temperature.

In the oversaturation experiment, the effect of oversaturating the CO_2 with some condensation (to avoid any desiccation) was analyzed. First, body temperature, pneumoperitoneum temperature, and RH were evaluated using nonhumidified CO_2 (group I), and humidified CO_2 corresponding to 75% (group II), 100% (group III), and 125% BTS (group IV), respectively (4 groups, n = 3 per group). Subsequently, the effect of oversaturating the CO_2 on adhesion formation was evaluated using the same discrete levels of humidification (4 groups, n = 10 per group).

Statistics

Statistical analyses were performed with the SAS System (SAS Institute, Cary, NC) and the GraphPad Prism (GraphPad Software Inc., San Diego, CA). Differences in body temperature were evaluated with two-way ANOVA. Differences between pneumoperitoneum and body temperatures were evaluated with Proc Univariate. Differences in adhesion formation were evaluated with Wilcoxon test for the univariate analysis and with General Linear Methods (proc GLM) for the multivariate analysis to evaluate simultaneously the effect of flow and body temperature. All data are presented as the mean ± standard error of the mean (SE).

RESULTS

In the desiccation experiment, the heating blanket kept body temperature constant at 37.5°C in groups I, II, and III throughout the experiment (between T_{20} and T_{80}) without intergroup differences (data not shown). In group IV body temperature increased up to 39°C and was higher than in groups I ($P<.0001$), II ($P<.0001$), and III ($P<.0001$). In group V body temperature decreased progressively to 31°C and was lower than in groups I ($P<.0001$), II ($P<.0001$), III ($P<.0001$), and IV ($P<.0001$) (two-way ANOVA).

The differences between peritoneum and body temperatures (δT) measured after an equilibration period (T_{40}) were not significant (Proc Univariate) except for group IV ($P=.03$), being 0.2; ± 0.1°C, −0.5 ± 0.3°C, −0.6 ± 0.4°C, 0.6 ± 0.1°C, and 0.5 ± 0.2°C for groups I, II, III, IV, and V, respectively. The RH of the pneumoperitoneum remained 100% in all groups throughout the experiment, also when nonhumidified CO_2 was used for insufflation reflecting the high humidification capacity of the peritoneal cavity up to the end of the experiment (data not shown).

In the desiccation experiment, adhesion formation was first evaluated in the mice that maintained their body temperature at 37°C (n = 9 per group). Desiccation without affecting body temperature increased adhesion formation (Fig. 2, Table 1). In comparison with group I, adhesion formation increased slightly in group II (P = not significant [NS]) and significantly in group III (proportion: $P=.01$, total: $P=.01$, extent: $P=.02$, type: $P=.04$, tenacity: $P=.05$, Wilcoxon test). As expected, this increase in adhesion formation was prevented by using humidified gas (group IV vs. III, proportion: $P=.004$, total: $P=.01$, extent: $P=.01$, type: $P=.01$, tenacity: $P=.01$). Hypothermia decreased adhesion formation caused by desiccation (group V vs. group III, proportion: $P=.01$, total: $P=.01$, extent: $P=.01$, type: $P=.02$, tenacity: $P=.04$), although not completely up to the level of the group with no desiccation (group I), possibly a consequence of the slightly higher temperature. Unexpectedly, comparing with group IV adhesion formation was lower than group I (proportion: $P=.04$, total: $P=.02$, extent: $P=.03$, tenacity: $P=.02$) notwithstanding the higher peritoneal temperature, suggesting that also in group I desiccation occurred in some animals due to leaks around the ports. No adhesions were found in the animals either in laparoscopic ports or in the nonoperative sites.

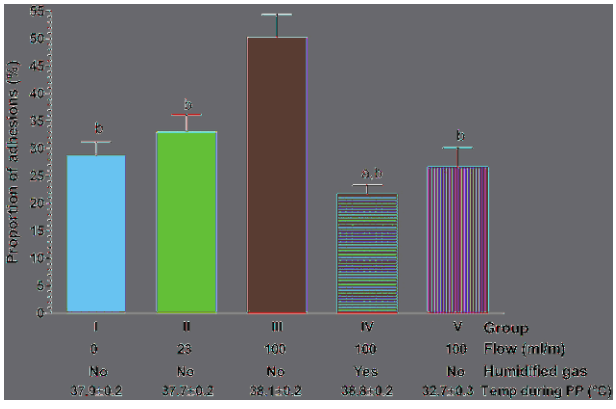
If all animals treated with nonhumidified CO_2, including those that were unable to maintain their body temperature at 37°C, were analyzed together (proc GLM; four groups, i.e., I, II, III, and V; two variables, i.e., desiccation [reflected by flow through the peritoneal cavity] and mean of body temperature), adhesions increased with desiccation (proportion: $P<.0001$; total: $P=.005$; extent: $P=.001$) and decreased with lower body temperature (proportion: $P<.0001$; total: $P=.0005$; extent: $P<.0001$; type: $P=.02$; tenacity: $P=.03$; Fig. 3). If only mice with body temperature close to 37°C were analyzed simultaneously (proc GLM; three groups; two variables, i.e., desiccation and temperature), adhesions increased with desiccation (proportion: $P<.0001$; total: $P=.001$; extent: $P=.001$; type: $P=.01$; tenacity: $P=.03$; Fig. 2) and, obviously, the effect of the minor differences of temperature around 37°C was not significant.

In the oversaturation experiment, as observed previously, that is, without the heating blanket (16), body temperature decreased from 37.5°C at T_0 to 35°C at T_{20}—the period before pneumoperitoneum was started. After this, body temperature further decreased to 33°C when nonhumidified CO_2 was used (group I). When humidified CO_2 was used temperature increased progressively to 36, 36.5, and 37°C in mice of group II (75% BTS), III (100% BTS), and IV (125% BTS), respectively (Fig. 4A). By ANOVA, body temperature between T_{20} and T_{80} was lower in mice of group I than in mice of groups II ($P<.0001$), III ($P<.0001$), and IV ($P<.0001$). Body temperature was also lower in mice of group II than in mice of groups III ($P=.02$) and IV ($P=.04$). Differences between groups III and IV were not significant (P=NS).

The pneumoperitoneum temperature in mice of group I was initially (T_{20}) almost identical to the body temperature at 35°C (Fig. 4B). Thereafter, the pneumoperitoneum temperature decreased slowly to 34.5°C, corresponding to the progressively decreasing body temperature. In the mice with humidified CO_2, pneumoperitoneum temperatures were higher around 37°C and increased slowly thereafter to 37.8°C, especially in group IV, reflecting the increase in body temperature (Fig. 4A). By ANOVA, pneumoperitoneum temperature was lower in mice of group I than in mice of groups II ($P<.0001$), III ($P<.0001$), and IV ($P<.0001$). It was also lower in mice of group II than in mice of groups III

FIGURE 2

Effect of desiccation and hypothermia during pneumoperitoneum on adhesion formation. Adhesions were induced during laparoscopy with 60 minutes of CO_2 pneumoperitoneum at 20 cm of water and quantitatively scored after 7 days during laparotomy. Nonhumidified gas at flows of 0 mL/min (group I), 23 mL/min (group II), 100 mL/min (groups III and V), and humidified gas at a flow of 100 mL/min (group IV) through the abdominal cavity were used. Mice were covered (groups I–IV) or not (group V) with a homeothermic blanket to ascertain body temperature within normal limits. Mean ± SE of body temperature during T_{20}–T_{80} is indicated. [a]P vs. group I <.05, [b]P vs. group III <.05 (Wilcoxon test).



Binda. Adhesions, temperature, and desiccation. Fertil Steril 2006.

TABLE 1

Effect of desiccation and hypothermia during pneumoperitoneum on adhesion formation.

Group	Pneumoperitoneum: Flow (mL/min)	Pneumoperitoneum: Humidified gas	Body Temp[c] (°C)	Adhesion scores (mean ± SE): Extent	Type	Tenacity	Total
I	0	No	37.9 ± 0.2	1.5 ± 0.1[b]	1.3 ± 0.1[b]	1.6 ± 0.1[b]	4.4 ± 0.2[b]
II	23	No	37.7 ± 0.2	1.7 ± 0.1[b]	1.3 ± 0.1	1.5 ± 0.1[b]	4.5 ± 0.3[b]
III	100	No	38.1 ± 0.2	2.3 ± 0.2	1.6 ± 0.1	1.9 ± 0.1	5.8 ± 0.4
IV	100	Yes	38.8 ± 0.2	1.1 ± 0.1[a,b]	0.9 ± 0.1[b]	1.3 ± 0.1[a,b]	3.3 ± 0.3[a,b]
V	100	No	32.7 ± 0.3	1.4 ± 0.1[b]	1.1 ± 0.1[b]	1.5 ± 0.1[b]	4.0 ± 0.3[b]

Note: Adhesions were induced during laparoscopy with 60 minutes of CO_2 pneumoperitoneum at 20 cm of H_2O and qualitatively scored after 7 days during laparotomy.

[a] *P* vs. group I <.05; [b] *P* vs. group III <.05.

[c] Mean of body temperature during T_{20}–T_{80} is indicated.

Binda. Adhesions, temperature, and desiccation. Fertil Steril 2006.

(*P*=.04) and IV (*P*=.004) and lower in mice of group III than in mice of group IV (*P*<.0001).

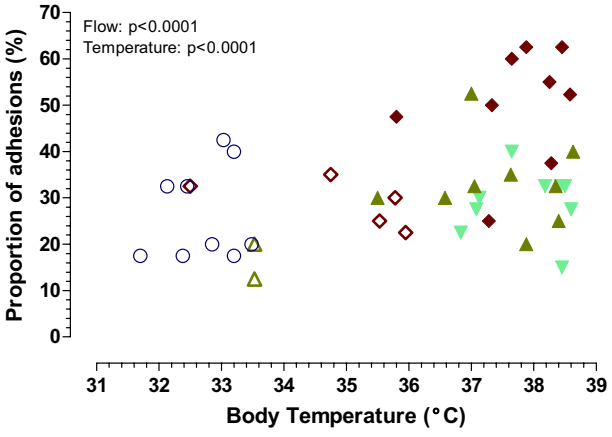
Peritoneum temperature was higher than body temperature (δT) after an equilibration period (T_{40}) (*P*<.05 for each group, Proc Univariate), being 1.4 ± 0.1°C, 1.2 ± 0.1°C, 1.4 ± 0.1°C, and 0.7 ± 0.1°C for groups II, III, IV, and I, respectively. The δTs remained constant up to T_{80}, being 1.3 ± 0.1°C, 1.0 ±0.1°C, 1.3 ±0.2°C, and 1.0 ± 0.2°C for groups II, III, IV, and I, respectively (*P*<.05 for each group, Proc Univariate).

The RH of the pneumoperitoneum remained 100% in all groups throughout the experiment, except for mice of group I. In this group RH of the pneumoperitoneum was initially (at T_{20}) 82.9% ± 1.9%, and decreased slightly thereafter to 80.8% ± 4.2%, reflecting the slightly lower humidification capacity of the peritoneum at lower temperatures (data not shown).

In the oversaturation experiment (Fig. 5, Table 2), adhesion formation in group I (important desiccation and much lower temperatures) was higher than in groups II (proportion: *P*=.02, total: *P*<.01, extent: *P*=.02, type: *P*<.01, tenacity: *P*<.01) and III (proportion: *P*=.05, total: *P*=.05), but not different from group IV (Wilcoxon). In group III, adhesion formation was lower than in group IV (proportion: *P*=.03, extent: *P*=.02). Adhesion formation in group II (slight desiccation and slightly lower temperatures) was not different from group III but lower than group IV (proportion: *P*<.01, total: *P*<.01, extent: *P*<.01, type: *P*<.01, tenacity: *P*=.03).

FIGURE 3

Relationship between adhesion formation and body temperature with different levels of desiccation. Individual values of mean of body temperature between T_{20} and T_{80} with their respective proportion of adhesions are depictured for pneumoperitoneum-enhanced adhesion for groups I (▼), II (▲), II with low temperature (△), III (♦), III with low temperature (◇), and V (○). Effect of flow: *P*<.0001, effect of temperature: *P*<.0001 (ProcGLM).



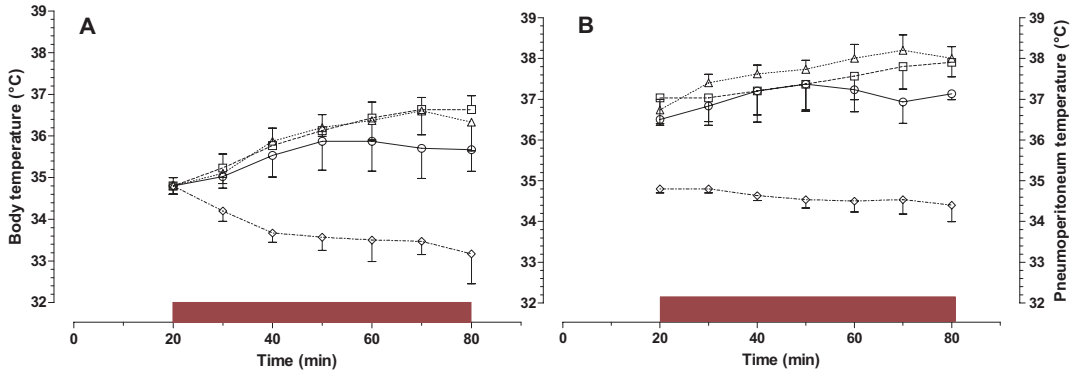
Binda. Adhesions, temperature, and desiccation. Fertil Steril 2006.

DISCUSSION

The peritoneal cavity has a high humidifying capacity, as in this study in all groups with nonhumidified gas (0% RH) the RH of the pneumoperitoneum was 100% (desiccation experiment) and 80.8% ± 4.2 % (oversaturation experiment), meaning that water content from the serous fluid was continuously being evaporated to humidify the pneumoperitoneum. This then leads to tissue dehydration and desiccation. This corresponds to a water loss from the peritoneum of 1 and 4.4 mg water/min for groups with a flow of 23 and 100 mL/min, respectively, and theoretically, no water loss for the

FIGURE 4

Effect of CO_2 pneumoperitoneum with discrete levels of humidification, expressed in relation to BTS conditions (37°C, 100% RH) on body (**A**) and pneumoperitoneum (**B**) temperature. Nonhumidified gas (group I) and humidified gas at 75% BTS (group II), 100% BTS (group III), and 125% BTS (group IV) conditions and a flow of 23 mL/min through the abdominal cavity were used. Symbols: group I (◇), group II (○), group III (□), and group IV (△); pneumoperitoneum (*shaded bar*). Means ± SE are indicated.



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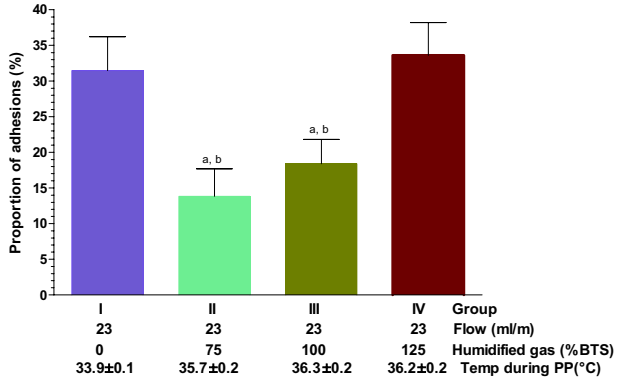
groups with no flow through the abdominal cavity or with humidified gas. This high humidifying capacity of the peritoneum was already shown in open surgery in humans; that is, when bowels are exteriorized, the water loss by evaporation is approximately 32 g/h and this causes their surface temperature to decrease by 3°–5°C (35).

FIGURE 5

Effect of CO_2 pneumoperitoneum with discrete levels of humidification, expressed in relation to BTS conditions (37°C, 100% RH) on adhesion formation. Nonhumidified gas (group I) and humidified gas at 75% BTS (group II), 100% BTS (group III), and 125% BTS (group IV) conditions and a flow of 23 mL/min through the abdominal cavity were used. Pneumoperitoneum-enhanced adhesions were induced during laparoscopy and quantitatively scored after 7 days during laparotomy. Means ± SE are indicated. [a]*P* vs. group I <.05, [b]*P* vs. group IV <.03 (Wilcoxon test).



Binda. Adhesions, temperature, and desiccation. Fertil Steril 2006.

As explained in the introduction, desiccation requires a high amount of energy. Taking into consideration energy calculations, whereas 1 cal is needed to heat 1 mL of water by exactly 1°C and 0.00003 cal is needed to heat 1 mL of CO_2 by 1°C, the energy to vaporize 1 mL of water at 37°C is 577 cal (63 cal to heat 1 mL to 100°C + 514 cal to vaporize) (31). This means that much more energy is needed to evaporate water than to heat water or CO_2 by 1°C. Applied to the desiccation experiment, using nonhumidified gas and assuming 100% RH in the pneumoperitoneum by evaporation of body water, body temperature of 37°C, and gas temperature of 37°C before entering the abdominal cavity, mice with a flow rate of 23 and 100 mL/min through the abdomen would lose 0.6 and 2.5 cal/min, respectively, whereas mice with no flow or with humidified gas (100% RH) would not require extra energy. The same calculations can be applied to the oversaturation experiment; the 0% BTS condition would require 0.6 cal/min, the 75% BTS 0.14 cal/min, and the 100% BTS 0 cal/min, whereas the 125% BTS would add 0.14 cal/min by condensation.

Animal body temperature changes in this study can, therefore, be explained by the energy required for evaporation or released at condensation. This decrease in body temperature was, however, masked by the homeothermic blanket in the desiccation experiment (groups I, II, and III), but fully evident when the homeothermic blanket was not used (group V). In that case body temperature decreased to 31°C, confirming observations in rats (27) and pigs (37). This cooling can be prevented by using warm and humidified gas, demonstrated in previous studies (27, 28, 36) and confirmed in this study (group IV). In the oversaturation experiment, we confirm that with warm and

TABLE 2

Effect of humidification and temperature during pneumoperitoneum on adhesion formation.

Group	Pneumoperitoneum: Flow (mL/min)	Pneumoperitoneum: Humidified gas (%BTS)	Body Temp[c] (°C)	Adhesion scores (mean ± SE): Extent	Type	Tenacity	Total
I	23	0	33.9 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	4.2 ± 0.4
II	23	75	35.7 ± 0.2	0.7 ± 0.2[a,b]	0.7 ± .01[a,b]	0.9 ± 0.1[a,b]	2.3 ± 0.3[a,b]
III	23	100	36.3 ± 0.2	0.9 ± 0.1[b]	1.0 ± 0.1	1.0 ± 0.2	2.9 ± 0.4[a]
IV	23	125	36.2 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	4.3 ± 0.5

Note: Discrete levels of humidification, expressed in relation to body temperature saturated (BTS) conditions (37°C, 100% RH, 44 mg of water/liter) were used. Adhesions were induced during laparoscopy with 60 minutes of CO_2 pneumoperitoneum at 20 cm of H_2O and qualitatively scored after 7 days during laparotomy.

[a] *P* vs. group I <.05; [b] *P* vs. group IV <.05.

[c] Mean of body temperature during T_{20}–T_{80} is indicated.

Binda. Adhesions, temperature, and desiccation. Fertil Steril 2006.

nonhumidified CO_2, animals cool down to 33°C, which can be explained solely by the evaporation of water. In the 100% BTS group, body temperature slightly increased, that is, returned to the initial 37°C before anesthesia, in the absence of any cooling. Moreover, some additional energy could have been provided if the gas was not completely cooled down in the trocar. As expected, this increase in temperature is more pronounced in the 100% and 125% BTS groups, particularly the 125% BTS group, where energy is released by the condensation due to the higher enthalpy of the gas.

The pneumoperitoneum temperature will be a function of the temperature of the insufflated gas, the flow through the abdominal cavity, the energy released or required by condensation or evaporation, the animal body temperature, and the surrounding environment. This explains why, in the desiccation experiment, in comparison with body temperature, pneumoperitoneum temperature was comparable in the group with no flow, slightly lower (NS probably because n = 3 only) in the groups with flows of 23 mL/min and 100 mL/min and nonhumidified gas, and significantly higher for the group with humidified gas. This shows that as the cold, nonhumidified gas flow increases, the temperature in the pneumoperitoneum decreases, whereas even at high flows the temperature remains close to body temperature when humidified gas is used. Also in the group with hypothermia the pneumoperitoneum temperatures were slightly higher than body temperatures. This is logical for the group with hypothermia because body temperature was approximately 31°C and insufflated gas temperature was approximately 37°C. The same holds true for the oversaturation experiment, explaining why in all the groups the pneumoperitoneum temperature remained higher than body temperatures, especially in the 75%, 100%, and 125% BTS groups.

This is to our knowledge the first direct demonstration that desiccation enhances adhesion formation. Unless great effort is taken to prevent the associated cooling, the effect will be underestimated, as the associated cooling will decrease adhesion formation (16). Even in the desiccation experiment, in which cooling was prevented with the homeothermic blanket, some underestimation by cooling cannot be ruled out. Pneumoperitoneum temperatures were, as expected, slightly lower when desiccation occurred; moreover, we can speculate that in the peritoneum, where desiccation occurred, the temperature was probably even lower. In all previous published experiments, desiccation was always associated with cooling. Also for effects such as alteration of mesothelium morphology, destruction of microvilli, and bulging up of cells with exposure of the basal lamina (27–30), it is difficult to judge the independent effects of desiccation and cooling.

Desiccation-enhanced adhesions are clearly prevented by using humidified gas. Adhesions were even slightly lower in the group with high flow and humidified gas than in the group with no flow and nonhumidified gas. This can be explained by the gas leakage during the surgical procedure to induce adhesions, a problem we were not aware of during the experiments. Leakage occurred from the 14-gauge catheters between their insertion and the insertion of the surgical instruments; slight leakage occurred during the surgery; more important leakage occurred after removal of the catheters until suturing was finished. The difficulty of avoiding leakage varies with the expertise of the surgeon. Considering the diameter of the 14-gauge catheter, leakage for 1 minute only can easily amount to more than 500 mL of CO_2, which accounts for nonnegligible desiccation. The relative importance of this leakage is huge in group I, considered as without desiccation; still important in group II, with total leakage of 1,380 mL; and less important in group III, with leakage of 6,000 mL. Thus, groups I, II, and III had desiccation of 500, 1,880, and 6,500 mL instead of 0, 1,380, and 6,000 mL. Without this leakage during surgery, we can speculate that adhesions in group I would have been consid-

erably less and in group II slightly less. In future experiments this leakage during surgery must be controlled.

These experiments confirm and extend previous observations that adhesions decrease with hypothermia (16). It remains surprising, however, that quantitatively this effect, at least under these experimental conditions, seems as important as using humidified gas. Also mice of groups II and III, which could not maintain their body temperature, had fewer adhesions (Fig. 3). It is unclear whether this decrease in body temperature was a consequence of a leakage and thus enhanced desiccation or of an insufficient metabolic capacity to maintain the body temperature at 37°C. In the former hypothesis, the decrease in body temperature would have a more important effect on adhesions than the increased desiccation. We can only speculate today that cooling might to some extent prevent the deleterious effect of desiccation as it does for the hypoxia. This also might explain why the effects of warm and humidified gas on mesothelium morphology are still controversial (27, 28).

To interpret the adhesion formation data in the oversaturation experiment, the opposing effects of desiccation and hypothermia should also be considered, knowing that both are intimately linked and that although the former increases adhesion formation (desiccation experiment), the latter reduces adhesion formation (16; desiccation experiment). Because adhesion formation was much higher in the 0% BTS group (oversaturation experiment), the effect of desiccation on adhesion formation was clearly confirmed. Because in this group body temperature was much lower, the adhesiogenic effect of desiccation must be clearly underestimated. Adhesions were slightly lower in the 75% BTS group than in the 100% BTS group, which can only be interpreted by the slightly lower temperature, as some evaporation must have occurred, considering the 100% RH in the peritoneal cavity. In the 75% BTS group, the effect of temperature is underestimated, as without desiccation adhesions would even have been less. Adhesions were slightly higher in the 125% BTS group than in the 100% BTS group, which can only be explained by the slightly higher temperature, as desiccation can be ruled out. It is unlikely that excess condensed water poses a hypotonicity challenge, causing cellular damage in the 125% BTS group (43), because of the limited amount of condensation produced.

The effect of heating and humidifying the gas during laparoscopy has been studied in clinical trials. Compared with cold and nonhumidified gas, warm and humidified CO_2 is claimed to reduce postoperative pain after laparoscopy (28, 44, 45), but this observation is still controversial (46). It should be stressed that in all these evaluations the effect of warm and humidified gas was always compared with that of cold and nonhumidified gas. The effect in reducing the pain therefore might be due to prevention of desiccation rather than to the heating of the gas.

In summary, we demonstrate the complex relationship between cooling and desiccation on adhesion formation. Desiccation clearly increases adhesion formation, and the effect is generally underestimated as the associated cooling decreases adhesion formation. We confirm the effect of hypothermia in reducing adhesion formation, an effect that at 32°C is quantitatively as pronounced as humidification. Slight cooling together with slight desiccation (oversaturation experiment) decrease adhesion formation, but this effect of cooling is overruled when desiccation becomes important. These data moreover extend the previous data demonstrating that increased pneumoperitoneum temperatures (above 37°C) increase adhesion formation even further. The initial hypothesis that oversaturation of the insufflated gas would be beneficial for adhesion formation, as all desiccation would be prevented, thus proved wrong because of the associated increase in peritoneal temperature and enthalpy of the gas. This in effect is consistent with the physiologic map (43) in that nonphysiologic gas conditions affect the normal physiologic state (lower than BTS or above BTS). From these data we anticipate that insufflators, which provide only a heating option that will warm the gas to body temperature without humidification, could be more deleterious for adhesion formation than using an insufflator without a heating option, because of higher temperature and higher desiccation. These data will have to be confirmed in larger animals. Moreover, in larger animals a decrease in pneumoperitoneum temperature is not necessarily associated with a decrease in body temperature. If confirmed in larger animals, these results may have very important clinical implications for the design of insufflators and humidifiers, which would minimize adhesion formation. The potential clinical implications of preventing adhesion formations in human surgery are important, especially if the prevention of hypoxia by adding a few percent of oxygen, preventing desiccation, and cooling the pneumoperitoneum to approximately some 32°C would have additive effects.

Acknowledgments: The authors thank Lisbeth Vercruysse, M.Sc., Silvia Caluwaerts, Ph.D., Salwan Al-Nasiry, M.D., Ms. Rita Van Bree, Adriana Bastidas, M.D., Jasper Verguts, M.D., Robert Pijnenborg, Ph.D. (Department of Obstetrics and Gynaecology, University Hospital Gasthuisberg), and Michael Blackhurst, B. Eng (Hons.) (Fisher & Paykel Healthcare Ltd.) for their help. The authors also thank Fisher & Paykel Healthcare Ltd. for the development and supply of the humidifier.

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