Prevention of Adhesions with Crystalloids during Laparoscopic Surgery in Mice

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Abstract

Study Objective. To evaluate the effect of saline and Ringer's lactate solutions in preventing adhesions during laparoscopic surgery in mice.
Design. Prospective, randomized trial (Canadian Task Force classification I).
Setting. Academic research center.
Subjects. Ninety-two female Naval Medical Research Institute mice.
Intervention. Adhesions were induced laparoscopically by opposing bipolar lesions in the uterine horns and pelvic sidewalls, and saline or Ringer's lactate solution was added at different times during the procedure.
Measurements and Main Results. Adhesions were scored quantitatively and qualitatively for extent, type, and tenacity after 7 days under microscopic vision during laparotomy. After 45 minutes of pneumoperitoneum, neither solution reduced adhesion formation, but when added immediately after surgery they did ($p = 0.002$). Coagulation was not significantly different with addition of either solution immediately after coagulation. In the third experiment the presence of fluid during pneumoperitoneum decreased adhesion formation ($p = 0.0001$) but Ringer's lactate was more effective than saline ($p = 0.0005$).
Conclusion. Crystalloids reduced CO_2 pneumoperitoneum-enhanced adhesion formation in a laparoscopic mouse model, but Ringer's lactate solution was more effective than saline.

(J Am Assoc Gynecol Laparosc 9(4):447–452, 2002)

Postoperative adhesion formation is an important clinical problem. Good surgical technique, that is, atraumatic and bloodless surgery, and prevention of desiccation are emphasized as preventing adhesions. Mechanical barriers and hydrofloatation with fluids help reduce adhesion formation in humans and animals. After laparotomy both Interceed and Gore-Tex were effective in humans[1–5] and in animal models such as rats,[6] rabbits,[7,8] and monkeys.[9] After laparoscopic surgery these materials were effective in humans but not in animal models.[4] Separation of injured surfaces by hydrofloatation with viscous substances such as dextran reduced adhesions after laparotomy in humans[10] and in rats,[11] and after laparoscopy in humans.[10,12] Hydrofloatation with crystalloids such as saline or Ringer's lactate solution in humans has a

controversial effect,[13,14] but was effective after laparotomy in rats[15–17] and rabbits.[18] No data are available regarding crystalloids after laparoscopy.

Materials and Methods

We investigated the effect of saline and Ringer's lactate solutions on adhesion formation in a mouse model of CO_2 pneumoperitoneum-enhanced adhesion formation.[19] The study was approved by the institutional review animal care committee.

Animals

Ninety-two Naval Medical Research Institute mice (age 10–12 wks, weight 25–35 g) were kept under standard laboratory conditions (temperature 20–25° C, relative humidity 40–70%, 14 hrs light, 10 hrs dark). They were fed a standard laboratory diet (Hope Farm, Woerden, The Netherlands) and had free access to water and food before and after the laparoscopic procedure.

Anesthesia and Endotracheal Intubation

After intramuscular anesthesia with pentobarbital 0.06 mg/g, the abdomen was shaved and the animal was secured to the table in supine position. For intubation, the animal was placed with the neck under a light source; the tongue was grasped with a hemostatic clamp and pulled out, and vocal cords were visualized by transillumination. A 20-gauge catheter with a blunt guidewire was inserted into the trachea. After removing the wire, the mouse was ventilated with room air using a mechanical respirator (Rodent Ventilator, Harvard Apparatus, Hollison, MA) with 1.5 ml tidal volume and 85 strokes/minute.

Induction of Adhesions

Laparoscopic surgery was performed under aseptic conditions as described elsewhere.[19] After disinfection, a 3.5-mm abdominal incision was made caudal to the xiphoid process. A 2.1-mm zero-degree endoscope with an outer sheath for protection and insufflation of 3.3 mm (Karl Storz, Tuttlingen, Germany), connected to a single-chip videocamera and light source, was introduced into the abdominal cavity. The endoscope was secured in a holder and the incision was sutured around the endoscope with 6-0 polyglycolic acid suture (Dexon II; Davis+Geck, Gosport, UK) to avoid gas leakage.

Pneumoperitoneum was maintained with an insufflator (Thermoflator Plus, Karl Storz) delivering carbon dioxide (CO_2) heated to 37° C and humidified with vapor (Drager, Lubeck, Germany). Since most insufflators have intermittent delivery of gas, a water valve with free escape of gas was used to provide continuous flow and constant pressure. An elastic balloon was placed next to the water valve to eliminate virtually all pressure changes in the animal's tiny abdominal cavity. Since peritoneum has a high exchange capacity, continuous removal of oxygen that could diffuse from the circulation is required to maintain the predefined concentration of gas inside the abdominal cavity. Therefore, a 26-gauge needle was inserted next to the endoscope to obtain continuous flow of some 10 ml/minute of gas through the animal.

After establishing pneumoperitoneum, two 14-gauge catheters were inserted under direct laparoscopic guidance for working instruments in both right and left flanks. With a 1.5-mm grasper the bicornuate uterus was individualized by removing surrounding fat tissue and grasped in the midline. Using the bicap forceps (Circon ACMI, Stanford, CT) with a 1.6-mm ball electrode, a linear bipolar coagulation lesion of 10 mm was created in the antimesenteric border of both uterine horns for 3 to 5 seconds and in ipsilateral pelvic sidewalls.

The procedure took 3 to 4 minutes. Pneumoperitoneum was maintained for 45 minutes. In groups I to III, macroscopic lesions were made with 10 W in standard coagulation mode (Autocon 350, Karl Storz), whereas in group IV (under fluid) 30 W had to be used for 3 to 5 seconds. These settings were obtained in previous pilot experiments. At the end of surgery the incisions were sutured with 6-0 polyglycolic acid in single layers.

Scoring Adhesions

After 7 days laparotomy was performed to evaluate adhesions under an operative microscope. The whole abdominal cavity was visualized through xyphopubic midline and bilateral subcostal incisions. After evaluating ports sites and viscera for de novo adhesions, fatty tissue surrounding the uterus was carefully removed. The lengths of visceral and parietal lesions and adhesions were measured. Adhesions, when present, were lysed to evaluate their type and tenacity. They were scored by the surgeon and an independent observer in random order, and codes of

the intervention were broken only at the end of the experiment. The qualitative evaluation assessed extent (0 = no adhesions, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%), type (0 = no adhesions, 1 = filmy, 2 = dense, 3 = capillaries present), tenacity (0 = no adhesions, 1 = essentially fall apart, 2 = require traction, 3 = require sharp dissection), and totals of these (extent + type + tenacity).

For quantitative evaluation the proportion of adhesions was calculated by measuring the length of the line covered by adhesions and the length of the lesions. Calculation was made according to the formula: adhesions (%) = (sum of the length of the individual attachments/length of the lesion) × 100. Adhesions in visceral and parietal peritoneum were evaluated separately. The animal was sacrificed immediately after evaluation.

Experimental Design

All experiments were performed using block randomization by day. Therefore, a block of animals, consisting of one animal of each group, was always operated on the same day, avoiding day-to-day variability.

The first experiment evaluated the effect of adding saline at the end of pneumoperitoneum, immediately after surgery or before surgery in 20 animals. In group I, no fluid was added; in group II, 20 ml of NaCl 0.9% (normal saline) was added at the end of the procedure; in group III, 20 ml of normal saline was added immediately after coagulation; and in group IV, 20 ml of normal saline was added before induction of pneumoperitoneum, and coagulation was performed under water.

The design of the second experiment was the same, but Ringer's lactate was used instead of saline in 40 mice.

The third experiment in 32 animals was designed to evaluate simultaneously (factorial 2 × 2 design) differences between saline and Ringer's lactate and the effect of fluid during coagulation. In the first two experiments a different power setting had to be used for normal and underwater coagulation to obtain similar macroscopic lesions. Differences in tissue damage thus could not be ruled out with certainty. To avoid differences caused by depth of coagulation, all coagulations were performed under fluid, which was either left or drained. Saline was left in the peritoneal cavity (group I) or evacuated at the end of coagulation (group II) by inserting a 14-gauge catheter through one of the laparoscopic ports. Ringer's lactate was left in situ (group III) or evacuated (group IV). Two, three, and three mice died after surgery in the first, second, and third experiments, respectively.

Statistical Analyses

Statistical analysis was performed with the SAS system (SAS Institute, Cary, NC) using a nonparametric two-way analysis of variance (Proc GLM), both giving similar results. Only significances obtained by regression analysis are given. Since the design of experiments I and II was identical, and since both were randomized, the data of these experiments were analyzed together to evaluate differences among the four groups. During this analysis, results were corrected for eventual differences between saline (experiment I) and Ringer's lactate (experiment II) as follows: adhesion scores are influenced simultaneously by the experimental group and by type of fluid.

Since data were obtained in two experiments and thus were obviously nonrandomized, differences between fluids were disregarded. Therefore, a third experiment was performed using a 2 × 2 factorial design to evaluate and ascertain differences between solutions and to confirm results of the first two experiments. In the analysis, parietal and uterine and right and left sides of adhesions were averaged and differences were not taken into account.

Means and standard errors are indicated unless indicated otherwise.

Results

Addition of saline or Ringer's lactate after 45 minutes of pneumoperitoneum (group II) did not reduce adhesion formation (Figure 1). Addition of either solution immediately after surgery decreased adhesion formation (group III) expressed as the total adhesions (p = 0.002) or as quantitative adhesion score (p = 0.007). Coagulation under saline or Ringer's lactate (group IV) was not significantly different with addition of the solutions immediately after coagulation (group III). Similar observations were seen for extent, type, and tenacity of adhesions. Scores for extent in group I were 2.5 ± 0.4 and 2.1 ± 0.2 for saline and Ringer's lactate, respectively; in group II, 2.6 ± 0.3 and 1.7 ± 0.2, respectively; in group III, 1.8 ± 0.3 and 1.2 ± 0.2, respectively (p = 0.003 vs group I, p = 0.02 vs group II); and in group IV, 1.5 ± 0.3 and 0.9
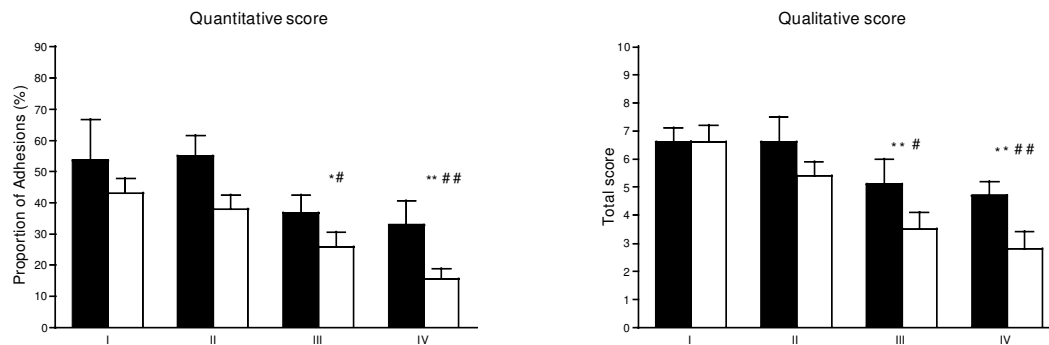
FIGURE 1. Quantitative and total adhesion scores in mice 7 days after induction of peritoneal adhesions by bipolar lesions and 45-minute pneumoperitoneum (group I). Saline (dark bars) or Ringer's lactate solution (light bars) 20 ml was added at the end of pneumoperitoneum, immediately after or before coagulation, respectively. Mean ± SEM together with significances (p vs group I * ≤0.02, ** ≤0.002; p vs group II # ≤0.02, ## ≤0.002) between groups are shown.

± 0.2, respectively (p = 0.0003 vs group I, p = 0.001 vs group II).

Scores for type were group I, 2 ± 0.1 and 2.3 ± 0.2 for saline and Ringer's lactate, respectively; group II, 2.1 ± 0.4 and 1.9± 0.2, respectively; group III, 1.6 ± 0.3 and 1.6 ± 0.3, respectively (p = 0.003 vs group I, p = 0.02 vs group II); and in group IV, 1.6 ± 0.2 and 1.1± 0.2, respectively (p = 0.002 vs group I, p = 0.008 vs group II). Scores for tenacity were group I, 2.1 ± 0.1and 2.2 ± 0.2 for saline and Ringer's lactate, respectively; group II, 2.0 ± 0.3 and 1.8 ± 0.1, respectively; group III, 1.8 ± 0.3 and 1.1 ± 0.2, respectively (p = 0.003 vs group I, p = 0.02 vs group II); and group IV, 1.6 ± 0.1 and 0.9 ± 0.2, respectively (p = 0.0002 vs group I, p = 0.0008 vs group II).

In the third experiment (Figure 2) both the presence of fluid during pneumoperitoneum and type of fluid (Proc Logistic) strongly affected adhesion formation as assessed by quantitative evaluation (p = 0.0001 and 0.005 for presence and type of fluid, respectively), total adhesions score (p = 0.0001 and 0.0005, respectively), extent (p = 0.0001 and 0.003, respectively), type (p = 0.0001 and 0.003, respectively), and tenacity (p = 0.0001 and 0.01, respectively) of adhesions. The same factors were important for total parietal (p = 0.005 and 0.0001, respectively) and total visceral (p = 0.007 and 0.0001, respectively) adhesion scores. For experimental groups I, II, III, and IV, scores for extent were 1.4 ± 0.2, 2.6 ± 0.2, 0.8 ± 0.1, and 2.0 ± 0.1, respectively; scores for type were
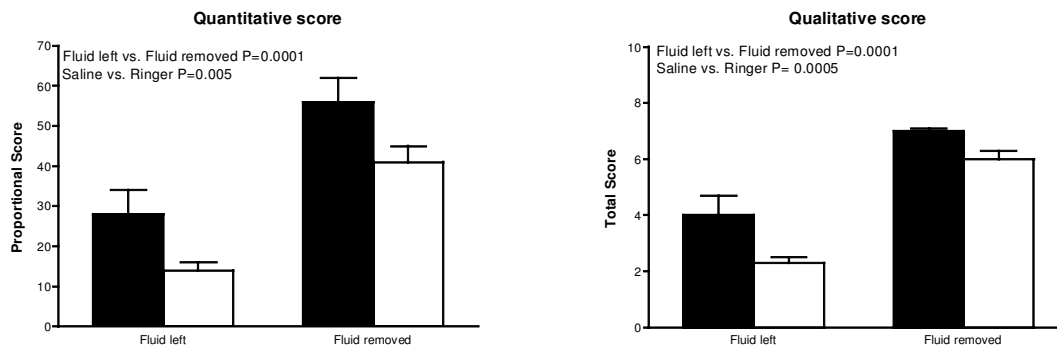

FIGURE 2. Quantitative total adhesion score in mice 7 days after induction of peritoneal adhesions. Bipolar lesions were created under saline (dark bars) or Ringer's lactate solution (light bars) at laparoscopy. Fluid was left or evacuated at the end of creating lesion. Mean ± SEM are indicated.

1.4 ± 0.3, 2.4 ± 0.1, 0.8 ± 0.1, and 1.9 ± 0.2, respectively; and for tenacity 1.3 ± 0.3, 2.4 ± 0.2, 0.7 ± 0.7, and 2.0 ± 0.2, respectively.

Discussion

Our data failed to confirm that saline or Ringer's lactate solution prevents primary adhesions (group I vs group II) when instilled at the end of surgery. This is in contradiction with data in rats for Ringer's lactate[15,17,20] and saline.[16] In humans, however, a review and meta-analysis could not confirm the effectiveness of either solution. The discrepancy between our data and previous animal reports could be explained by different models. In all previous reports fluid was instilled after laparotomy. We used a laparoscopic model. This also is the first report in mice, which could respond slightly differently from other animals. This mouse model was validated in previous experiments,[19–22] in which CO_2 pneumoperitoneum was a cofactor in adhesion formation. Duration of pneumoperitoneum therefore was standard at 45 minutes.

Our data clearly show that the two crystalloids are ineffective in reducing CO_2 pneumoperitoneum-enhanced adhesion formation. This could be extremely important for future investigations on adhesion prevention. It indeed suggests that mechanisms involved in adhesion formation might not be identical after laparotomy and laparoscopy. Data for laparotomy therefore should not be extrapolated to laparoscopy without evidence.

Both crystalloids prevented CO_2 pneumoperitoneum-enhanced adhesions if instilled immediately after the lesion was created. During that time the lesions were covered by fluid. This observation is fully consistent with our hypothesis that CO_2 pneumoperitoneum induces hypoxia in superficial mesothelial layers.[19] Partial pressure of oxygen in superficial mesothelial layers will be decreased during CO_2 pneumoperitoneum, and this obviously will be less if peritoneum is covered by layers of fluid. The importance of this observation is that any substance covering peritoneum during laparoscopic surgery has the potential of reducing adhesion formation, at least those enhanced by CO_2 pneumoperitoneum.

Ringer's lactate was superior to saline in this study, but it is unlikely that pH and buffering capacity could explain this difference. Indeed we clearly showed that the effects of helium and CO_2 pneumoperitoneum were identical.[23] Although differences in thermal injury after coagulation under saline or Ringer's lactate cannot be ruled out, this is highly unlikely and we suggest that Ringer's lactate will more effectively protect cells from hypoxia-induced cell damage. The importance of this for preventing adhesions is that solutions that better support cell viability, such as those used during cell culture (phosphate-buffered saline), could be superior.

The advantage of the 2×2 factorial design is that to achieve the same statistical precision as with a one-at-a-time approach, twice as many observations would have been required. This increase in power by factorial design is valid only when the effects of the two factors are additive; that is, when no interaction between factors is present. The possibility to detect an interaction, a different effect of one factor at different levels of the other factor, can also be considered an advantage of the 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) can be missed, especially when between-subject variability is high.[24]

These data extend our previous observation on CO_2 pneumoperitoneum as a cofactor in adhesion formation. Prevention of adhesions is fully consistent with the concept of superficial mesothelial hypoxia as the driving mechanism. Absence of an effect when instilled after pneumoperitoneum suggests that the mechanisms involved after laparoscopic surgery might be at least partly different from those after laparotomy. These concepts require further experiments to clarify the exact mechanisms. If substantiated, however, this will be clinically extremely important for adhesion prevention since results after laparotomy cannot be extrapolated to laparoscopy. In addition, new approaches to adhesion prevention after laparoscopic surgery could be developed.

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