Postoperative inflammation in the abdominal cavity increases adhesion formation in a laparoscopic mouse model

Roberta Corona, M.D.,[a,b] *Jasper Verguts, M.D.,*[a] *Ron Schonman, M.D.,*[a] *Maria Mercedes Binda, Ph.D.,*[a] *Karina Mailova, M.D.,*[a] *and Philippe Robert Koninckx, M.D., Ph.D.*[a]

[a] Department of Obstetrics and Gynaecology, University Hospital Gasthuisberg, Leuven, Belgium; and [b] Department of Obstetrics and Gynaecology, University Hospital S. Giovanni di Dio, Cagliari, Italy

Objective: To investigate acute inflammation in the peritoneal cavity in adhesion formation.
Design: Prospective randomized, controlled trial.
Setting: University laboratory research center.
Animal(s): 9- to 10-week-old BALB/c female mice.
Intervention(s): In a laparoscopic mouse model, acute inflammation in the peritoneal cavity evaluated in CO_2 pneumoperitoneum enhaced adhesions, by CO_2 pneumoperitoneum plus manipulation, and in the latter group plus dexamethasone.
Main Outcome Measure(s): Qualitative and quantitative adhesion scores and an acute inflammation score (neoangiogenesis, diapedesis, and leukocyte accumulation).
Result(s): Adhesions at the lesion site were enhanced by the CO_2 pneumoperitoneum, further enhanced by manipulation, and decreased by the administration of dexamethasone. The acute inflammation scores (total, neoangiogenesis, diapedesis, and leukocyte accumulation) strongly correlated with the total adhesion score. Inflammation scores were similar at both the surgical lesion and the parietal peritoneum.
Conclusion(s): Acute inflammation of the entire peritoneum cavity is an important mechanism involved in adhesion formation and enhances adhesion formation at the lesion site. (Fertil Steril® 2011;95:1224–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: Adhesions, acute inflammation, laparoscopy, dexamethasone, inflammation score

Postoperative adhesion formation results from a series of local events at the trauma site. Peritoneal injury by surgery, infection, or irritation initiates a local inflammatory reaction, exudation, and fibrin deposition into which white blood cells, macrophages, fibroblasts, and mesothelial cells can migrate, proliferate, and/or differentiate. Within a few hours the lesion is covered by macrophages and other tissue repair cells whose exact precursors are still unclear (1–3). The local interplay between inflammatory cells, macrophages, and cytokines is not well understood.

These events at the trauma site are modulated by factors derived from the peritoneal cavity. Adhesions at the lesion site are enhanced in a dose-dependent way by pure CO_2 pneumoperitoneum (4), pneumoperitoneum with more than 10% O_2 (5, 6), by desiccation (7), and by bowel manipulation at a remote site (8) believed to act through mesothelial hypoxia, mesothelial hyperoxia and reactive oxygen species (ROS), desiccation, and trauma, respectively.

Because the inflammatory reaction at the lesion site is widely believed to be a driving mechanism of adhesion formation (9–19), it was surprising that neither nonsteroidal anti-inflammatory drugs (NSAIDs) such as cyclooxygenase-1 (COX-1) or COX-2 inhibitors, nor antibodies neutralizing anti-tumor necrosis factor-α (TNF-α) had any effect upon adhesion formation in hypoxia-enhanced adhesion (pure CO_2 pneumoperitoneum) (20) or hyperoxia-enhanced adhesion (pneumoperitoneum with more than 12% O_2) (6) models. In contrast, dexamethasone, a steroidal anti-inflammatory drug, reduced adhesions by 30% and 62% in the hypoxia- and hyperoxia-enhanced adhesions models, respectively. We therefore investigated the relationship between adhesion formation and acute inflammation in the entire peritoneal cavity.

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Reprint requests: Roberta Corona, M.D., Department of Obstetrics and Gynaecology, University Hospital Leuven, University Hospital Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium (E-mail: coronaroberta@gmail.com).

MATERIALS AND METHODS

Laparoscopic Mouse Model for Adhesion Formation

The experimental setup (i.e., animals, anesthesia, ventilation, laparoscopic surgery, and induction and adhesion scoring) has been described in detail elsewhere (4, 5, 21–25). The model consisted of a bipolar lesion made at the begining of a 60 minutes pneumoperitoneum during laparoscopy. The pneumoperitoneum was induced using the Thermoflator (Karl Storz, Tuttlingen, Germany) through a 2 mm endoscope with a 3.3 external sheath for insufflation (Karl Storz) introduced into the abdominal cavity through a midline incision caudal to the xiphoid appendix. The incision was closed gas tight around the endoscope to avoid leakage. The insufflation pressure was 15 mm Hg. For humidification, the Storz Humidifier 204320 33 (Karl Storz) was used.

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 bipolar coagulation (20W, standard coagulation mode, Autocon 350; Karl Storz). Because temperature is critical for adhesion formation, animals and equipment were placed in a closed chamber at 37°C (heated air, WarmTouch, Patient Warming System, model

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5700; Mallinckrodt Medical, Hazelwood, MO). Because anesthesia and ventilation may influence body temperature (7), the timing from anesthesia (T0), to intubation (at 10 minutes, T10), to the onset of the experiment (at 20 minutes, T20) was strictly controlled.

After 7 days, adhesions were scored quantitatively (proportion and total) and qualitatively (extent, type, tenacity) blindly by laparotomy under a stereomicroscope. The entire abdominal cavity was visualized using a xiphopubic midline and bilateral subcostal incision. After the evaluation of port sites and viscera (omentum, large and small bowels) for de novo adhesions, the fat tissue surrounding the uterus was carefully removed. The length of the visceral and parietal lesions and adhesions were measured. Adhesions, when present, were lysed to evaluate their type and tenacity. Pouly and Seak-San (44) terminology was used to describe the de novo adhesion formation for the adhesions formed at nonsurgical sites and adhesion formation for adhesions formed at the surgical site.

Animals

The study used 9- to 10-week-old female BALB/c mice of 20 to 24 g. Animals were kept under standard laboratory conditions and diet at the animal facilities of the Katholieke Universiteit Leuven (KUL). The study was approved by the Institutional Review Animal Care Committee.

Histologic Inflammation Score

A scoring system of acute inflammation was based upon the known pathophysiology of acute inflammation (26–30). Acute inflammation has three major components: [1] alterations in vascular caliber leading to an increase in blood flow, [2] neoangiogenesis and structural changes in the microvasculature permitting plasma proteins and leukocytes to leave circulation, and [3] diapedesis of leukocytes from the microcirculation leading to their accumulation and activation in the lesion (31). We scored [1] the number of vessels, reflecting neoangiogenesis; [2] the number of polymorphonuclear cells (PMNs) in diapedesis, reflecting the increased permeability of vessels; and [3] the PMN accumulation at the site of the injury. Scoring was as follows: neoangiogenesis (0 when $\leq$3 vessels, 1 when 4–8 vessels, 2 when 9–12 vessels, 3 when $\geq$12 vessels), vessel permeability (0 when 0 PMNs in diapedesis, 1 when $\leq$2 PMNs, 2 when 3–4 PMNs, 3 when $\geq$4 PMNs), and leukocyte activation and accumulation (0 when $\leq$3 activated PMNs are present, 1 when 4–8 PMNs, 2 when 9–12 PMNs, 3 when $\geq$12 PMNs). Total inflammation score was the sum of the neoangiogenesis, permeability, and leukocyte activation scores.

Histology and Immunohistochemistry

Under stereomicroscopic vision, biopsy samples were obtained during laparotomy. After dissecting skin and muscles from the peritoneum, a tissue adherent (Lyostypt; B. Braun Melsungen AG, Mesungen, Germany) was applied over the whole length of the lesion plus 5 mm of peritoneum on each side of the lesions. The specimens were then fixed with JB fix (32) for 24 hours, embedded in paraffin, oriented, and four 4 to 6 μm sections were taken perpendicularly to the surface and perpendicularly to the lesion. Each section permitted evaluation of the changes in the lesion and the surrounding peritoneum from the surface to the depth of the biopsy sampling. Sections were immunohistochemically stained for CD45 to detect activated leukocytes (LCA, Ly-5, T200) (BD Biosciences Pharmingen, San Diego, CA) in citrate bluffer 80°C, pH 6.0, dilution 1/400.

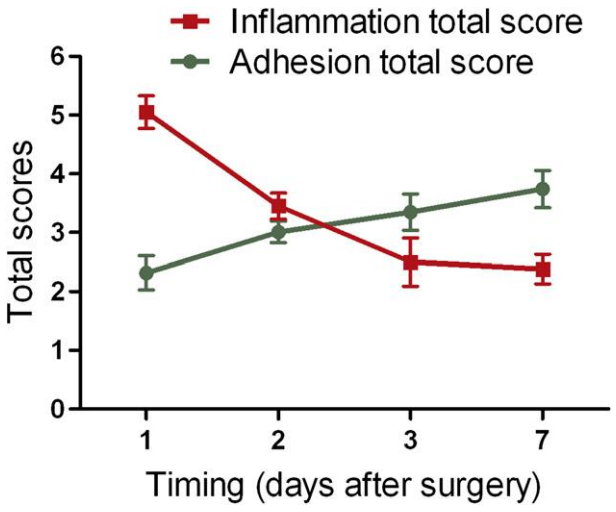
Inflammation parameters were blindly scored under a microscope with a camera (Axio scope, AxioCam MRc 5, KS 400 imaging system; Carl Zeiss MicroImaging, Jena, Germany). For each slide, four high-power fields were randomly chosen, two of the lesion and two of the surroundings (one for each side), and we counted the number of vessels, PMNs in diapedesis, and leukocyte stained by CD45.

Experiment Design

The experiments were designed to investigate the effect on the inflammation score of factors known to enhance adhesions (CO_2 pneumoperitoneum and manipulation) or to decrease adhesions (dexamethasone). Experiment 1 was designed to determine which day after surgery acute inflammation and adhesion formation should be scored. After 60 minutes of CO_2 pneumoperitoneum (n = 8), the inflammation and adhesion scores were evaluated after 1, 2, 4, and 7 days (two mice per day) (Fig. 1). Based on these results, we decided to evaluate inflammation and adhesions 48 hours after surgery in all further experiments.

FIGURE 1

Total adhesion and inflammation score during the first 7 days after a surgical bipolar lesion and 60 minutes of CO2 pneumoperitoneum.



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Experiment 2 (16 mice: 4 mice per group) was designed to evaluate the inflammation score and adhesions in control animals after 60 minutes of CO_2 pneumoperitoneum with 4% O_2 (group 1), after 60 minutes CO_2 pneumoperitoneum (group 2), and after 60 minutes CO_2 pneumoperitoneum plus manipulation (group 3); group 4 underwent the same manipulation-enhanced adhesion formation as group 3 but with added dexamethasone, which is known to decrease adhesions (8). Manipulation consisted of manipulating fat and bowels in the upper abdomen: the omentum and large and small bowels were moved gently up and down across the abdomen for 5 minutes with a 1.5-mm nontraumatic grasper for 5 minutes as described elsewhere (8). Dexamethasone (Aacidexam, 5 mg for injection; Organon, Brussels, Belgium) was administered by intraperitoneal injection of the medication through the midline of the lower abdominal wall with a 31-gauge, 8 mm (5/16-in) BD Ultra-Fine Short Needle (BD, Franklin Lakes, NJ) holding the mouse in an upside down position to avoid bowel injuries, at the dose of 40 μg at the end of pneumoperitoneum and 40 μg after 24 hours.

Experiment 3 (16 mice: 4 mice per group) was similar to experiment 2; inflammation was scored at the parietal peritoneum in the upper abdomen at distance from the surgical site. Experiments 2 and 3 were block randomized by day, meaning that one mouse from each group was treated randomly the same day.

Statistics

Statistical analyses were performed with the SAS System (SAS Institute, Cary, NC) using a Wilcoxon test for differences in adhesion formation, and Spearman correlation between adhesion and total inflammation scores. All the data are presented as mean $\pm$ standard deviation.

RESULTS

Experiment 1

The time courses of both adhesion scores (total and proportion) were similar. Total adhesion scores after 24, 48, and 72 hours and 7 days

FIGURE 2

Correlation between total adhesion and total inflammation scores at the lesion ($P<.0001$, Spearman test; see Table 1).

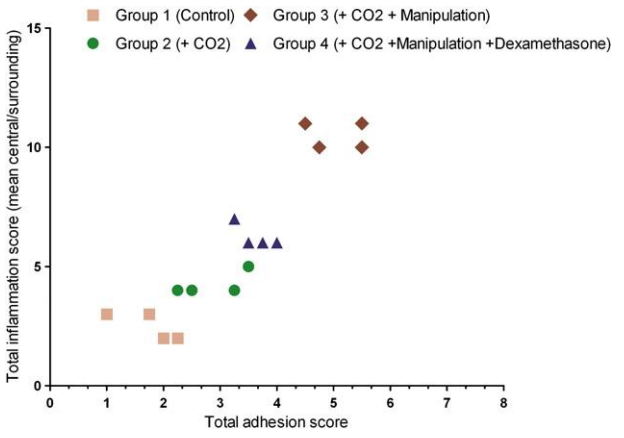

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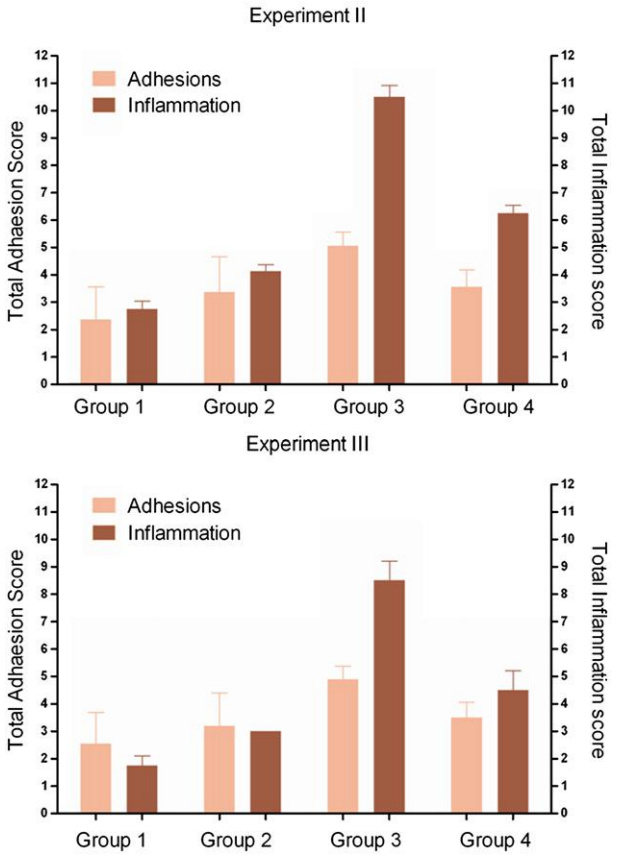
FIGURE 3

Total adhesion and total inflammation scores at the surgical lesion (mean of scores of the lesion and 5 mm from the lesion; experiment 2) and at the parietal peritoneum (experiment 3).



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were 2.32 ± 0.29, 3.01 ± 0.18, 3.35 ± 0.31, and 3.74 ± 0.32, respectively. Total adhesion scores and the total inflammation, the former increasing and the latter decreasing progressively, are shown in Figure 1. Also the scores of neoangiogenesis, diapedesis, and leukocytes accumulation decreased progressively. We therefore decided to evaluate acute inflammation and adhesion formation on day 2 in all subsequent experiments.

Experiment 2

Adhesion scores on day 2 confirmed the observations from day 7 after surgery (8) (Figure 2 and Table 1). In comparison with the control group (group 1), both total and proportion of adhesions increased when a pure CO_2 pneumoperitoneum was used (group 2; $P=.007$ and $P=.0053$, respectively). Adhesions further increased in group 3 ($P<.0001$ for both total and proportion). The addition of dexamethasone (group 4) decreased adhesion formation ($P<.0001$ for both adhesion scores).The total inflammation score at the central part of the surgical lesion was slightly higher in comparison with the inflammation score at 0.5 cm from the lesion: 1.5 ± 0.40 and 2.625 ± 0.25 ($P=.0203$), 0.625 ± 0.25 and 2.125 ± 0.25 ($P=.0154$), 4.125 ± 0.25 and 6.375 ± 0.25 ($P=.0321$), 2.375 ± 0.25 and 3.875 ± 0.25 ($P=.0591$) for groups 1, 2, 3, and 4, respectively.

Depth of the inflammatory reaction spanned 2 mm to a maximum of 4 mm in group 3 which had the highest inflammation score. Changes in total inflammation scores at the lesion (mean of the inflammation scores in the central part and in the periphery) and in adhesions scores were strikingly similar (Figs. 3 and 4). At the level of the surgical lesion, pure CO_2 pneumoperitoneum in comparison with group 1 slightly increased the total inflammation score ($P=.07$), neoangiogenesis ($P=.0577$), and lymphocyte accumulation ($P=.0796$). In group 3, the inflammation score further increased: total inflammation (group 2 vs. group 3, $P<.0001$), neoangiogenesis ($P=.0007$), vasodilatation and permeability ($P=.0052$), and lymphohistiocytic activation and accumulation ($P=.0022$). When dexamethasone was added after surgery, the total mean inflammation score decreased (group 3 vs. group 4, $P<.0001$), an effect observed for all parameters: neoangiogenesis ($P=.0154$), diapedesis ($P=.0016$), and lymphocytes activation-accumulation ($P=.001$). Most strikingly, however, was the strong correlation between adhesion scores and inflammatory parameters (Table 1). Total acute inflammation score (Fig. 2), neoangiogenesis, diapedesis, and leukocyte accumulation (see Table 1) strongly correlated with total adhesion scores.

Experiment 3

Experiment 3 confirmed the results of experiment 2. In addition, the inflammation scores of the parietal peritoneum were found to be comparable with those at the surgical lesion (see Fig. 4). Indeed, the use of pure CO_2 pneumoperitoneum in comparison with $CO_2 + 4\%$ O_2 increased slightly the total inflammation score (not statistically significant, $P=.06$); manipulation further increase the total inflammation score (group 2 vs. group 3, $P<.0001$), and dexamethasone decreased the total inflammation score (group 3 vs. group 4, $P<.0001$).

DISCUSSION

These data confirm and extend previous observations on adhesion formation in our laparoscopic mouse model. Indeed, as observed on day 7, pure CO_2 pneumoperitoneum increased adhesions on

TABLE 1

Correlation between adhesion and inflammation scores.

Inflammatory parameters	Biopsy location	Adhesion score (*P* value)				
		Total	Extent	Type	Tenacity	Proportion (%)
Neoangiogenesis	Central	.0002	NS	<.0001	.0032	NS
	Surrounding	.0002	NS	<.0001	.0021	NS
Permeability	Central	.0391	NS	.0411	NS	NS
	Surrounding	.0286	NS	.0382	NS	NS
Leukocytes accumulation	Central	.0003	.0043	.0029	<.0001	.0031
	Surrounding	.0002	.0032	.0021	<.0001	.0028
Total score	Central	<.0001[a]	.0017	<.0001	<.0001	.0005
	Surrounding	<.0001[a]	.0005	<.0001	<.0001	.0005

Note: Spearman correlation was performed. NS = not statistically significant.
[a] Data shown in Figure 2.

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day 2 in comparison with a pneumoperitoneum with 96% CO_2 + 4% O_2, (4, 5, 24). When, in addition, mechanical trauma was added, the adhesions further increased at the lesion site (8), whereas dexamethasone decreased the adhesions (8). Also the absence of de novo adhesions was confirmed. The parallelism between changes in adhesion formation and in acute inflammation scores was striking, with a linear correlation between adhesion and inflammation scores. This suggests that acute inflammation is an important common driving mechanism modulating adhesion formation. The similarity of acute inflammation at the lesion, in the peritoneum 5 mm from the lesion, and in the entire peritoneal cavity strongly supports the concept that the entire peritoneal cavity is a cofactor affecting adhesion formation at the lesion site. In particular, the effect of bowel manipulation in the upper abdomen (i.e., at a distance from the bipolar lesion) strongly supported the concept that some peritoneal cavity factors stimulated by mesothelial trauma with exposure of the basal membrane may enhance adhesion formation and also inflammation at the lesion site. The absence of de novo adhesions confirmed that adhesion formation requires a peritoneal lesion, but that quantitatively the inflammatory reaction in the entire peritoneal cavity is the most important factor.

The exact mechanism by which the inflammatory reaction of the entire peritoneal cavity affects adhesion formation at the lesion site is still unclear. Because mesothelial cells are known to retract and to bulge during CO_2 pneumoperitoneum without affecting the basal membrane, and because bowel manipulation was done very gently, a very superficial mesothelial cell trauma is suggested as causing an inflammatory reaction of the entire peritoneal cavity. Subsequently, some substances or cells could be released, activated, or attracted into the peritoneal fluid affecting adhesion formation at the lesion site. We speculate that chemokines could be involved as they are known to be inflammatory mediators involved in the activation and migration of leukocytes into the tissue (33). Indeed, according to the position of the first two cysteine residues (34), some are chemoattractants and activators of non-PMNs leukocytes, and others attract neutrophils. Also, macrophages and leukocytes attracted into the peritoneal cavity and their secretion products as cytokines are likely to be involved.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, tenoxicam, nimesulide, parecoxib, and TNF-α-neutralizing antibodies do not affect adhesion formation (20), but dexamethasone has been confirmed to decrease adhesion formation (20, 35) while decreasing the acute inflammatory reaction. The mechanism by which dexamethasone decreases the inflammatory reaction in the peritoneal cavity can only be speculated upon. Dexamethasone has a wide range of effects such as inhibiting fibroblast proliferation and procollagen gene expression through a decreased transforming growth factor secretion (36) or cytokines (37).

Moreover, dexamethasone has an anti-inflammatory effect by inducing mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), decreasing cytokines in innate immune cells (38) and MAPKs (38). The MAPKs include the extracellular signal-regulating kinase (ERK), p38 MAPK, and c-Jun N-Terminal protein Kinase (JNK), and they all play an important role in cell proliferation, apoptosis, and many other nuclear events. In particular, MKP-1 has been shown to inhibit a number of cellular responses mediated by ERK and p38 MAPK. The MAPKs regulate metalloproteinases (MMPs), thus affecting fibrinolysis and nitric oxide (NO) production and angiogenesis (39–43). Our data strongly suggest that acute inflammation in the entire peritoneum cavity is an important mechanism involved in adhesion formation and enhancing adhesion formation at the lesion site.

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