Title:

The role of acute inflammation at the peritoneal cavity-enhanced adhesion formation

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Capsule:

The strong correlation between adhesion score and acute inflammation in the peritoneal cavity suggests that acute inflammation is an important driving mechanism enhancing adhesion formation.

Abstract:

Objective: To investigate the role of acute inflammation of the peritoneal cavity in adhesion formation.

Design: Prospective randomized, controlled trial

Setting: University laboratory research center

Animals: 9-10 weeks old BALB/c female mice

Interventions: In our laparoscopic mouse model acute inflammation of the peritoneal cavity and adhesion formation were evaluated in a control group with CO_2 pneumoperitoneum (PP)-enhanced adhesions, in CO_2 PP plus manipulation-enhanced adhesions and in the latter group + dexamethasone-reduced adhesions. Adhesions and acute inflammation were assessed by neoangiogenesis, diapedesis and leukocytes accumulation on the 2nd day after surgery.

Main outcome measure(s): Qualitative and quantitative adhesion scores and an acute inflammation score.

Results: Adhesions were enhanced by the CO_2 PP ($p=0.007$) , further enhanced by manipulation ($p<0.0001$ versus CO_2 PP) and decreased by the administration of dexamethasone ($p<0.0001$ versus CO_2 PP+manipulation). Acute inflammation scores strongly correlated with total adhesion score whether assessed as total inflammation score ($p<0.0001$) or as neoangiogenesis ($p<0.0002$), diapedesis ($p<0.03$) or leukocyte accumulation ($p<0.0002$). Inflammation scores, moreover, were strikingly similar at the surgical lesion and at the parietal peritoneum.

Conclusions: These data strongly suggest that acute inflammation in the entire peritoneum cavity is the driving mechanism of adhesion formation at the lesion site.

Key words: adhesions, laparoscopy, acute inflammation, inflammation score, dexamethasone, metalloproteasis.

Introduction

Postoperative adhesion formation is believed to result 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' for which it is still unclear what their exact precursors are (diZerega, 1997;diZerega, 2000;diZerega and Campeau, 2001). The local interplay between inflammatory cells, macrophages and cytokines, is not well-understood.

These local events are modulated by factors derived from the peritoneal cavity. Adhesions at the lesion site are enhanced in a dose dependent way by pneumoperitoneum (PP) with pure CO_2 (Molinas, C. R., Mynbaev, O. *et al.* 2001), or PP with more than 10% O_2(Elkelani, Binda *et al.*, 2004;Binda, Molinas *et al.*, 2003), by desiccation (Binda, M. M., Molinas, C. R. *et al.* 2006) and by mesothelial trauma at a remote site (Schonman, Corona *et al.*, 2009) believed to act through mesothelial hypoxia, mesothelial hyperoxia and reactive oxygen species (ROS), desiccation and trauma, respectively. Interestingly none of these factors did induce *de novo* adhesions in our model

Since the inflammatory reaction at the lesion site is widely believed to be a driving mechanism of adhesion formation (Guvenal, Cetin *et al.*, 2001;Siegler, Kontopoulos *et al.*, 1980;Luciano, Hauser *et al.*, 1983;Aldemir, Ozturk *et al.*, 2004;Celebioglu, Eslambouli *et al.*, 1999;Golan, Bernstein *et al.*, 1991;Tayyar and Basbug, 1999;Nishimura, Nakamura *et al.*, 1983;Nishimura, Nakamura *et al.*, 1984;Rodgers, Girgis *et al.*, 1990;Greene, Alwayn *et al.*, 2005), it was surprising that neither non-steroidal anti-inflammatory drugs (NSAIDs) such as cyclooxygenase (COX)-1 or COX-2 inhibitors, nor anti-TNF alpha neutralizing antibodies had any effect upon adhesion formation neither in our pure CO_2 pneumoperitoneum enhanced

adhesion model (Binda, M. M., Molinas, C. R. *et al.* 2007), nor in the hyperoxia-enhanced adhesion model (PP with more than 12% O_2) (Binda Koninckx BJOG 2010) (Binda, M. M., Molinas, C. R. *et al.* 2003). The absence of effect of anti-TNF alpha antibodies is surprising given the strong anti inflammatory effects found both in animals and in humans. In contrast, dexamethasone, a steroidal anti-inflammatory drug, reduced adhesions by 30% and 62% in the hypoxia- and hyperoxia-enhanced adhesions, respectively. Thus other inflammatory aspects than those controlled by COX-1, COX-2 and TNF alpha must be involved.

We therefore wanted to investigate the relationship between adhesion formation and acute inflammation in the entire peritoneal cavity.

Material and Methods

The laparoscopic mouse model for adhesion formation

The experimental setup (i.e., animals, anesthesia and ventilation, laparoscopic surgery and induction and scoring of peritoneal adhesions) has been described in detail previously (Molinas, Mynbaev *et al.*, 2001;Binda, Molinas *et al.*, 2004;Elkelani, Binda *et al.*, 2004;Molinas, Campo *et al.*, 2003b;Molinas, Campo *et al.*, 2003a;Molinas, Elkelani *et al.*, 2003;Elkelani, Molinas *et al.*, 2002). Briefly, the model consisted of a bipolar lesion made by laparoscopy followed by a pneumoperitoneum for 60 min. Since temperature affect adhesion formation, animals and all equipment were placed in a closed chamber at 37°C (heated air, WarmTouch, Patient Warming System, model 5700, Mallinckrodt Medical, Hazelwood, MO). Since anaesthesia and ventilation may influence body temperature (Binda, M. M., Molinas, C. R., Hansen, P., and Koninckx, P. R. 2006), the timing between anaesthesia (T0), intubation (at 10 min, T10)and the onset of the experiment (at 20 min, T20) was strictly controled.

Animals

The present study was performed in 9-10 weeks-old female BALB/c c mice weighting 20 to 24g. Animals were kept under standard laboratory conditions (temperature 20°C–22°C, relative humidity 50%–60%, 14 hours light and 10 hours dark) at the animal facilities of the Katholieke Universiteit Leuven (KUL). They were fed with a standard laboratory diet (MuraconG, Carsil Quality, Turnhout, Belgium) with free access to food and water at any time. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia/Intubation and ventilation

Animals were anesthetized at T0 with i.p. 0.08 mg/g pentobarbital (Nembutal, Sanofi Sante Animale, Brussels, Belgium). Animal preparation, intubation and ventilation with humidified air with a tidal volume of 250 µl at 160 strokes/min (Mouse Ventilator MiniVent, Type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) was started after 10 min exactly (T10).

Laparoscopic surgery

The surgical procedure to induce adhesions was the same for all groups as reported before. Briefly, at T20, the CO_2 pneumoperitoneum was initiated (Thermoflator, Karl Storz, Tuttlingen, Germany) and under direct vision with a 2 mm endoscope (Karl Storz, Tuttlingen Germany standardized 10 mm x 1.6 mm lesions were performed in the antimesenteric border of both right and left uterine horns and in both right and left pelvic side walls with bipolar coagulation (20W, standard coagulation mode, Autocon 350, Karl Storz, Tuttlingen Germany) through two 14-gauge catheters (Insyte-W, Vialon, Becton Dickinson, Madrid, Spain). For humidification, the Storz Humidifier 204320 33 (Karl Storz, Tuttlingen, Germany) was used.

Scoring of adhesions

Adhesions were scored qualitatively and quantitatively blindly under a stereomicroscope. 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), type (0: no adhesions; 1: filmy; 2: dense; 3: capillaries present) and tenacity (0: no adhesions; 1: easily fall apart; 2: require traction; 3: require sharp dissection) of adhesions, the sum of extent, type and tenacity, which was the total score, was calculated . The quantitative scoring system assessed the proportion

175 of the lesions covered by adhesions. The results are presented as the average of the adhesions
176 formed at the four sites (right and left visceral and parietal peritoneum), which were
177 individually scored.
178
179 *The histological inflammation score.*
180 We developed a scoring system of acute inflammation based upon the known
181 pathophysiology of acute inflammation (Krenn, Morawietz *et al.*, 2002;Krenn, Morawietz *et*
182 *al.*, 2006;Haywood, McWilliams *et al.*, 2003;Pessler, Dai *et al.*, 2008). Acute inflammation is
183 a rapid response to an injury and serves to deliver mediators of host defense such as
184 leukocytes and plasma proteins to the site of injury. Acute inflammation has three major
185 components: (a) alterations in vascular caliber that lead to an increase in blood flow, (b) new
186 blood vessel formation and structural changes in the microvasculature that permit plasma
187 proteins and leukocytes to leave the circulation, and (c) diapedesis of leukocytes from the
188 microcirculation and their accumulation in the focus of injury and their activation (Robbins
189 and Cotran 2005). In our scoring system, we evaluated (a) the number of vessels, reflecting
190 the neoangiogenesis, (b) the number of PMNs in diapedesis, reflecting the increased
191 permeability of the vessels; and (c) the PMNs accumulation on the site of the injury. Each
192 parameter was scored as follows: neoangiogenesis (0 = number of vessels < 3, 1 = number of
193 vessels 4-8, 2 = number of vessels 9-12, 3 = number of vessels >12), increasing of
194 permeability (0 = number of PMNs in diapedesis 0, 1 = number of PMNs in diapedesis <2, 2
195 = number of PMNs in diapedesis 3-4, 3 = n° PMNs in diapedesis >4) and leukocytes
196 activation and accumulation (0 = n° PMNs <3, 1 = number of PMNs 4-8, 2 = number of
197 PMNs 9-12, 3 = number of PMNs >12). Total inflammation score was considered as the sum
198 of the neoangiogenesis, permeability and leukocyte activation scores.
199

Histology and immunohistochemistry.

Under microscopic vision a biopsy of the lesions was taken during laparotomy. The skin and muscles were dissected from the peritoneum and, in order to take a biopsy of the lesion and of the surrounding peritoneum, a tissue adherant (Lyostipt, Braun) was applied covering 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 (Beckstead J.H. 1995, J. Histochem. Cytochem. 43,345, letter) for 24 hours, embedded in paraffin, oriented and four 4-6µm sections were taken perpendicularly to the surface and perpendicularly to the lesion. Each section thus permitted to evaluate changes of the lesion and of the surrounding peritoneum from the surface to the depth of the biopsy. Sections were immunohistochemically stained for CD45 to detect leukocytes (LCA, Ly-5, T200) (BD Pharmigen) in citrate bluffer 80°C, pH 6, dilution 1/400.

Inflammation parameters were blindly scored at the biopsies under a microscope with a camera for imaging system (Axio scope, Axio cam MRc5, KS 400 imaging system, Zeiss, Germany) . For each slide, 4 high power fields were randomly chosen, 2 centrally at the level of the lesion and 2 at the surrounding of the lesion (1 for each side) and each vessel (the number of vessels indicating neoangiogenesis), each PMN in diapedesis (permeability of vessels) and each leukocyte stained by CD45 (number of activated leucocytes) were counted.

Experiment Design

These experiments were designed to investigate the effect of factors known to enhance adhesion formation (CO_2 pneumoperitoneum and manipulation) or to decrease adhesions (dexamethasone) upon the inflammation score. After anaesthesia, a laparotomy was performed to score adhesions and to take biopsies at the level of the lesion and at the parietal peritoneum.

Experiment I. It was designed to determine which day after surgery acute inflammation and adhesion formation should be scored. After 60 min of CO_2 pneumoperitoneum (n=8) inflammation and adhesion scores were evaluated after 1, 2, 4 and 7 days following surgery (2 mice per day) (Fig. 1). Based upon the results of this experiment, we decided to evaluate inflammation and adhesions on the second day i.e. 48 hours after surgery in all further experiments.

Experiment II**.** It was designed to evaluate the inflammation score and adhesions in control animals (Group I, 60 min of CO_2 pneumoperitoneum with 4% O_2), in animals with 60 min CO_2 pneumoperitoneum-enhanced adhesions (group II), in mice in which the CO_2 pneumoperitoneum-enhanced adhesions were further enhanced with manipulation (group III) and in mice with enhanced adhesion formation as in group III, which received in addition dexamethasone (Group IV) known to decrease adhesions. Manipulation enhanced adhesions consisted of manipulating fat and bowels in the upper abdomen with a 1.5 mm non-traumatic grasper for 5 min as previously described (Schonman, Corona *et al.*, 2009). Dexamethaxone (Aacidaxim 5 mg for injection; Organon, Bruxelles, Belgium) was given intraperitoneally, 40 µg immediately after the end of pneumoperitoneum and 40 µg 24 hours later . The experiment was block randomised by day meaning that one mouse of each group was done randomly the same day (16 mice: 4 mice per group). After 48 hours adhesion formation was scored and biopsies were taken at the level of the lesion.

Experiment III. It was similar to experiment II, but the inflammation was score besides at the lesion site, in the parietal peritoneum.

Statistics

Statistical analyses were performed with the SAS System (SAS Institute, Cary, NC). Differences in adhesion formation were evaluated with the Wilcoxon test. The correlation between adhesion and inflammation total scores was evaluated with the Spearman's correlation test . All the data are presented as the mean ± standard deviation of the mean.

274 **Results**

275 Experiment I. The time courses of both the total adhesion and the quantitative adhesion scores
276 were similar (fig. 1). Whereas the quantitative adhesion scores increased progressively, being
277 after 24, 48, 72 hrs and 7 days, 2.32 ±0.29, 3.01±0.18, 3.35±0.31 and 3.74±0.32, respectively,
278 the total inflammation scores decreased progressively being 5.05±0.27, 3.45±0.22, 2.5±0.40,
279 and 2.38±0.25 respectively (Fig 1). Also the scores of neoangiogenesis, diapedesis and
280 leukocytes accumulation decreased progressively. With these data, we decided to evaluate
281 acute inflammation and adhesion formation on day 2 in all subsequent experiments.
282 Experiment II. The adhesion scores on day 2 confirmed previous observations on day 7 after
283 surgery(Schonman, Corona *et al.*, 2009) (Fig. 2). In comparison with the control group (group
284 I: CO_2 pneumoperitoneum with 4% O_2), both total and proportion of adhesions increased
285 when a pure CO_2 pneumoperitoneum was used (group II: hypoxia-enhanced adhesions ;
286 P=0.007 and P = 0.0053, respectively). Adhesions further increased in group III (P<0.0001 for
287 both total and proportion). The addition of dexamethasone (group IV) decreased adhesion
288 formation (P<0.0001 for both total adhesion score and proportion).
289 The total inflammation score at the central part of the surgical lesion was slightly higher in
290 comparison to the inflammation score at 0.5 cm from the lesion, being 1.5±0.40 and 2.625
291 ±0.25 (p=0.0203), 0.625±0.25 and 2.125±0.25 (p= 0.0154), 4.125±0.25 and 6.375±0.25
292 (p=0.0321), 2.375±0.25 and 3.875±0.25 (p= 0.0591) for groups I, II, III and IV, respectively.
293 We therefore used the mean of the inflammation scores in the central part and in the
294 periphery of the lesion to correlate inflammation with adhesion formation.
295 Depth of the inflammatory reaction spanned 2 mm till a maximum of 4 mm in group III
296 which had the highest inflammation score.
297

The total inflammation score at the lesion strongly correlated with and indeed were strikingly similar to the adhesion scores (Fig. 3 and 4).At the level of the surgical lesion the use of pure CO_2 pneumoperitoneum in comparison with group I slightly increased the total inflammation score ($p=0.07$), neoangiogenesis ($p=0.0577$) and lymphocytes accumulation ($p=0.0796$). In group III, the inflammation score further increased i.e. the total inflammation (group II versus group III $P<0.0001$), neoangiogenesis ($P=0.0007$), vasodilatation and permeability ($P=0.0052$) and lymphohistiocytic activation and accumulation ($p=0.0022$). When dexamethasone was added after surgery total mean inflammation score decreased (group III vs group IV: $P<0.0001$), an effect observed for all parameters i.e. neoangiogenesis ($p=0.0154$), diapedesis ($P=0.0016$) and lymphocytes activation-accumulation ($P=0.001$). Most strikingly, however, was the strong correlation between adhesion scores and inflammatory parameters (table 1). Total acute inflammation score (fig. 2), neoangiogenesis, diapedesis and leukocytes accumulation (table 1), strongly correlated with total adhesion scores.

Experiment III. This experiment confirmed that inflammation scores of the parietal peritoneum were comparable to the inflammation score at the surgical lesion for the 4 groups (Fig 4). Indeed the use of pure CO_2 pneumoperitoneum in comparison with CO_2 + 3% O_2 increased slightly the total inflammation score although the comparison was not statistically significant ($P= 0.06$). When in addition to the pure CO_2 pneumoperitoneum, manipulation was added the total inflammation score increased further (group II versus group III: $P<0.0001$). When dexamethasone was added total inflammation score decreased (group III vs group IV: $P<0.0001$) (Fig. 4).

Adhesion scores were also comparable to the adhesion scores of experiment II (Fig. 4).

Discussion

These data confirm and extend our previous observations on adhesion formation in our laparoscopic mouse model. Indeed, in comparison with a pneumoperitoneum with 96%CO_2 + 4% O_2, pure CO_2 pneumoperitoneum increase adhesions also on day 2 (Elkelani, Binda *et al.*, 2004;Elkelani, Binda *et al.*, 2004;Binda, Molinas *et al.*, 2004;Molinas, Mynbaev *et al.*, 2001). When a mechanical trauma is added in addition to the pneumoperitoneum, , adhesions are increased at the lesion site even further without causing *de novo* adhesions (Schonman, Corona *et al.*, 2009). We also confirmed that dexamethasone decrease adhesions(Schonman, R., Corona, R. *et al.* 2009). The similarity between adhesion formation and acute inflammation scores is striking, especially the linear correlation between adhesion and inflammation scores in all the groups. This suggests that acute inflammation is an important common and driving mechanism for adhesion formation. The similarity of acute inflammation at the lesion level, at its periphery 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. Especially the effect of bowel manipulation in the upper abdomen i.e. at a distance from the bipolar lesion strongly supports the concept that some peritoneal cavity factors stimulated by the mesothelial trauma can enhance adhesion formation at the lesion site. The absence of *de novo* adhesions confirms the concept that adhesion formation requires a peritoneal trauma. The extend and severity of adhesions, however, largely vary with the inflammatory reaction in the entire peritoneal cavity.

The mechanism by which the inflammatory reaction of the entire peritoneal cavity affects adhesion formation at the lesion site is still unclear. Since mesothelial cells are known to retract and to bulge during CO_2 pneumoperitoneum without affecting the basal membrane, and since 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 further affecting adhesion formation at the lesion site. The first candidate, to be investigated in further experiments are chemokines known as important inflammatory mediators involved in the activation and migration of leukocytes into the tissue (Adams, D. H. and Lloyd, A. R. 1997).Indeed according to the position of the first two cysteine residues (Murphy, P. M. 1994), some chemokines are chemoattractants and activators of non-PMNs leukocytes while others attract neutrophils.

The second candidates are cells such as macrophages, leucocytes attracted into the peritoneal cavity and their secretion products as cytokines. Since non-steroidal anti-inflammatory drugs(NSAIDs), as ibuprofen, tenoxicam, nimesulide, parecoxib, and anti-TNF-alpha anti-TNF alpha neutralizing antibodies were ineffective in reducing adhesions in our model, we postulate that these drugs did not affect the acute inflammatory reaction (Binda, Molinas *et al.*, 2007b), something to be confirmed in the future.

In this study, dexamethasone did decrease the acute inflammatory reaction and to a similar extend adhesion formation as previously demonstrated (Binda, Molinas *et al.*, 2007; Binda and Koninckx, 2009). This suggest that dexamethasone is acting through other mechanisms such as inhibition of fibroblast proliferation, depression of procollagen gene expression through a decreased transforming growth factor secretion (Bladh, Johansson-Haque *et al.*, 2009), or by immunosuppressive effects or by cytokines (Brunton, L. L., Lazo, J. S. *et al.* 2006) .

Our data are tempting to conclude that in the process of adhesion formation acute inflammation of the peritoneal cavity is quantitatively the most important factor. Although the exact mechanism remains unclear the following mechanisms could be involved. Since it was demonstrated that dexamethasone produces its anti-inflammatory effect by inducing the expression of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), we

postulate that this pathway could be involved in the adhesion formation process. The MAP kinase phospatase (MKP)-1 is a negative regulator of cytokines production in innate immune cells (Wang, X. X., Nelin, L. D. *et al.* 2008) and it has a negative regulation effect on the mitogen-activated proteine kinases (MAPKs) production (Wang, X. X., Nelin, L. D., Kuhlman, J. R., Meng, X. M., Welty, S. E., and Liu, Y. S. 2008). MAPKs include the extracellular signal-regulating kinase (ERK), p38 MAPK and c-Jun N-Terminal proteine Kinase (JNK) and they all play an important role in cell proliferation, apoptosis and many other nuclear events. MKP-1 has been shown to inhibit a number of cellular responses mediated by ERK and p38 MAPK.

MAPKs also regulate the metalloproteinases (MMPs) and MMPs have a role in fibrinolysis and in adhesion formation. A key factor in adhesion prevention is fibrinolysis, which is regulated by the plasminogen system. The inactive proenzyme plasminogen is converted into plasmin by tissue-type plasminogen activator (tPA) and/or urokinase type plasminogen activator (uPA). The fibrin matrix serves as a scaffold for fibroblasts and capillary ingrowth and for extracelullar matrix (ECM) deposition. During normal healing, the fibrin matrix is rapidly removed and the ECM will be degraded by MMPs. The traditional concept is that when the fibrin matrix persists too long, or when the ECM degradation is inhibited, peritoneal adhesions will be formed. In addition, MMP-2 was demonstrated to be expressed in mature human peritoneal adhesions (Binnebosel, Klinge *et al.*, 2008).

Moreover, MMPs have also been implicated as important factor in the control of the tumor implantation . MMPs plays roles in pathological conditions involving untimely and accelerated turnover of extracellular matrix, including inflammation, angiogenesis and metastasis (Nakano, Tani *et al.*, 1995;Price, Farrar *et al.*, 2001;Rao, 2003). Among MMPs, we focus our attention on matrix metalloproteinase 2, a secreted endopeptidase homologous

with interstitial collagenase but which possesses an additional fibronectin-like domain. Specific cell surface receptors bind to fibronectines. These receptors include the traditional fibronectin receptor, also called integrin alpha5 beta1, the major fibronectin receptor on most cells, and several other integrins. Several studies have shown that the adhesive extracellular matrix protein fibronectin and its integrin receptors function in certain types of adhesive contact as well as playing a major role in matrix assembly (Yubero, S., Ramudo, L. *et al.* 2009). Integrin ligands, such as fibronectin, are not passive adhesive molecules but are active participants in the cell adhesive process that leads to signal transduction (Ruoslahti, E. 1999). MMPs secretion is stimulated also by nitric oxide (NO), induced by the expression of the gene nitric oxide synthase (i-NOS), also associated to the angiogenesis process (Yubero, S., Ramudo, L., Manso, M. A., and De Dios, I. 2009).

This enzyme is associated to the angiogenesis process (Yubero, S., Ramudo, L., Manso, M. A., and De Dios, I. 2009) and, in addition its expression, like the MPK-1expression , is also inhibited by dexamethasone (Lin, Jan *et al.*, 2008). These two important effects of dexamethasone, not observed using NSAIDs, would explain why dexamethasone is the only anti-inflammatory drug strongly effective on adhaesion prevention.

To summarise, the inflammation at the peritoneal cavity level, due to the mesothelial trauma, causes an activation of a signal transduction pathway like MAPKs that induces an expression of MMPs; at the same time, due to a local wound following the surgery, there is afibrin matrix and extracelullar matrix (ECM) deposition. We speculate that this two simultaneous events bring to an over production of MMP- 2 that, miming the effect of the fibronectin, increase the fibrin and the extracellular matrix assembling that lead to adhaesion formation, confirming the traditional concept of fibrin matrix persisting and insufficient ECM degradation.

In conclusion, these data strongly suggest that acute inflammation in the entire peritoneum cavity is the driving mechanism of adhesion formation at the lesion in our laparoscopic mouse

model. In addition, MMPs may play an important role being a link between the two processes. Of course, new experiments should be done to confirm our hypothesis.

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Figure 1: Total adhesion and inflammation score during the first 7 days after a surgical bipolar lesion and 60 minutes of CO_2 pneumoperitoneum.

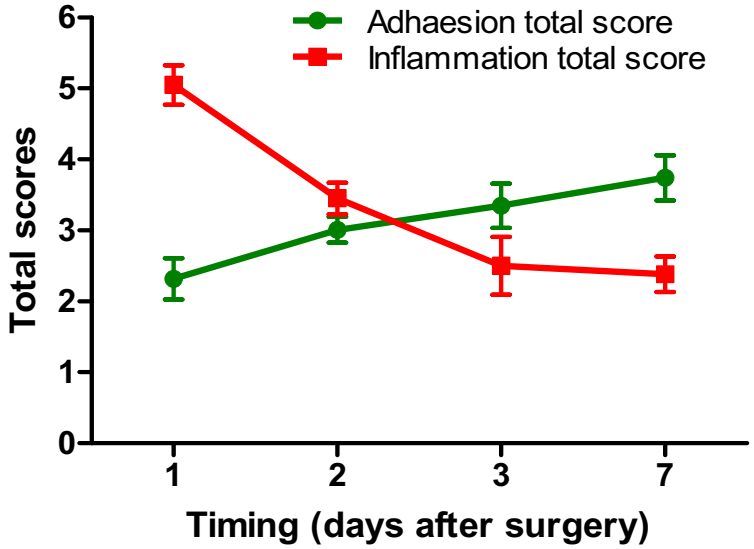

Figure 2: Correlation between total adhesion and inflammation scores (Statistics: $p < 0.0001$, Spearman Test, for details see table 1 yellow highlighted).

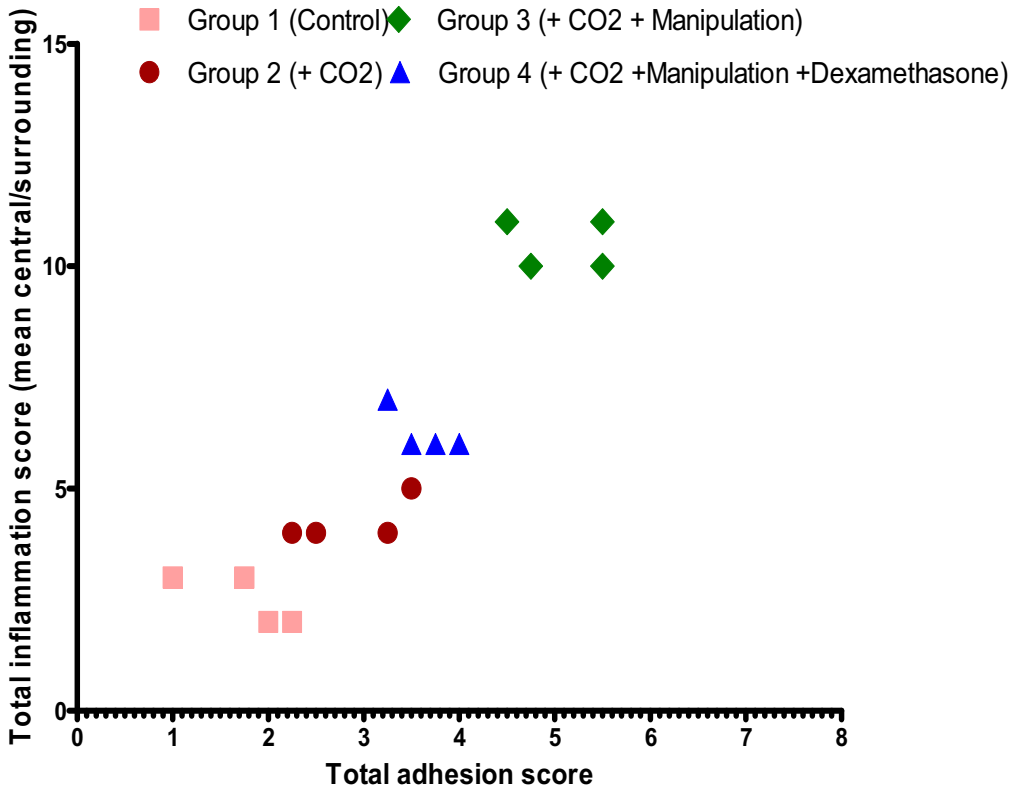

Fig. 3. The inflammation scores (mean and SD) for the different parameters analyzed.

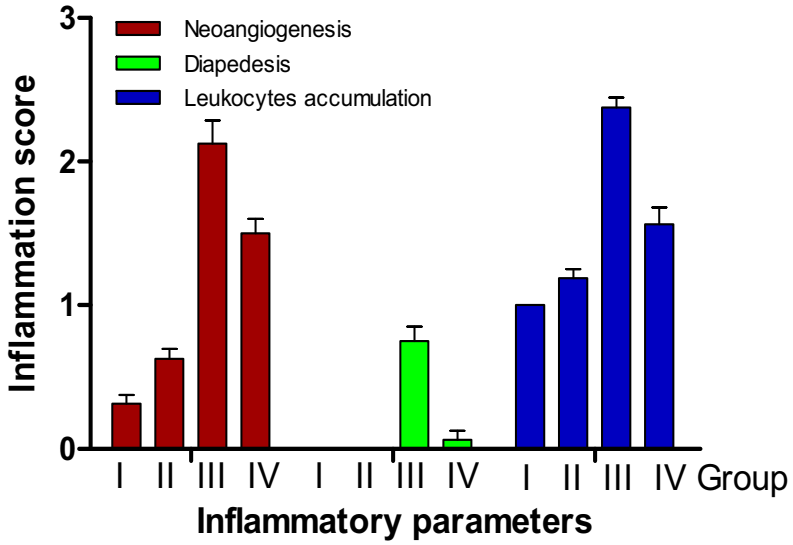

Fig. 4. Total adhesion and inflammation score at the surgical lesion (experiment II) and at the parietal peritoneum (experiment III).

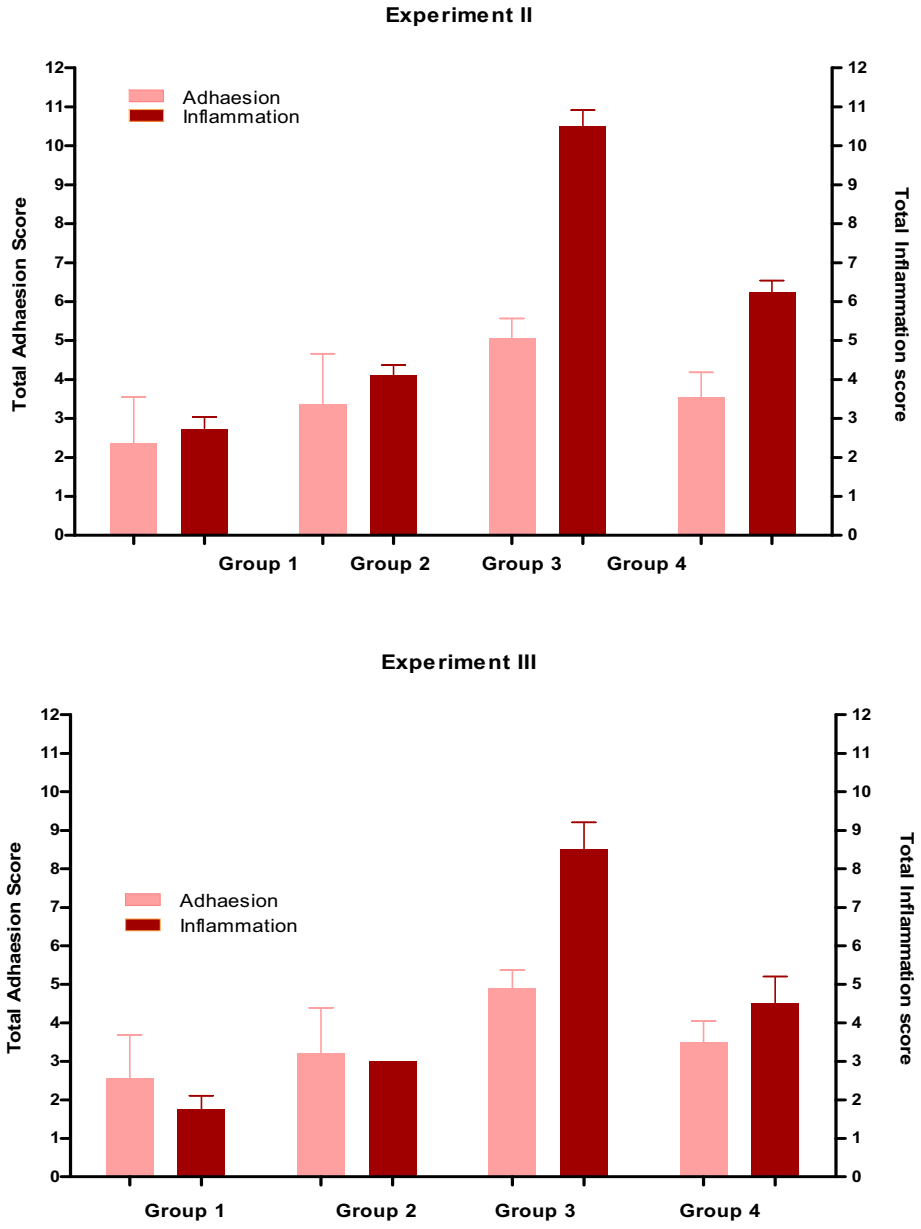

Table 1. The table shows the p values for the correlations between the quantitative and qualitative adhesions scoring system and the inflammation scoring. (for yellow fields see Fig. 2). Statistics: Spearman correlation.

Inflammatory parameters	Biopsy Location	Adhesion score (P Value)				
		Total	**Extent**	**Type**	**Tenacity**	**Proportion (%)**
Neoangiogenesis	Central Surrounding	0.0002 0.0002	NS NS	<0.0001 <0.0001	0.0032 0.0021	NS NS
Permeability	Central Surrounding	0.0391 0.0286	NS NS	0.0411 0.0382	NS NS	NS NS
Leucocytes accumulation	Central Surrounding	0.0003 0.0002	0.0043 0.0032	0.0029 0.0021	<0.0001 <0.0001	0.0031 0.0028
Total score	Central Surrounding	<0.0001 <0.0001	0.0017 0.0005	<0.0001 <0.0001	<0.0001 <0.0001	0.0005 0.0005