

Original Article

Surgical trauma and CO₂-insufflation impact on adhesion formation in parietal and visceral peritoneal lesions

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Abstract: CO₂-insufflation and electrocoagulation were advanced as causative factors of postsurgical adhesions. We assumed that severe tissue reaction due to electrocoagulation might obscure CO₂-insufflation impact on adhesion formation. Therefore, the purpose of this study was to evaluate the effects and interactions of surgical trauma and CO₂-insufflation on adhesion formation. Prospective-randomized study with 60 rats, equally divided into 3 groups. In the control group, the sidewall adhesion model was induced by monopolar coagulation of the uterine horn and ipsilateral parietal peritoneum and by mechanical damaging – in the opposite side through open laparoscopy without CO₂-insufflation. In two other groups, CO₂ was insufflated for 60 min at 15 cm of water, either before or after the sidewall model-induction. Parameters of sidewall and lesion site adhesions of parietal peritoneum and uterine horns were evaluated by scoring system and analyzed by two-way ANOVA with Bonferroni posttests, one-way ANOVA Student-Newman-Keuls multiple comparisons test, as well as by two-tailed unpaired Mann-Whitney test. Monopolar coagulation significantly increased peritoneal lesion site adhesion scores, as compared with the scores for mechanical damaging (p=0.0001). Visceral peritoneal lesion sites were more predisposed to adhesion formation than parietal peritoneal lesion sites (p=0.0009), whereas CO₂ did not affect parameters of either sidewall or peritoneal lesion site adhesions, regardless of the insufflation mode (p>0.05). The data suggest that both surgical trauma and peritoneal lesion sites had a substantial impact on adhesion formation, whereas CO₂ did not interfere with adhesion parameters irrespective of its insufflation mode. These findings may improve our insights into adhesion formation pathophysiology and open new perspectives in developing future adhesion prevention strategies.

Keywords: Sidewall, lesion site adhesions, CO₂-pneumoperitoneum, surgical trauma, parietal, visceral peritoneum

Introduction

Adhesion formation is a major unfavorable outcome of surgical procedures. It is a health care concern because it triggers the necessity for repeated surgical procedures in order to treat

inherent complications such as intestinal obstructions, abdominal/pelvic pain and infertility. When laparoscopy was introduced as a surgical approach in the 80s, it was expected that laparoscopy in itself would substantially decrease adhesion formation since it was to

exclude macrosurgery “adhesiogenic” factors. Then, when observations, presenting adhesion formation after laparoscopic surgical procedures accumulated, researchers started to study the impact of laparoscopic conditions on adhesion formation resulting in controversial conclusions [1]. Setting up randomized clinical trials studying the impact of CO₂-pneumoperitoneum is questionable due to the need for repeated laparoscopy and the ethical justification restrictions in their design, therefore, mostly animal models were used [2-5].

Impact of CO₂-insufflation on adhesion formation was proved in several studies [4, 6, 7] and disproved in other research [5]. As a mechanism of CO₂-related adhesion formation, it was suggested that CO₂-insufflation impact on intraperitoneal tissue and parenchymatous organs is accompanied by disturbances in local cell protective immunity [8] and by depression of abdominal tissue plasminogen activator (tPA) activity [9, 10] and expression of plasminogen activator inhibitor type1 (PAI-1) in mesothelial cells [10].

However, these hypotheses were contradicted with results of other studies. So, Lee et al [11, 12] demonstrated similarities of macrophages' function in rats after laparoscopic-assisted cecectomy with that of macrophages in rats suffered anesthesia alone. Ziprin et al [12] presented that CO₂ environment enhances mesothelial cell fibrinolytic activity via down-regulation of PAI-1. Whereas Hanly et al [13] proposed pneumoperitoneum-mediated attenuation of the inflammatory response after laparoscopic surgery occurs via a mechanism of peritoneal cell acidification, confirming findings by Kopernik et al [14], who showed suppressed inflammatory and metabolic responses of peritoneal cells exposed to a high CO₂ insufflation pressure environment.

Indeed, Hirota et al [15] presented significant adhesion formation extension after monopolar coagulation (MC), as compared with adhesion formation by manipulations with other surgical instruments in a juvenile pig model with laparoscopic bilateral resection of the uterine horns under general anaesthesia and pneumoperitoneum. These findings were in accord with results of several teams comparing different modes of surgical trauma and adhesion prevention adjuvants in animal models [16-18].

It was shown that surgical trauma, induced by electrocauterization, causes the largest and deepest ischemic peritoneal tissue damage [19, 20], tissue carbonization [21], impaired healing patterns [22], severe inflammation [23], ultrastructural cell alterations [24], resulting in a severe and extended adhesion formation in experiments, either with CO₂-insufflation or without.

According to these findings, CO₂-insufflation impact on adhesion formation may be obscured because surgical trauma also considerably contributes to this process. Subsequently, we assumed that the effect of gases used for the pneumoperitoneum would be correctly examined if the adhesion formation models were to be established by different types of surgical trauma.

Proceeding from this reasoning, we undertook the present study to determine surgical trauma type and CO₂-insufflation impact and their interactions on sidewall and peritoneal/visceral lesion site adhesion formation.

Materials and methods

The experiment was done in the Laboratory of Pathologic Physiology, Faculty of Basic Medicine and the Laboratory of Fermentative Fibrinolysis, Faculty of Biology of the M.B. Lomonosov Moscow State University, by permission (and ethical approval) of the University Review Board and Animal Care Committee in accord with Basic Principles for all Medical Research of the Declaration of Helsinki by the World Medical Association – (1964-2008, www.wma.net).

Animals

60 white adult female Wistar rats (6 months old), in weight 210-230 g, were acclimated to 20 - 25°C, with air humidity 40 - 70%, with a 14-hour day and 10-hour night cycle on a standard laboratory diet with free approach to food and water for 10 days before the onset of this study at the Laboratory for Animal Care, Faculty of Basic Medicine, M.B. Lomonosov Moscow State University. Animals were kept in vivarium according to the Guide for the Care and Use of Laboratory Animals [25].

For six hours before every operation, 15 rats were prepared by food fasting whereas water was not restricted.

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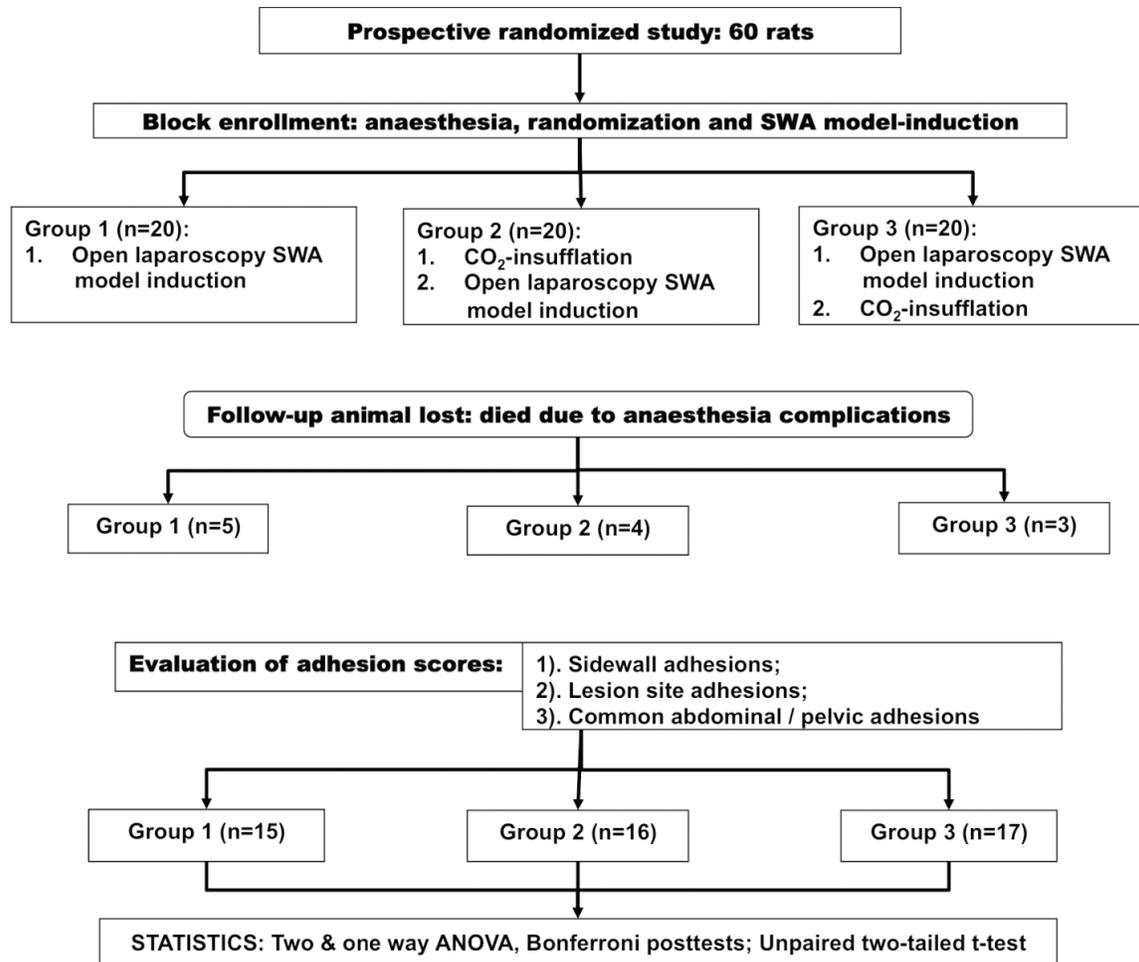


Figure 1. Study design (an explanation in the text).

Anaesthesia

Anaesthesia was achieved by intramuscular fractional injection of 50 mg/kg of Thiopentalum Sodium in the Musculus Femoralis. The amount of injected Thiopentalum Sodium and the duration of the anaesthesia were identical in all groups.

Study design

In this prospective-experimental study, the anaesthetized animals were randomly and equally divided into three groups (**Figure 1**). The sample size was estimated as 15 animals per group for two-way ANOVA with 3 fixed groups (columns) and 2 levels (rows) at the power 80% and $\alpha=0.05$ by means of the online Java applets for power and sample size. Therefore, taking into account possible animal losses due to

anaesthesia and surgery we included 20 animals per group. In the 1st group, the sidewall adhesion (SWA) model was created by mechanical damaging (MD) in one side of the parietal peritoneum (PP) and uterine horns (UH) and by MC in the opposite side without CO₂-insufflation. In the two other groups, in addition to the SWA model induction, CO₂ was insufflated for 60 min with intraperitoneal pressure at 15 cm of water. In the 2nd group CO₂ was insufflated before the SWA model induction and in the 3rd group – after the SWA model induction. Time of surgery in all groups was standardised by prolonged anaesthesia for 60 min in the 1st group. Our choice of this study design was predetermined by a similar successful study design by Sahakian et al [26] with analogous sample size, 3 surgical groups and CO₂-insufflation after adhesion model-induced via laparotomy incision. Adhesions were evaluated after 7 days.

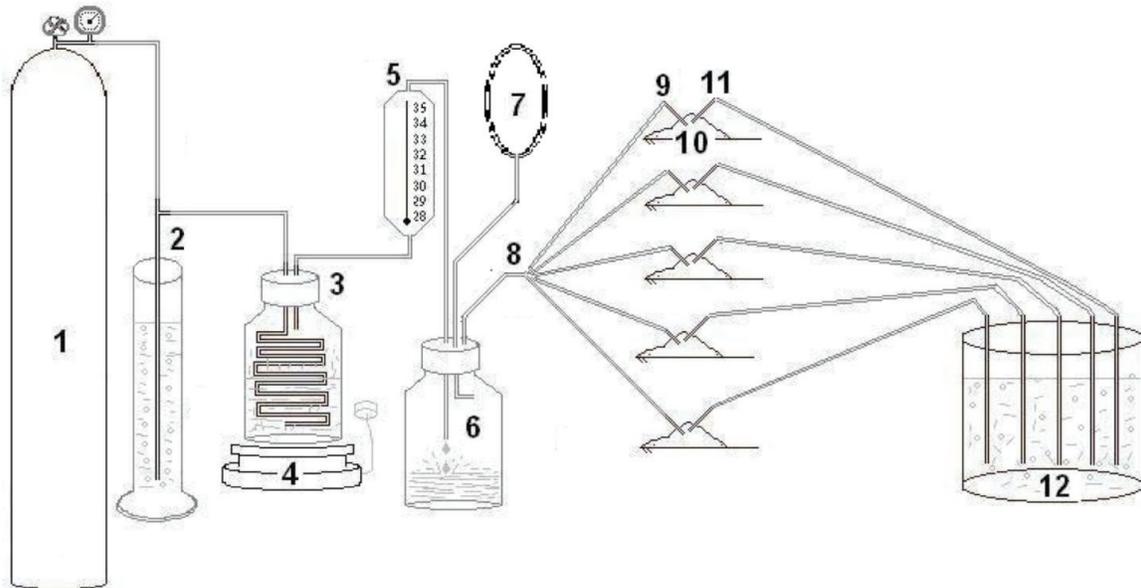


Figure 2. CO₂-insufflation setup (an explanation in the text).

CO₂-insufflation setup

Our modified from Pismensky et al [27] homemade CO₂-insufflation setup (**Figure 2**) included the CO₂ cylinder (1) connected sequentially with a water valve (2), a humidifier (3), a heating device (4) with thermometer (5), the excess water reservoir (6), an elastic balloon (7) and a distributor (8). Functions of the water valve, the humidifier, the heating device, the excess water reservoir and the balloon were meant for regulation of CO₂ gas temperature and humidity, its flow rate and to dampen insufflation pressure fluctuations. The distributor evenly allocated the CO₂ gas current into 5 small tubes, which were connected with the let in 18 G catheter (9) HELMFLON®/HELMSYTE® (HELM India PVT Ltd) inserted into the rats' abdomen (10). Another 5 outlet 20 G catheters (11) were inserted into the abdominal cavity of the rats, which were connected with the second water valve (12) to control intraabdominal pressure. When the intraperitoneal pressure was achieved at 15 cm of water, the excess amount of CO₂ was deflated through the second water valve. The CO₂ was simultaneously insufflated in 5 animals and the temperature in the system was kept at 37°C.

The sidewall adhesions model-induction

In aseptic conditions, on the middle line of the abdomen, a two-centimeter incision was made

and expanded by eye speculum (Murdock titanium eye speculum, "Microsurgical Instruments" CJSC, Kazan, Russian Federation). Then, all surgical procedures were performed by open laparoscopy using a fiber headlight magnifying glass (HLMG) LNH400 (Scaner, Cherkassy city, Ukraine). We gently manipulated using two fine, long tissue forceps De Bakey, 240 mm ("Microsurgical Instruments" CJSC, Kazan, Russian Federation). In addition, during SWA model-induction, CO₂ from an extra source was constantly and slowly insufflated into the abdominal cavity, so as to make a local CO₂ environment acting as a barrier to exclude room air contact with peritoneal tissue. For this reason, a wet, air-tight, rolled-up surgical drape was fixed round the surgical field borders. Since carbon dioxide molecules are heavier than oxygen molecules, we assumed that CO₂ accumulates within the confinement of the rolled drape surrounding the surgical field.

The target UH was exposed and fixed for manipulation by gentle straightening. This procedure was done by the assistant who, using fine tissue forceps gently grasped the UH's proximal part at the bifurcation of two horns and with another forceps gently grasped the distal part of the UH close to its oviduct's end.

The laparoscopic ball electrode, bent, Ø2 mm, length 100 mm (ERBE Elektromedizin GmbH, Germany) by the standard coagulation mode

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Table 1. Scoring system to evaluate the extent, type and tenacity of adhesions

Adhesion score		Scores
Attributes	Parameters	
Extent of adhesions	The size of adhesion induced area covered by adhesions	0
		<25
		26-50
		51-75
		>76
Type	No adhesions	0
	Individual (1) filmy, thin, avascular, transparent, short adhesions	1
	Individual (up to 3) thin, filmy avascular, semi-diaphanous or thick opaque, filmy adhesions	2
	Multiple, dense thick opaque, filmy adhesions with visual, but thin vessels	3
	Multiple, dense thick opaque, filmy adhesions with visual, large (rasping) vessels or capillaries	4
Tenacity	No adhesions	0
	The adhesions freely separated during research	1
	The adhesions separated with traction	2
	The separation of the adhesions required sharp dissection	3
Total score		0-11

with 35 watts energy (Autocon 200, Karl Storz Endoscope, Germany) was applied for MC. With one hand, the surgeon held up the wound edges by the eye speculum. Holding the electrode in the other hand, he performed a visible, linear destruction with necrosis in the middle of the lesion; blanching and contracting its edges while gentle touching with a tip of the electrode and moving straight along the antimesenteric serosal surface of the target UH. Then, the ipsilateral sidewall PP was exposed by gently lifting the ipsilateral border of the abdominal wound and three parallel linear lesions were thus made with their length of 2.5 cm on the sidewall PP.

The special, homemade device (brush for scratching) was developed for a MD. Our choice of this technique was predetermined because scratching both contacting peritoneal surfaces triggered adhesion formation in 100% cases in a murine SWA model by Haney and Doty [28]. In the opposite side both ipsilateral UH and PP in turn was similarly exposed for manipulation. Then, an antimesenteric serosal surface along the target UH was gently scratched with one touch, whereas sidewall PP was gently scratched producing 3 parallel lesion lines with their length of 2.5 cm on the sidewall PP. MD triggered diffuse hemorrhages without bleeding in both UH and PP surfaces.

Thus, the SWA inducing model was performed, using one of the two methods (MC or MD) in the

opposite sites in each animal. Both ipsilateral sidewall PP and UHs' visceral peritoneum wounds were accurately tracked. Lesion sites were chosen randomly, either in the left or in the right side in turn. Standardization of surgical trauma was controlled by surgical trauma size as well as by the severity of the procedures inducing visible macroscopic changes in lesion sites being equal in size and appearance.

The abdominal wall was closed by two layers of continuous sutures (Vicril 5/0, Ethicon, Johnson and Johnson). The first layer included the peritoneum, muscle and aponeurosis of the abdomen and the second – the skin. All surgical procedures were performed by one surgeon and the duration of all surgical procedures was registered.

Main outcome measures

5, 4 and 3 animals died within the first day after surgery, respectively in the 1st, 2nd and 3rd groups; all due to anesthesia complications [29]. These rats were excluded from the study. The remaining animals were euthanized on the 7th day after surgery by intramuscular injection of an overdose of Hexenalm.

Overall adhesion severity was evaluated by a scoring system (Table 1) under 4.5-fold magnification by means of fiber headlight magnifying glass. Adhesions depending on their localization and side attachments were classified (Figure 3) in the following way:

Surgical trauma impact on adhesion formation

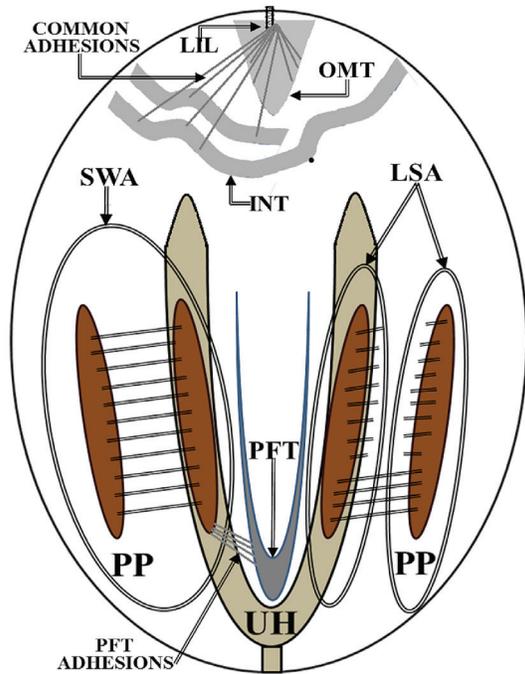


Figure 3. The schematic representation and classification of adhesions, which were registered after sidewall adhesion (SWA) model-induction. LIL - laparotomy incision line; OMT - omentum; INT - intestine; LSA - lesion site adhesions; PFT - pelvic fat tissue; PP - parietal peritoneum; UH - uterine horns. Common adhesions between LIL and OMT and INT; SWA between PP and UH; LSA within PP or UH, as well as part of SWA, corresponding to PP or UH.

- a). SWA – simultaneously attached with one side to UH and with another side to ipsilateral PP, presented as SWA: MD and MC.
- b). Lesion site adhesions (LSA) – which occurred within parietal or visceral peritoneal lesion sites (PP/HU) without both side attachments. Into this category of adhesions we also included the part of SWA (attached to PP or to UH) corresponding to PP or UH lesion sites and registered them as LSA: PPMD/PPMC and UHMD/UHMC.
- c). Common abdominal adhesions attached with one side to the laparotomy incision line (LIL) and with the other - to the abdominal organs.
- d). Common pelvic adhesions occurred between UH and other pelvic organs and structures were defined as pelvic fat tissue (PFT) adhesions.

Randomization

The experiment was done by block randomization: 15 animals were operated daily, 5 rats in each group. The assistants marked the anesthetized animals and randomly assigned them to the 1st, 2nd or 3rd group (Figure 4). At the end of each experiment, an assistant filled in the first individual protocol N1 for each rat. Then, protocols were each put in a separate envelope, which was immediately sealed.

Seven days later, the animals were randomly picked for evaluation. Then, adhesions were evaluated and the second protocol N2 was filled in for each animal with adhesion scoring results.

Finally, after the experiment had ended, the protocol N1 was matched with the protocol N2 for each rat by the outsider participant managing these procedures.

Statistics

Statistics were performed with an independent and experienced statistician, who was also blinded. All results were analyzed by Graph Pad Prism 5. Mean with SD or lower and upper 95% confidence intervals (CI) were given unless stated otherwise and differences less than ($p < 0.05$) were considered to be significant.

Following hypotheses (null - H_0 and alternative- H_a) were tested: $H_{01}(=)/H_{a1}(\neq)$ to test an impact of type of surgical trauma on adhesion parameters via: MD vs MC; $H_{02}(=)/H_{a2}(\neq)$ to test an impact of CO_2 insufflation on adhesion values via: group 1 vs group 2 vs group 3; $H_{03}(=)/H_{a3}(\neq)$ to test interactions between type of surgical trauma and CO_2 insufflation on adhesion parameters; $H_{04}(=)/H_{a4}(\neq)$ to test an impact of lesion sites on adhesion parameters via: PP vs UH; $H_{05}(=)/H_{a5}(\neq)$ to test interactions between lesion sites and type of surgical trauma and CO_2 insufflation on adhesion parameters; $H_{06}(=)/H_{a6}(\neq)$ to test interactions between lesion sites and CO_2 insufflation on adhesion parameters.

Parameters of sidewall and lesion site adhesions of parietal peritoneum and uterine horns were evaluated by scoring system and analyzed by two-way ANOVA with Bonferroni posttests, and one-way ANOVA Student-Neuman-Keuls multiple comparisons test, as well as by two-

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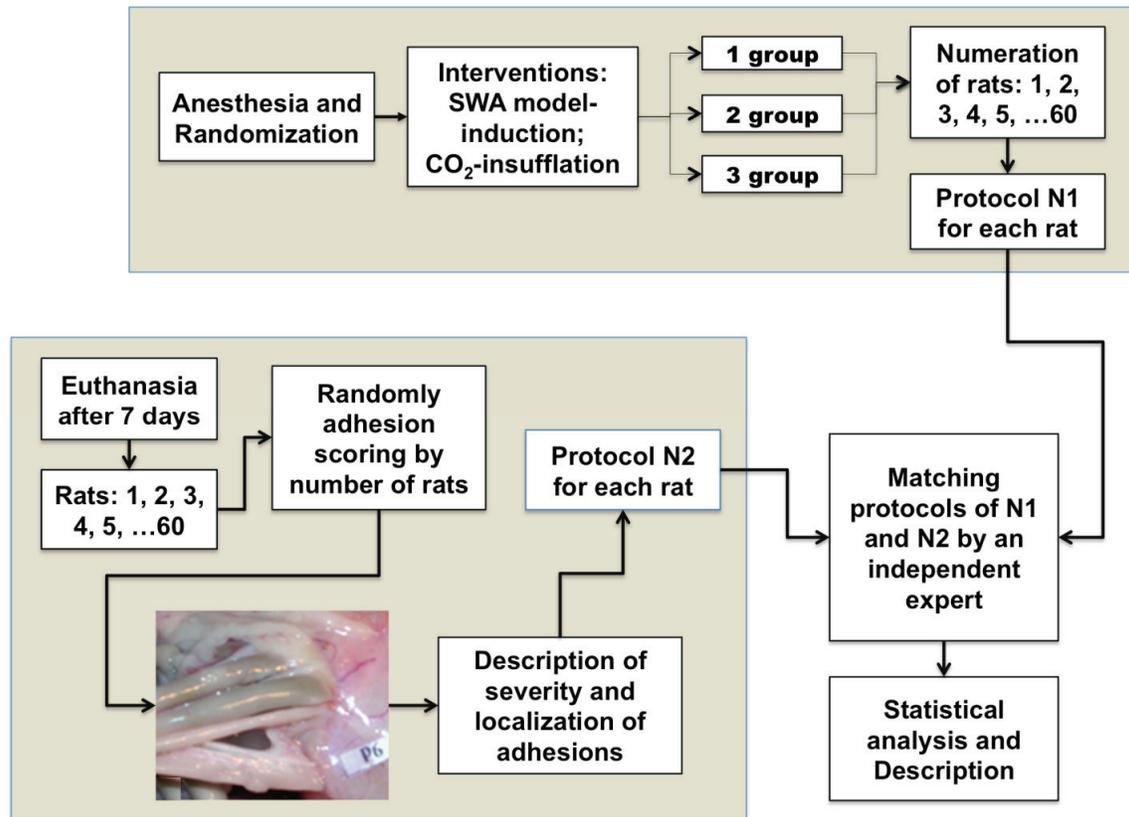


Figure 4. The schematic representation of block randomization steps (an explanation in the text).

Table 2. Hypotheses proposing factors, working hypotheses and results of their examination

Hypotheses proposing factors and working hypotheses	Null (Ho) and alternative (Ha) hypotheses	Sidewall adhesions (SWA)	Lesion site adhesions (LSA)	Pelvic fat tissue adhesions	Common abdominal adhesions*
Surgical trauma type (STT)	Ho1	confirmed	rejected	confirmed	NA
impact: MC vs MT	Ha1	rejected	confirmed	rejected	NA
CO ₂ insufflation impact; group 1 vs group 2 vs group 3	Ho2	confirmed	confirmed	confirmed	confirmed
	Ha2	rejected	rejected	rejected	rejected
Interactions between STT and CO ₂ insufflation	Ho3	confirmed	confirmed	confirmed	NA
	Ha3	rejected	rejected	rejected	NA
Peritoneum lesion site (PLS)	Ho4	NA	rejected	NA	NA
impact: PP vs UH	Ha4	NA	confirmed	NA	NA
	Ho5	NA	rejected	NA	NA
Interactions between: PLS and STT	Ha5	NA	confirmed	NA	NA
	Ho6	NA	confirmed	NA	NA
	Ha6	NA	rejected	NA	NA

Notes: *adhesions involved omentum and intestine. monopolar coagulation (MC); mechanical damaging (MD); parietal peritoneum (PP); uterine horns (UH).

tailed unpaired Mann-Whitney test. SWA parameters were analyzed to test an impact of type of surgical trauma (MD vs MC) and CO₂ insufflation (group 1 vs group 2 vs group 3) on adhesion values as well as interactions

between these hypotheses proposing factors, whereas LSA values (PPMD vs PPMC; UHMD vs UHMC; PPMD vs UHMD; PPMC vs UHMC) were additionally evaluated to test an impact of lesion sites on adhesion parameters and inter-

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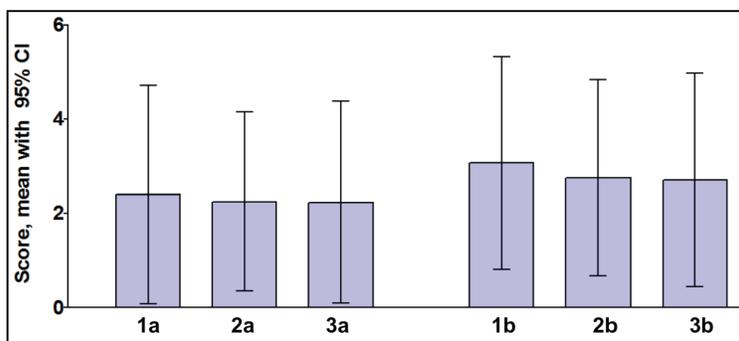


Figure 5. Scores of the sidewall adhesions (SWA) after mechanical damaging (1a, 2a, and 3a) and monopolar coagulation (1b, 2b and 3b). X - Groups and Y - Adhesion Score (Means with 95% confidence intervals are shown).

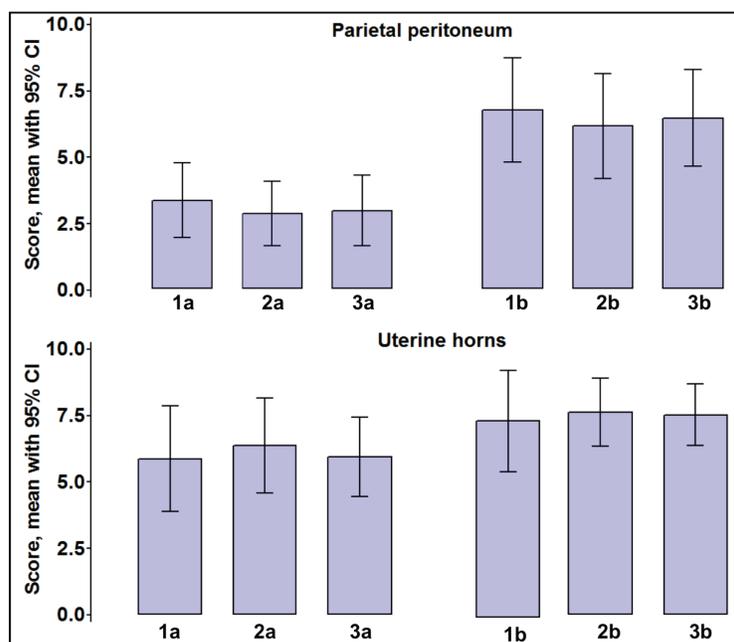


Figure 6. Scores of lesion site adhesions (LSA) after mechanical damaging (1a, 2a, and 3a) and monopolar coagulation (1b, 2b and 3b). X - Groups and Y - Adhesion Score (Means with 95% confidence intervals are shown).

actions of lesion sites with type of surgical trauma and CO₂ insufflation. Incorporated values of LSA were also analyzed to test differences between surgical trauma types (MDPP/MDUH vs MCPP/MCUH) as well as between parietal and visceral peritoneal lesion sites (PPMD/PPMC vs UHMD/UHMC).

Results

The SWA model-induction, including abdominal incision line suturing, took in an average of 9.58 with CI (8.69-10.47) min, 9.61 with CI (8.74-10.48) min and 9.57 with CI (8.70-10.44)

min in groups 1, 2 and 3 respectively without significant differences between groups ($p > 0.05$).

Sidewall adhesions

Null hypotheses (Ho1, 2, 3) were confirmed, whereas alternative ones (Ha1, 2, 3) – rejected overall (Table 2). It means that by two-way ANOVA analysis differences between CO₂-insufflation groups (group 1 vs 2 and 3; group 2 vs 3) and between types of surgical trauma (MD vs MC) were not found showing no effects nor interactions on SWA variables (Figure 5) overall ($p > 0.05$).

Lesion site adhesions

Following hypotheses (Ho1, 4, 5 and Ha2, 3) were rejected and other hypotheses vice versa (Ha1, 4, 5 and Ho2, 3) – confirmed (see Table 2). It means that by two way ANOVA analysis were estimated differences in LSA scores between CO₂-insufflation groups (group 1 vs 2 and 3) were not significant overall PP and UH lesion sites ($p > 0.05$). Neither were the differences between group 2 and group 3 ($p > 0.05$) nor were interactions found between CO₂-insufflation and type of surgical trauma (Figure 6).

In contrast, there were significant differences between LSA scores depending on surgical trauma type. So, LSA scores in PP after MD were significantly lower than those after MC ($p = 0.0001$ for total score, $p = 0.05$; $p = 0.01$ and $p = 0.01$, respectively for 1, 2 and 3 groups). In addition, the total score of visceral LSA in UH after MD was also significantly lower than that after MC ($p = 0.019$), but individual values of groups did not vary significantly ($p > 0.05$). The incorporated values of LSA after MD, including PP and UH values, were significantly lower than those after MC by nonparametric tests ($p = 0.0001$).

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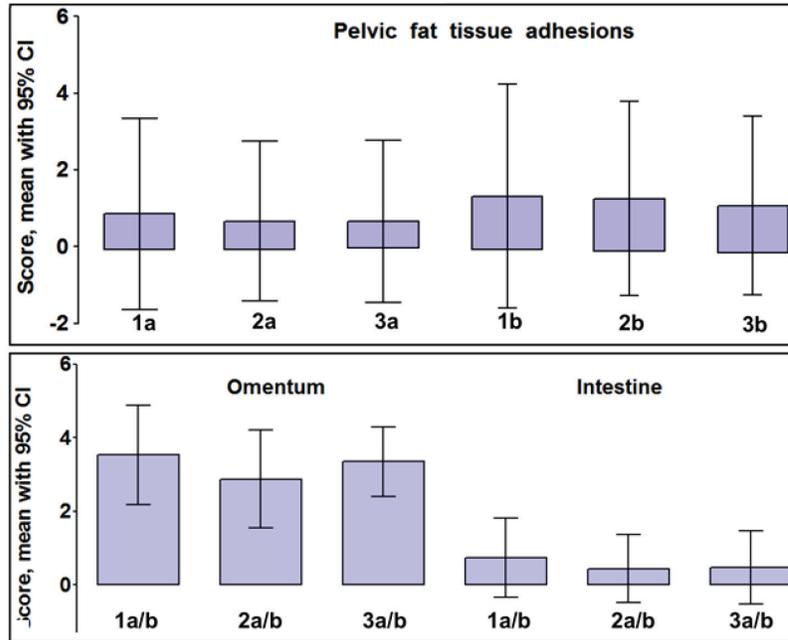


Figure 7. Scores of common abdominal (omentum and intestine) and pelvic fat tissue adhesions. X - Groups and Y - Adhesion Score (Means with 95% confidence intervals are shown).

Surprisingly, there were significantly lower PP LSA scores after MD than those in UH LSA after the same type of surgical trauma ($p=0.0001$) for total score, whereas analogous comparisons after MC were not significant ($p>0.05$), showing significant interactions between an impact of surgical trauma type and lesion sites, but there were not differences depending on CO_2 insufflation impact (group 1 vs group 2 vs group 3) and its interactions with lesion sites ($p>0.05$).

The incorporated values of LSA in PP, including both MD and MC parameters, were significantly lower than those of UH by two-tailed unpaired Mann-Whitney test ($p=0.0009$).

Pelvic and common abdominal adhesions

All tested null hypotheses (Ho 1, 2, 3) were confirmed according to CO_2 insufflation impact on pelvic fat tissue adhesion parameters' variations and subsequently alternative ones (Ha 1, 2, 3) were rejected (see **Table 2**). It means that manifestations of pelvic fat tissue adhesions, attached to UH visceral peritoneal lesion sites (**Figure 7**), were also insignificant in regard to type of trauma (MD vs MC), to impact of CO_2 -insufflation and its insufflation modes ($p>0.05$).

For common abdominal adhesions, involving omentum and intestine null hypothesis (Ho2) was confirmed and alternative hypothesis (Ha2) - rejected, presenting an absence of significant differences in regard to CO_2 -insufflation impact ($p>0.05$). Adhesions between LIL and omentum occurred considerably often than between LIL and intestine ($p=0.01$, $p=0.05$ and $p=0.01$), respectively for 1, 2 and 3 groups (see **Figure 7**).

In addition, uncommon and irregular adhesions between LIL and liver, pancreas and colon, as well as between UH visceral lesion sites and bladder were also registered. However, there were no significant

differences overall concerning to an impact and interactions of both CO_2 -insufflation modes and type of surgical trauma on variables of these common abdominal/pelvic adhesions ($p>0.05$). Therefore they were not presented.

Discussion

Adhesions occurring after SWA model-induction were characterized and classified according to their localization, side attachments and their involving intraperitoneal structures, such as SWA, LSA and common abdominal and pelvic adhesions.

To test the initial hypothesis, we thoroughly analyzed the differences between overall adhesion parameters, including SWA, parietal and visceral LSA, common abdominal/pelvic adhesions both with CO_2 -insufflation and without (see **Table 2**). We also tested the differences between two groups with CO_2 -insufflated before and after SWA model-induction, taking into account surgical trauma type impact. A secondary hypothesis was proposed since the analysis of the variables of PP and UH LSA within parietal and visceral peritoneal wounds produced evidence of a possible lesion site dependent predisposition to adhesion formation.

In spite of the limitations of our study, an early postsurgical loss of animals due to anaesthesia complications [29] and the SWA model-induction in a manner of open laparoscopy through midline laparotomy incision in a CO₂ environment, the following hypotheses were confirmed: 1). Adhesion formation is pronounced after MC in comparison with that after MD; 2). The absence of CO₂-insufflation impact itself, as well as its insufflation before or after traumatization of parietal and visceral peritoneum on variables of postsurgical adhesions; 3). Severe and extended LSA were more pronounced in the UH i.e., visceral peritoneum than those in the PP lesion sites, regardless of CO₂-insufflation mode.

Surprisingly, in our study, CO₂ impact and its insufflation before and after traumatization of the parietal and UH visceral peritoneum did not manifest itself in SWA scores at all. Inversely, we observed highly differentiated effects of surgical trauma types on variables of PP LSA after MD. In addition, differentiated surgical trauma impact on variables of UH LSA was less manifest, even absent after MC. These results contradict with findings of previous studies by Molinas and Koninckx [4], Yesildaglar et al [7] and others showing a high sensitivity of adhesion score assessment based on a SWA model-induced by electrocoagulation to study the impact of CO₂ insufflation and pneumoperitoneum with small-scale gas mixtures.

In contrast, there were significant differences between LSA variables depending on type of surgical trauma and lesion sites. We found that MD triggers a significantly lower LSA than MC. So a substantially lower adhesion scores were observed in the PP lesions than in the UH lesions, but there were no significant differences between the adhesion parameters regarding CO₂-insufflation mode. In our experiment, CO₂ was insufflated before and after the SWA model-induction to stress the possible impact of CO₂ absorbed via damaged and intact peritoneal tissue on adhesion formation parameters. Consequently, CO₂-insufflation did not increase adhesion variables in spite of the fact that we used CO₂-insufflation with intraperitoneal pressure at 15 cm of water, longer time (60 min) and high insufflated gas temperature (37°C) in spontaneous breathing rats to stress its maximum impact on the damaged and intact peritoneum of small laboratory animals.

The absence of intraperitoneal CO₂-insufflation impact on adhesion formation observed in our study is in accord with results of several researches [2, 3, 5, 12, 27]. This can be linked to the immunological changes with suppressed inflammatory and metabolic responses of the peritoneal cells [13, 14]. However, these results contradict with a hypothesis supporting CO₂-insufflation impact on adhesion formation [4, 6, 7].

Pronounced adhesion severity after MC, was observed in this study, correspond with literature data concerning electrocauterization [15]. It is well known that electrocoagulation produces severe trauma with subsequent acute and prolonged inflammatory reactions [20, 24] triggering postsurgical adhesion formation. These findings were supported by the results of a German team comparing different modes of surgical trauma [17]. They concluded that additional trauma to the underlying tissues, either by deeper electrocoagulation or suturing, leads to significantly increased adhesion formation.

Moreover, results of current study support our previous hypothesis that severe inflammatory reaction, induced by peritoneal trauma, is the key driving mechanism of postoperative adhesion formation [27]. Noteworthy, recently, the KULeuven team also suggested that acute inflammation of the entire peritoneum cavity is an important mechanism involved in adhesion formation, enhancing adhesion formation at the lesion site after bipolar coagulation and manipulation [23].

In this study we observed a visceral peritoneal lesion-site dependent predisposition to the formation of adhesions. Incorporated PP LSA scores were significantly lower than those of the UH visceral peritoneal LSA. If we take into account the 3-fold wider size of lesion sites in the PP than analogous parameters of the UH, then the role of this observation is very significant. These results correspond with conclusions by Wallwiener et al [30], presenting a significantly higher potential of the visceral peritoneal lesions to form adhesions than those of the PP. An explanation for this event would be that in normal physiological conditions the UH and other abdominal and pelvic structures are in very close and permanent contact. Fibrin depositions are formed immediately after surgery and cover wounds. These

adhesiogenic depositions will easily fix neighboring organs and structures to the traumatized areas. Then, according to the current adhesion formation theory, these fibrin depositions (fibrinogenous adhesions) will be converted into permanent fibrotic adhesions when the tPA is inhibited. We assume that in our experiments a severe trauma-induced by MC could be a triggering factor of tPA inhibition. However, a feature of the sidewall PP lesion sites is that the abdominal and pelvic structures in these lesions do not permanently touch and contact. Therefore, for the abdominal and pelvic structures to adhere to the PP lesions, a more potent adhesive effect is necessary, which is achieved by MC with subsequent significantly higher adhesion scores than those after MD.

Common abdominal adhesions were observed between the LIL and other abdominal structures (omentum and intestine) as well as between UH visceral peritoneal lesion sites and pelvic fat tissue. These findings correspond with results by Haney and Doty [28], presenting adhesion formation between contacting peritoneal surfaces in a murine SWA model as well as by Rappaport et al [19] when incisions made using coagulation current to study the effect of electrocautery on midline fascial wound healing in rats produced more extensive tissue necrosis and inflammation as well as adhesion formation between the incision and abdominal visceral peritoneum. An explanation for this phenomenon is the anatomical structure of the anterior wall and intraperitoneal structures in small animals. So, in rats, in normal physiological conditions, the anterior wall is in a horizontal position in the ventral side and all the abdominal structures are in close contact with LIL, especially the omentum and intestine. Therefore, these abdominal structures easily adhered to the underlying LIL by fibrin depositions with their subsequent conversion into permanent adhesions. As usual, adhesion formation between PFT and UH lesion sites occurs by an analogous scenario. The lower adhesion involvement frequency of the intestine compared with that of the omentum is, probably, related with intestinal peristaltic movements, which will detach adhered intestinal loops from the LIL.

In summary, substantial impact depending upon the type of surgical trauma on adhesion formation was observed, whereas CO₂ did not

trigger any significant effect nor interactions, irrespective of its insufflation before or after sidewall adhesion model-induction. Monopolar coagulation resulted in more severe and extended adhesion formation than that triggered by mechanical damaging. Severe and extended lesion site adhesions were more pronounced in the visceral peritoneal lesion sites than in those of the parietal peritoneal lesion sites, regardless of CO₂-insufflation mode. These findings may improve our insights into adhesion formation pathophysiology and open new perspectives in developing future adhesion prevention strategies.

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