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SURGICAL ENDOSCOPY
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Effect of reactive oxygen species scavengers, antiinflammatory drugs, and calcium-channel blockers on carbon dioxide pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model

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Abstract
Background: Postoperative adhesions are a clinical problem. They can cause female infertility, intestinal obstruction, chronic pelvic pain, and difficulties at the time of reoperation. A variety of approaches described to prevent adhesions have shown variable and inconsistent results. Therefore, this study aimed to evaluate most known substances in a laparoscopic mouse model to obtain quantitative and comprehensive information on adhesion prevention. Specifically, this first study aimed to investigate the effects of reactive oxygen species (ROS) scavengers, antiinflammatory agents, and a calcium-channel blocker on pneumoperitoneum-enhanced adhesions.
Methods: Adhesions were induced during laparoscopy in BALB/c female mice by creation of a bipolar lesion. Carbon dioxide (CO_2) pneumoperitoneum was maintained for 60 min using humidified CO_2. Six experiments were conducted to evaluate the effects of ROS scavengers (superoxide dismutase [SOD], catalase, melatonin, and ascorbic acid), antiinflammatory agents (dexamethasone, tenoxicam, ibuprofen, parecoxib, nimesulide, anti–tumor necrosis factor [TNF]-alpha), and a calcium-channel blocker (diltiazem). Adhesions were scored after 7 days during laparotomy.
Results: Adhesions were reduced by SOD ($p < 0.01$, proc general linear methods (GLM) of experiments 1 and 2), diltiazem ($p = 0.05$, Wilcoxon), and dexamethasone ($p < 0.03$), but not by nonsteroidal antiinflammatory drugs (NSAIDs) nor by anti–TNF-alpha. When all the experiments were grouped for analysis, adhesions also decreased with one and three doses of SOD ($p < 0.01$ and $p < 0.01$, respectively) and with one and three doses of ascorbic acid ($p < 0.02$ and $p = 0.05$, respectively).
Conclusions: These experiments confirm that SOD, diltiazem, and dexamethasone can decrease adhesion formation. The absence of effect from the other antiinflammatory drugs and anti-TNF-alpha is surprising.

Key words: Antiinflammatory agents — Calcium-channel blockers — Laparoscopy — Pneumoperitoneum-enhanced adhesions — Prevention — ROS scavengers

Postoperative adhesion formation remains an important clinical problem because it causes intestinal obstruction [1], chronic pelvic pain [2], female infertility [3], and difficulties at the time of reoperation. However, adhesion prevention still is inadequate and poorly understood overall.

In animal models, adhesion prevention has been studied during both open and laparoscopic surgery. During open surgery, many products have been demonstrated to decrease postoperative adhesion formation, including corticosteroids, [4–7], nonsteroidal antiinflammatory drugs (NSAIDs) [6, 8–19], fibrinolytic agents [20], surfactants [21–25], flotation agents and semisolid barriers [23, 26–28], mechanical barriers [26, 29–32], hormones [33], calcium-channel blockers [34–38], reactive oxygen species (ROS) scavengers [39–45], and antiangiogenesis therapy [46, 47]. It should be stressed that these observations generally were reported with the use of only one drug in different models with different species and using different scoring systems. Therefore, a comprehensive quantitative evaluation of efficacy in one model still is lacking.

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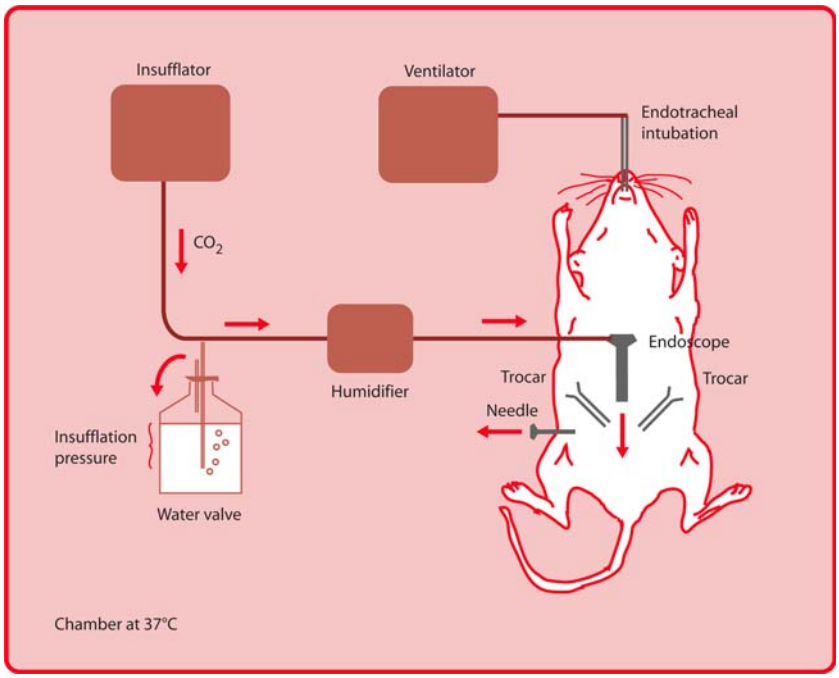

Fig. 1. Laparoscopic mouse model.

Prevention of adhesion formation after laparoscopic surgery has been poorly addressed. Some products have been demonstrated to decrease postoperative adhesions during laparoscopy including antibodies against vascular endothelial growth factor receptor 1 (VEGF-R1) [48], crystalloids [49, 50], 4% icodextrin [50], ferric hyaluronate gel [50–52], Sepracoat [53], a crosslinked hyaluronan solution [54], and hyaluronate membrane [55].

Effectiveness after open surgery for humans was demonstrated in clinical trials for the SprayGel adhesion barrier system [56], Seprafilm [57], Intergel solution [58], 0.5% ferric hyaluronate [59, 60], Sepracoat (HAL-C) solution [61], Interceed [62–64], and glycerol hyaluronate/carboxymethylcellulose [65]. In addition, the following products also were effective in preventing adhesions after laparoscopic surgery in clinical trials: SprayGel adhesion barrier system [56], Interceed [66], Viscoelastic gel [67] and Hyalobarrier Gel [68].

During laparoscopy, a pneumoperitoneum is necessary. This carbon dioxide (CO_2) pneumoperitoneum has been identified as a cofactor in adhesion formation. Mesothelial hypoxia is suggested as the driving mechanism because adhesion formation increased with insufflation pressure and with duration of pneumoperitoneum, because similar effects were observed with CO_2 and helium pneumoperitoneum, and because the addition of 2% to 4% of oxygen to both CO_2 and helium pneumoperitoneum decreased adhesion formation [69, 70]. This hypothesis also was supported by the observation that pneumoperitoneum-enhanced adhesion formation was absent in mice deficient in genes encoding for factors upregulated by hypoxia, such as hypoxia-inducible factors [71], vascular endothelial growth factor (VEGF) and placental growth factor (PiGF) [72], and plasminogen activator 1 [73].

In addition, we demonstrated that both temperature and desiccation increased adhesion formation [74, 75]. These observations led to the conclusion that the mechanisms involved in adhesion formation after CO_2 pneumoperitoneum may be different from those observed after laparotomy. This also led to new concepts of adhesion prevention such as the addition of 3% oxygen to the pneumoperitoneum [76, 77], the use of anti–VEGF-R1 antibodies [48], using anti-PiGF antibodies [72], and lowering of body temperature [74]. In this model, we demonstrated that avoidance of desiccation prevented adhesion formation [75], whereas the addition of more than 3% oxygen to the pneumoperitoneum increased adhesions [77]. This led to the hypothesis that ROS could be another cofactor in adhesion formation [78].

Because ROS scavengers, antiinflammatory drugs, and calcium-channel blockers have not been investigated for the prevention of pneumoperitoneum-enhanced adhesions, this study aimed to evaluate these drugs in our laparoscopic mouse model. These experiments are part of a series intended to evaluate most known substances in one model to obtain quantitative and comprehensive information on adhesion prevention.

Materials 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 intraperitoneal adhesions) has been described in detail previously [49, 71–77]. Briefly, the model consisted of pneumoperitoneum-enhanced adhesions induced during laparoscopy by creation of a mechanical lesion. The pneumoperitoneum was kept for 60 min using pure and humidified CO_2 at 15 mmHg of insufflation pressure. Gas and body temperatures were kept strictly at 37°C using a heated chamber (Fig. 1).

Animals

This study used 192 female 9- to 10-week-old BALB/c mice weighing 20 g. The animals were kept under standard laboratory conditions. They were fed using a standard laboratory diet with free access to food and water any time. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia and ventilation

The mice were anesthetized with intraperitoneal pentobarbital 0.08 mg/g, intubated with a 20-gauge catheter, and mechanically ventilated (Mouse Ventilator MiniVent, Type 845; Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) using humidified room air with a tidal volume of 250 μl at 160 strokes/min. Humidified air for ventilation was used to prevent cooling as occurs during ventilation with nonhumidified air [74].

Laparoscopic surgery

A midline incision was performed caudal to the xyphoides. A 2-mm endoscope with a 3.3-mm external sheath for insufflation (Karl Storz, Tuttlingen, Germany) was introduced into the abdominal cavity, and the incision was closed gas tight around the endoscope to avoid leakage.

Pneumoperitoneum was created with pure CO_2 at 15 mmHg insufflation pressure using the Thermoflator Plus (Karl Storz) and a water valve to damper pressure changes. The gas was humidified (Storz Humidifier 204320 33; Karl Storz), and the whole setup was kept in a 37°C chamber to obtain CO_2 at 37°C and with 100% relative humidity. As described previously, we maintained a controlled 23-ml/min flow of CO_2 through the abdominal cavity using a 26-gauge needle to ascertain a continuous 100% CO_2 environment by constant removal of any oxygen that might have diffused from the capillaries.

Induction of intraperitoneal adhesions

Pneumoperitoneum-enhanced adhesion formation was induced by maintaining the pneumoperitoneum for 60 min and creating standardized 10 × 1.6-mm lesions in the antimesenteric border of both the right and left uterine horns and the pelvic sidewalls with bipolar coagulation (BICAP bipolar hemostasis probe, BP-5200A, 5 Fr, 200 cm; IMMED Benelux, Linkebeek, Belgium) at 20 W (Autocon 200; Karl Storz, standard coagulation mode).

Scoring of adhesions

Adhesions were qualitatively and quantitatively scored. Scoring was performed blindly, with the investigator not informed of the group being evaluated, after 7 days during laparotomy under microscopic vision. The qualitative scoring system assessed extent (0 [no adhesions], 1 [1–25% of the injured surface involved], 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 [easily falls apart], 2 [requires traction], 3 [requires sharp dissection]), and total (extent + type + tenacity).

The quantitative scoring system assessed the proportion of the lesions covered by adhesions using the following formula: adhesion (%) = (sum of the lengths of the individual attachments/length of the lesion) × 100. The results are presented as the average of the adhesions formed at the four individual sites (right and left visceral and parietal peritoneum), which were individually scored.

Products

ROS scavengers

Superoxide dismutase from bovine erythrocytes and catalase from murine liver (Sigma, Bornem, Belgium) were dissolved in saline (NaCl 0.9%) to 3,000 U/ml and kept at −20°C and 4°C until used, respectively. Melatonin (Sigma) was dissolved in ethanol (60 mg/ml) and kept protected from the light at −20°C. The day of the experiment, it was diluted in saline to 2 mg/ml immediately before use. Ascorbic acid (AA) (Sigma) was dissolved to 20 mg/ml in saline before use.

Antiinflammatory drugs

Dexamethasone (Aacidexam 5 mg for injection; Organon, Bruxelles, Belgium) was prepared the day of the experiment as indicated in the product data sheet, diluted to 80 μg/ml in phosphate-buffered saline (PBS), and kept at 4°C. Nimesulide (Sigma) was dissolved in dimethyl sulphoxide (30 mg/ml), kept at −20°C, and diluted to 0.2 mg/ml in PBS the day of the experiment. Parecoxib (Dynastat injectable; Pfizer, Bruxelles, Belgium) was prepared the day of the experiment as indicated in the product data sheet, diluted to 0.2 mg/ml in PBS, and kept at 4°C. Ibuprofen 10 mg/ml (Office Chimique-Certa S.P.R.L., Braine l'alleud, Belgium) was diluted to 2.8 mg/ml in PBS and kept at 4°C until used. Intravenous (IV) tenoxicam (Tilcotil; Roche, Bruxelles, Belgium) was prepared the day of the experiment as indicated in the product data sheet, diluted to 0.4 mg/ml in PBS, and kept at 4°C.

Anti–TNF-alpha antibody

A neutralizing antibody against mouse tumor necrosis factor (TNF)-alpha and a nonneutralizing antibody (used as a control condition) were kindly provided by Centocor B.V., Leiden, The Netherlands. The concentration of both antibodies was 10 mg/ml. Neutralizing antibodies were diluted to 1 mg/ml and to 0.1 mg/ml in saline and kept at −20°C.

Calcium-channel blockers

Diltiazem hydrochloridum (Tildiem 25 mg IV; Sanofi-Synthelabo S.A.N.V., Bruxelles, Belgium) was prepared the day of the experiment as indicated in the product data sheet, diluted to 0.2 mg/ml in saline, and kept at 4°C.

All the drugs were diluted in saline except the drugs of the experiment, which were diluted with nimesulide. In this experiment, the dilutions were made in PBS because nimesulide precipitated in saline (pH of 5.5) and was completely dissolved in PBS (pH of 7.4).

At least minimally effective doses, according to the literature, were chosen. For superoxide dismutase (SOD) and catalase,15,000 U/kg, administered intraperitonealy (IP) was chosen because this dose given intravenously (IV) was shown to be effective in rats [39]. For melatonin, 10 mg/kg IP was used because it was shown to be effective in rats [42]. For AA, 100 mg/kg IP was used because it was shown to be effective in guinea pigs [79]. In addition, AA 80 and 250 mg/kg IP also were effective in mice [80, 81]. For the diet supplement, AA 8% was used because this concentration was shown to increase the dehydroascobate plasma concentration [82]. For dexamethasone, 2 mg/kg IP was used because 1 mg/kg was effective in rabbits [6]. For nimesulide, 5 mg/kg IP was used because 2.5 mg/kg was effective in rats [9]. For parecoxib, the same dose as with nimesulide was used because they both are a selective NSAID. For Ibuprofen, 70 mg/kg IP was used because this dose was shown to be effective in rabbits [14]. For tenoxicam, 10 mg/kg IP was used because 5 mg/kg IP was shown to be effective in mice [10]. For anti–TNF-alpha, 0.01, 0.1, and 1 mg IP were used because 1 mg IP was shown to be effective in mice [83]. For diltiazem, two daily doses 5 mg/kg IP were used because one daily dose of 10 mg/kg IP was shown to be effective in rats [38]. This drug was strictly controlled because overdoses can produce hypotension and bradycardia [84].

Experimental design

The time of anesthesia injection was considered time 0 (T_0). The animal preparation and ventilation started after exactly 10 min (T_{10}). The pneumoperitoneum was started at 20 min (T_{20}) and maintained for 60 min, until T_{80}. For all the experiments, eight animals per group were used.

Table 1. Prevention of pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model[a]

Experiment	Group	No. of doses	Route of administration	Dose	Quantitative scoring (Proportion, %)	Qualitative scoring (Total)
1	Control (saline)	1	IP	—	27.8 ± 2.8	3.5 ± 0.4
	SOD	1	IP	300 U	17.5 ± 4.2[b,c]	2.6 ± 0.5[c]
	Catalase	1	IP	300 U	31.3 ± 5.0	3.7 ± 0.5
	Melatonin	1	IP	200 μg	27.2 ± 2.5	3.6 ± 0.3
	AA	1	IP	2 mg	22.1 ± 3.2[c]	3.5 ± 0.4
2	Control (saline)	3	IP	—	30.6 ± 3.1	3.4 ± 0.3
	SOD	3	IP	300 U	21.8 ± 3.1[b,c]	3.1 ± 0.1[c]
	AA	3	IP	2 mg	23.1 ± 4.6[c]	3.3 ± 0.5
	SOD + AA	3	IP	300 U; 2 mg	27.2 ± 3.8	3.5 ± 0.3
3	Control (PBS)	2	IP	—	34.6 ± 2.0	5.0 ± 0.3
	Control (PBS)	4	IP	—	33.7 ± 4.1	4.2 ± 0.4
	Dexamethasone	2	IP	40 μg	23.4 ± 2.3[c,d]	3.2 ± 0.1[c,d]
	Nimesulide	4	IP	100 μg	26.8 ± 3.7	3.8 ± 0.2
	Parecoxib	4	IP	100 μg	34.1 ± 4.4	4.1 ± 0.4
	Ibuprofen	4	IP	1.4 mg	28.8 ± 2.2	4.0 ± 0.4
	Tenoxicam	2	IP	200 μg	36.4 ± 2.8	3.9 ± 0.7
4	Control (Ab)	1	IP	1 mg	29.9 ± 3.8	3.3 ± 0.3
	Anti-TNFα Ab, 0.01	1	IP	0.01 mg	23.0 ± 7.6	3.2 ± 0.6
	Anti-TNFα Ab, 0.1	1	IP	0.1 mg	34.6 ± 6.6	3.4 ± 0.5
	Anti-TNFα Ab, 1	1	IP	1 mg	37.1 ± 5.4	3.6 ± 0.4
5	Control (Ab)	1	IV	1 mg	26.9 ± 3.4	3.6 ± 0.8
	Anti-TNFα Ab, 1	1	IV.	1 mg	28.1 ± 2.8	2.5 ± 0.8
6	Control (saline)	4	IP	—	35.7 ± 3.4	3.9 ± 0.3
	Diltiazem	4	IP	100 μg	22.5 ± 4.6[c,d]	3.0 ± 0.5[c]

IP, intraperitoneal; SOD, superoxide dismutase; AA, ascorbic acid; PBS, phosphate-buffered saline; TNF, tumor necrosis factor; IV, intravenous; Ab, antibody

[a] Carbon dioxide (CO_2) pneumoperitoneum using humidified gas at 15 mmHg was maintained for 60 min. Adhesions were induced during laparoscopy by performing a bipolar lesion. Six experiments were performed evaluating the effects of reactive oxygen species (ROS) scavengers (SOD, catalase, melatonin and ascorbic acid), antiinflammatories (dexamethasone, tenoxicam, ibuprofen, parecoxib, nimesulide and anti–TNF-alpha antibodies) and a calcium channel blocker (diltiazem). In this table, quantitative (proportion) and qualitative (total) scores are presented (mean ± standard error of the mean). The volumes administrated per dose were 100 μl for experiments 1, 4, and 5; 200 μl for experiment 2; and 500 μl for experiments 3 and 6

[b] $p < 0.05$: Experiments 1 and 2 analyzed together (proc general linear methods [GLM], 2 groups, 2 variables [i.e., experiment and treatment])

[c] $p < 0.05$: Interexperiment comparisons (each group compared with a control group grouping all the control subjects) (Wilcoxon test)

[d] $p < 0.05$: Intraexperiment comparisons (each group compared with its own control group) (Wilcoxon test)

To make the table clearer, only the comparisons to the control groups were placed

Experiment 1 (5 groups) was designed to evaluate the effect of ROS scavengers on adhesion formation. Because it is well known that ROS scavengers are produced during reperfusion, 100 μl of SOD, catalase, melatonin, and AA were injected IP at T_{75} (i.e., 5 min before the pneumoperitoneum was ended). Similarly, 100 μl of saline was injected in the control group.

Because some inhibitory effect was shown with SOD and AA, experiment 2 (4 groups) was designed for a more detailed study on the effect from higher doses of SOD, AA, and the combination of both on adhesion formation. In this experiment, 100 μl of SOD + 100 μl of saline (SOD group), 100 μl of AA + 100 μl of saline (AA group), 100 μl of SOD + 100 μl of AA (SOD + AA group), or 200 μl of saline (control group) were administrated IP at the beginning (T_{20}), before completion (T_{75}), and 30 min after completion of the pneumoperitoneum (T_{110}). In addition, the mice of groups AA and SOD + AA received AA orally (PO) in their food (Harlan Special Diet 2018 containing 8% ascorbic acid; Horst, The Nederland) 1 week before and after the surgery.

Experiment 3 (7 groups) was designed to evaluate the effect of steroidal (dexamethasone) and cyclooxygenase-2 (COX-2) nonselective (ibuprofen and tenoxicam) and COX-2 selective (nimesulide, parecoxib) NSAIDs on adhesion formation. For the dexamethasone and tenoxicam groups, the mice received two IP doses of 500 μl (immediately after creation of the lesion and on the day after the surgery) because these drugs have a very long half-life (36–72 h and 67 h, respectively) [85, 86]. The mice in the nimesulide, parecoxib, and ibuprofen groups received four IP doses of 500 μl (immediately after creation of the lesion, 6 h later, the day after the surgery in the morning, and 6 h later) because those drugs have a shorter half-life (1.80–4.73 h, 7 h, and 2–4 h, respectively) [85, 87, 88]. Two control groups were used, receiving two and four saline doses of 500 μl, respectively, in the same way as the treated groups.

Experiment 4 (4 groups) was designed to evaluate the effect of anti–TNF-alpha antibodies administered IP on adhesion formation. Immediately after induction of the pneumoperitoneum, 100 μl of either nonneutralizing antibodies 1 mg (control group) or anti–TNF-alpha antibodies 0.01 mg, 0.1 mg, or 1 mg were injected IP under laparoscopic vision.

Experiment 5 (2 groups) was designed to evaluate the effect of anti–TNF-alpha antibodies administered IV on adhesion formation. The day before the surgery, either nonneutralizing antibodies 1 mg/100 μl (control group) or anti–TNF-alpha antibodies 1 mg/100 μl were injected IV in the vein of the animal tail.

Experiment 6 (2 groups) was designed to evaluate the effect of a calcium-channel blocker, diltiazem, on adhesion formation. Because diltiazem has a short half-life (1.5–7 h) [89], the mice received four IP doses of 500 μl (immediately after creation of the lesion, 6 h later, on the day after the surgery in the morning, and 6 h later). Similarly, four saline doses of 500 μl were administered in the control group.

Statistical analysis

Statistical analyses were performed using the SAS System (SAS Institute, Cary, NC, USA). Differences in adhesion formation were evaluated with the Wilcoxon test and with procedure general linear methods (proc GLM) for a two-way analysis of variance (ANOVA) of the data from experiments 1 and 2. In Table 1, all the data are presented as the mean ± standard error of the mean. To represent all data in one figure, proportion data (mean ± standard deviation) were divided by the mean of the their control group and multiplied by 100 to be expressed as a percentage of the control subjects.

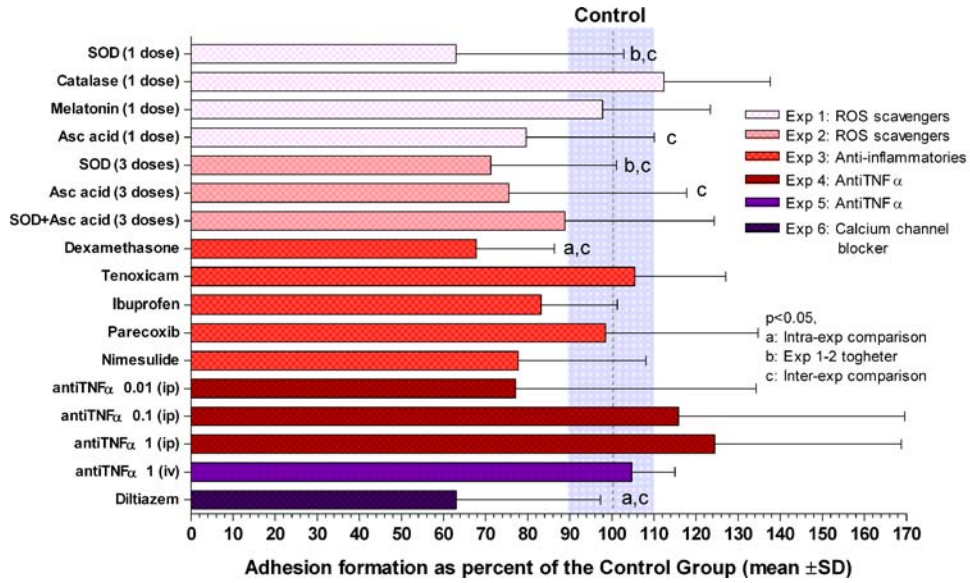

Fig. 2. Prevention of pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model. Carbon dioxide pneumoperitoneum (humidified gas at 15 mmHg) was maintained for 60 min. Adhesions were induced during laparoscopy by performing a bipolar lesion. Six experiments were performed evaluating the effects of reactive oxygen species (ROS) scavengers (superoxide dismutase, catalase, melatonin, and ascorbic acid), antiinflammatories (dexamethasone, tenoxicam, ibuprofen, parecoxib, nimesulide, and anti–tumor necrosis factor [TNF]-alpha antibodies), and a calcium-channel blocker (diltiazem). Proportions of adhesions are indicated. For visualization of the results for all the experiments in one graph, the percentage of change in comparison with the controls is given for each treatment. The coefficient of variation for all the controls is indicated as the shadowed area. $^{a}p < 0.05$: Intraexperiment comparisons (each group compared with its own control group) (Wilcoxon test). $^{b}p < 0.05$: Experiments 1 and 2 analyzed together (proc general linear methods [GLM], 2 groups, 2 variables [i.e., experiment and treatment]); p value of the variable treatment. $^{c}p < 0.05$: Interexperiment comparisons (each group compared with a control group grouping all the controls) (Wilcoxon test).

Results

The results of all six experiments are presented in Table 1. In experiments 1 and 2, the effect of the ROS scavengers, catalase, SOD, melatonin, and AA were evaluated. When both experiments were analyzed together (proc GLM, 2 groups, 2 variables [i.e., experiment and treatment]), SOD reduced adhesion formation: effect of treatment (proportion: $p < 0.01$; extent: $p < 0.01$); effect of experiment (not significant). None of the other drugs showed any important effect on adhesion formation, neither when the two experiments were analyzed together nor when they were analyzed as single experiments (Wilcoxon).

In experiment 3, the effect of antiinflammatory drugs was evaluated. First, no differences were found between two and four doses of saline injected IP in the control groups (Wilcoxon). Dexamethasone reduced adhesion formation (proportion: $p < 0.03$, total: $p = 0.01$; extent: $p < 0.02$, type: $p < 0.01$, tenacity: $p < 0.01$), but no significant effect was observed when ibuprofen, tenoxicam, nimesulide, and parecoxib were used. The mice treated with dexamethasone had less adhesions than the mice treated with tenoxicam (proportion: $p = 0.02$, total: $p < 0.04$, extent: $p < 0.04$), but not less adhesions than the mice treated with nimesulide, parecoxib, and ibuprofen.

In experiments 4 and 5, the effect of neutralizing anti–TNF-alpha antibodies was analyzed. In comparison with the control group, the IP administration of 0.01, 0.1, and 1 mg and the IV administration of 1 mg of the neutralizing antibodies did not have any effect on adhesion formation. In experiment 6, the effect of a calcium-channel blocker was analyzed. Diltiazem reduced adhesion formation in comparison with the control group (proportion: $p = 0.05$).

The results of all the experiments can be visualized in Fig. 2. Adhesion formation in the control groups during the six experiments was comparable (proc GLM, 2 variables [i.e., experiment and block, nonsignificant effect for both]), with a coefficient of variation of 10% only. Given this low variability of adhesion formation between the experiments, a reanalysis was performed grouping the control groups in all the experiments (total, 56) as the comparator. As expected, this confirmed the effects of SOD, dexamethasone, and diltiazem. In addition, adhesions decreased with one IP dose of SOD (proportion: $p < 0.01$, total: $p < 0.01$, extent: $p < 0.01$, type: $p < 0.01$) and with three IP doses of SOD (proportion: $p < 0.01$, total: $p < 0.01$, extent: $p < 0.01$). Moreover, adhesions decreased with one IP dose of AA (proportion: $p < 0.02$, extent: $p < 0.02$) and with the combined use of three IP ascorbic acid doses together with PO administration (proportion: $p = 0.05$).

Discussion

In open surgery models for adhesion formation effectiveness of ROS scavengers, antiinflammatory drugs, and calcium-channel blockers have been shown, albeit in isolated experiments with mice [10, 45], rats [4, 5, 7–9, 11–13, 16, 38–40, 42–44], hamsters [36], and rabbits [6, 14, 15, 17, 18, 31, 34, 37, 41]. In our pneumoperitoneum-

enhanced adhesion mouse model, ROS scavengers (only SOD was significant), dexamethasone and a calcium-channel blocker (diltiazem) decreased adhesion formation, whereas no effect was found for NSAIDs, even for specific COX-2 inhibitors or for anti–TNF-alpha antibodies. These data should be interpreted cautiously because results from one species do not necessarily apply to other species.

In open surgery, ROS scavengers have been described as reducing adhesion formation in mice [45], rats [39, 40, 42–44], and rabbits [41], but the effect is not consistent (e.g., vitamin E failed to decrease adhesion formation in rats) [90]. In our laparoscopic mouse model, we confirmed that ROS scavengers can decrease adhesion formation, although the overall effect was small. It is not surprising that not all products decreased adhesions significantly given the small number of animals in each group. Increasing the number of animals and repeating the experiments would not have changed the message that ROS scavengers can be effective but the effect is small. It is unlikely but cannot be excluded that IP administration such as we used is less effective than IV administration. Possibly, results differ between mice and rats. In rats the same dose of SOD and catalase (i.e., 15,000 U/kg IV) decreased intraperitoneal adhesions [39]. Also for melatonin, the same IP dose of 10 mg/kg body weight was effective in rats [42].

Most important, however, is that effectiveness is more pronounced after open surgery because exposure to air with approximately 21% of oxygen (i.e., a partial pressure of 160 mmHg) may generate much more ROS than the ischemia–reperfusion process after laparoscopy. In conclusion, we confirmed in our laparoscopic mouse model that ROS scavengers can reduce adhesion formation but the effect is small.

Antiinflammatory drugs are widely accepted for reducing adhesion formation in mice [10, 91], rats [4, 5, 7–9, 11–13, 16], and rabbits [6, 14, 15, 17–19]. However, these effects have not always been consistent in open surgery models. Ibuprofen failed to reduce adhesion formation in rats [7]. Anti–TNF-alpha antibodies also failed to reduce adhesions in a rat cecal serosal abrasion model [92]. In our laparoscopic mouse model, we confirmed the effectiveness of dexamethasone, but failed to demonstrate any important effects of NSAIDs as COX-1 and COX-2 inhibitors or of neutralizing anti–TNF-alpha antibodies.

Taken together, these data after both open surgery and laparoscopy suggest that this inflammatory reaction may be less important for adhesion formation. The absence of effect from anti–TNF-alpha antibodies with their strong effects on inflammation supports this suggestion. The fact that dexamethasone, nevertheless, had some effect on adhesion prevention suggests the involvement of other mechanisms. Glucocorticoids indeed can inhibit fibroblast proliferation and can have immunosuppressive effects on the production and release of cytokines [85].

Diltiazem, a calcium-channel blocker, reduced adhesion formation in our laparoscopic model, confirming the results for open surgery [34–38]. The suggested cause of this effect involves mechanisms such as interference with the inflammatory response [93], protection against the toxic effect of the ischemic–reperfusion cell injury [94], and activation of cellular processes [95].

The current understanding of adhesion formation comprises several mechanisms including the initial inflammatory reaction, with exudation and fibrin deposition, and fibroblast migration or differentiation, as well as a role for macrophages and later angiogenesis and collagen deposition. These experiments question the importance of the inflammatory reaction while confirming the effectiveness of ROS scavengers (SOD and AA) and calcium-channel blockers (diltiazem), at least in our model.

These obviously are screening experiments. It remains to be confirmed that the conclusion is valid for other species, especially primates, and for other adhesion formation models. Unfortunately the cost and effort associated with experiments of sufficient size with larger animals is prohibitive for screening experiments. Our screening data nevertheless warrant evaluating at least ROS scavengers such as SOD and AA, antiinflammatory drugs such as dexamethasone, and calcium-channel blockers such as diltiazem.

In conclusion, we confirmed with our laparoscopic mouse model the effects of ROS scavengers, calcium-channel blockers, and dexamethasone. The absence of effect from the other antiinflammatory drugs and anti–TNF-alpha antibodies is surprising. It is clear that we currently can begin to identify mechanisms and players, but we are far from a comprehensive understanding of the adhesion formation process and its prevention.

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