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Effect of Reteplase™ and PAI-1 antibodies on postoperative adhesion formation in a laparoscopic mouse model

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Abstract
Background Postoperative adhesions remain an important clinical problem, accounting for infertility, chronic pain and bowel obstruction. Its prevention is still inadequate and overall poorly understood. The aim of this study was to investigate the effect of Reteplase (a recombinant plasminogen activator, r-PA) and of PAI-1 antibodies upon adhesion formation in a laparoscopic model.
Methods Pneumoperitoneum-enhanced adhesions were induced by performing a bipolar lesion in female BALB/c mice and by using pure and humidified CO_2 as insufflation gas for 60 min. In experiment 1, four doses of 0.125, 0.25, 0.5 and 1 mg/0.5 ml r-PA and one and two doses of 1 mg r-PA were administrated i.p. Two control groups were included, one without any treatment and the second one receiving four times 0.5 ml of saline. In experiment 2, four doses of 0, 1, 10 and 100 μg/0.5 ml r-PA were administrated i.p. In experiment 3, PAI-1 neutralising and non-neutralising antibodies were injected i.p. after performing the lesion on day 0 and days 2 and 4. Adhesions were scored after 7 days.
Results Adhesion formation was less with the administration of four doses of 1 μg r-PA (proportion, $p < 0.04$, Wilcoxon). An increase in adhesion formation was observed when higher number of doses and amounts of r-PA were used (Proc GLM, eight groups, two variables, $p = 0.05$ for the amount of r-PA and $p < 0.02$ for the number of doses administrated). No effect was observed with the PAI-1 antibodies.
Conclusions Low-dose i.p. administration of rPA is effective in the prevention of adhesions in a laparoscopic mouse model.

Keywords Adhesion formation · Laparoscopy · Mouse model · PAI-1 antibodies · Reteplase · r-PA

Peritoneal adhesions are pathological bonds between surfaces within body cavities. They are formed when the parietal or visceral peritoneum is damaged and the basal membrane of the mesothelial layer is exposed to the surrounding tissues. This injury to the peritoneum, due to either surgery or infection, causes a local inflammatory reaction which leads to the formation of a serosanguineous exudate that is rich in fibrin. The fibrinous exudate is part of the hemostatic process and facilitates tissue repair by providing a matrix for invading fibroblasts and new blood vessels. On the one hand, this deposition of fibrin is an essential component of normal tissue repair, but on the other hand, resolution of this fibrin deposit is required to restore the preoperative conditions or conditions before inflammation. The degradation of fibrin is regulated by the plasminogen system. The inactive proenzyme plasminogen is converted into plasmin by tissue-type plasminogen activator (t-PA) and/or urokinase-type

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plasminogen activator (u-PA), which are inhibited by the plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2). Plasmin degrades fibrin, the matrix structure of fibrinous adhesions. When the fibrinolytic capacity is insufficient, persistence of deposited fibrin may occur and fibrinous adhesions may develop. Fibrinous adhesions become organized, characterized by deposition of collagen and concomitant vascular ingrowth, as a consequence of which the adhesions are changed into fibrous, permanent adhesions. Thus, imbalance between fibrin deposition and fibrin dissolution is the key event in the development of adhesion formation [1].

We previously demonstrated that adhesion formation increases with duration of the pneumoperitoneum [2]. This effect of pneumoperitoneum-enhanced adhesions was not observed in PAI-1 (−/−), u-PA (−/−), and t-PA (−/−) knockout mice [3]. Compared with wild-type mice, PAI-1 knockout mice developed fewer adhesions, whereas both u-PA and t-PA knock out mice developed more adhesions. This effect was expected since the lack of u-PA and t-PA reduces plasmin activation and fibrin degradation, thus leading to adhesion formation, whereas the lack of PAI-1 reduces the inactivation of u-PA and t-PA, increasing plasmin levels and fibrin degradation, thus reducing adhesion formation [3]. In addition, an increase in PAI-1 expression in the abdominal wall indicates that PAI-1 upregulation by CO_2 pneumoperitoneum is a mechanism of pneumoperitoneum-enhanced adhesion formation [3]. The same susceptibility to develop more adhesion was also observed in t-PA and u-PA knockout mice during open surgery [4].

After the initial use of streptokinase, continuing research has given rise to the development of second-generation (recombinant human tissue plasminogen activator, rt-PA, Alteplase™) and third-generation (mutants of rt-PA such as recombinant plasminogen activator or r-PA or Reteplase™) plasminogen activators [5]. Reteplase™ lacks the finger, epidermal growth factor, and kringle-1 domain. The slower clearance resulting from these changes in the molecule allows Reteplase to be given as a bolus. Fibrinolytic agents as streptokinase, urokinase, plasmin preparations and rt-PA have been reported to decrease adhesion formation and reformation in several animal models [5]. In these experiments, different numbers of doses, concentrations, vehicles and animal adhesion models were used, and adhesions were induced during laparotomy. However, nothing is known about the effect of fibrinolytic agents during laparoscopy. Specifically, Reteplase and PAI-1 antibodies have ever been tested neither during laparotomy nor during laparoscopy for their anti-adhesive properties. Therefore, the aim of the present study was to investigate the effect of Reteplase and PAI-1 antibodies upon adhesion formation in a laparoscopic mouse model.

Materials and methods

Laparoscopic mouse model for adhesion formation

Experimental setup, i.e. animals, anaesthesia and ventilation, laparoscopic surgery, induction and scoring of intraperitoneal adhesions, has been described in detail previously [2, 3, 6–15] (Fig. 1).

Animals

One hundred twelve 9–10-week old female BALB/c mice weighing 20 g were used. Animals were kept under standard laboratory conditions and were fed with a standard laboratory diet with free access to food and water at anytime. The study was approved by the Institutional Review Animal Care Committee.

Anaesthesia and ventilation

Mice were anaesthetised with intraperitoneal 0.08 mg/g pentobarbital, 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 (Fig. 1A, B) since adequate ventilation was found to be important, affecting adhesion formation [15]. Humidified air for ventilation was used in order to prevent cooling, as occurs during ventilation with nonhumidified air [8].

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 in order to avoid leakage (Fig. 1B).

The CO_2 pneumoperitoneum was created with the Thermoflator Plus (Karl Storz, Tuttlingen, Germany). Using humidified gas (Storz Humidifier 204320 33, Karl Storz, Tuttlingen, Germany) and the 37°C chamber, gas at 37°C and 100% relative humidity was obtained. A controlled flow of the gas was obtained using a 26-gauge needle placed in the abdomen, which at 15 mmHg insufflation pressure induced a 23 ml/min flow of gas through the abdominal cavity.

Induction of intraperitoneal adhesions

After the establishment of the pneumoperitoneum, two 14-gauge catheters were inserted under laparoscopic vision (Fig. 1B). Standardized 10 × 1.6 mm lesions were created

in the antimesenteric border of both right and left uterine horns and pelvic sidewalls with bipolar coagulation (BICAP™, bipolar hemostasis probe, BP-5200A, 5 Fr, 200 cm; IMMED Benelux, Linkebeek, Belgium) at 20 watts (Autocon 200, Karl Storz, Tuttlingen, Germany, standard coagulation mode) (Fig. 1C, D, E).

Scoring of adhesions

Adhesions were qualitatively and quantitatively scored blindly (i.e. the investigator was not informed of the group being evaluated) under microscopic vision during laparotomy 7 days after their induction (Fig. 1F, G). 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), tenacity (0: no adhesions; 1: easily fall apart; 2: require traction; 3: require 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 length of the individual attachments/length of the lesion) × 100. 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

Recombinant human PA: Reteplase (Rapilysin® 10 U, Roche) was prepared as indicated in the product data sheet and diluted to 0.25, 0.5, 1 and 2 mg/ml in saline (experiment 1) and to 2, 20 and 200 µg/ ml (experiment 2) and kept at −20°C.

PAI-1 neutralizing antibodies: PAI-1 monoclonal antibody MA-33H1F7 and PAI-1 non-neutralizing antibody MA-32K3 were used. MA-33H1F7 was chosen because it was shown to cross-react with murine PAI-1 [16]. The antibodies were diluted to 0.06 mg/ml with saline and kept at −20°C.

Dosages

Recombinant human PA: From existing literature it is difficult to assess the minimal dosage required to prevent adhesions. The treatment programs are hard to compare because different animal models, different methods of inducing adhesions and different routes of administration and dosages were used [5]. In addition, no studies in mice are available. For this reason, we decided to use in experiment 1 a relatively high but effective concentration found in the bibliography used in a rat model, i.e. 5 mg rt-PA/rat (or 25 mg rt-PA /kg b.w.) [17], and to double this concentration in order to be sure that enough PA would be available. Therefore, the highest dose used in experiment 1 was 1 mg r-PA /mouse (or 50 mg r-PA /kg b.w.). Moreover, the r-PA was injected i.p. since greater availability was demonstrated by this route [18]. With regard to length of treatment, we decided to administrate the r-PA for a period of 2 days. This was based on a study by Dunn et al. in which varying days of tPA therapy were studied in a rabbit adhesion model. Results showed that 2 days of treatment resulted in significant reduction in adhesions formation from 35–40% to 6.3 ± 1.57% (control versus rt-PA-treated groups) [19]. Consistent with this, Orita et al. concluded that effective adhesion prevention occurred with 2 days of rt-PA treatment, beginning on the day of operation in a study in rabbits [20].

From the results generated in experiment 1, we realized that the dosages used were most likely too high for our experimental model, after which we performed experiment 2 in which we used lower r-PA dosages.

PAI-1 neutralizing antibodies

The quantity of anti-PAI-1 antibody was 1.5 mg/kg b.w. based on Schoots et al. studies done in rats [21]. This corresponds to a dose of 30 µg per mouse.

Experimental design

Since anaesthesia and ventilation can influence body temperature, these were strictly controlled. The time of anaesthesia injection was considered time 0 (T_0). The animal preparation and ventilation started after 10 min (T_{10}). The pneumoperitoneum started at 20 min (T_{20}) and was maintained for 60 min (T_{20} to T_{80}) to induce pneumoperitoneum-enhanced adhesions.

Experiment 1 was designed to evaluate the effect of different doses of r-PA and the timing of the r-PA administration upon adhesion formation. Pneumoperitoneum- enhanced adhesions were induced and r-PA or saline were administrated i.p. depending on the groups: two doses the day of the surgery (immediately after performing the lesion and 6 h thereafter) and two doses the day after (24 h after surgery and 6 h thereafter). Four groups receiving four doses of 0.125, 0.25, 0.5 and 1 mg/0.5 ml of r-PA were performed. Another two groups in which one dose of 1 mg r-PA (and three times saline) or two times 1 mg r-PA (and two times saline) were administrated. Two control groups were used, one without any treatment and the second one receiving four times 0.5 ml saline (untreated control and saline control, respectively) (eight groups, eight mice per group, $n = 64$).

After completing experiment 1, it was hypothesized that the concentrations used might be too high and experiment 2

was designed in order to evaluate the effect of lower concentrations of r-PA upon adhesion formation. Pure CO_2 pneumoperitoneum was induced and four doses of 1, 10 and 100 μg/0.5 ml of r-PA were i.p. administrated: two doses the day of the surgery (immediately after performing the lesions and 6 h thereafter) and two doses the day after the surgery (24 h after surgery and 6 h thereafter). A control group receiving four times saline was also included (four groups, eight mice per group, $n = 32$).

Experiment 3 was designed to evaluate the effect of PAI neutralizing antibodies in pneumoperitoneum-enhanced adhesions. PAI neutralizing and non-neutralizing antibodies were injected i.p. at day 0 (after performing the lesion) and days 2 and 4 (two groups, eight mice per group, $n = 16$).

Each experiment was performed using block randomisation by days. Therefore, one block of mice, comprising one animal of each group, was operated during the same day, and within a block the animals were operated in random order. This model as used is simple and does not carry any mortality.

Statistics

Statistical analyses were performed by using the SAS System (SAS Institute, Cary, NC, USA). Differences in adhesion formation were evaluated with Wilcoxon test for the univariate analysis and with general linear methods (GLM procedure) for multivariate analysis to evaluate the effect of number of doses and amount of r-PA simultaneously. All data are presented as the mean ± standard error of the mean (SE).

Results

In experiment 1, the effect of different amounts and numbers of doses of r-PA were evaluated upon pneumoperitoneum-enhanced adhesions (Fig. 2, Table 1). When four doses of r-PA were administrated, adhesion formation increased with the amount of r-PA (proportion: $p = 0.05$; total: $p < 0.0004$; extent: $p < 0.02$; type: $p < 0.0001$; tenacity: $p < 0.002$; Fig. 2, left). When the dosage of 1 mg was administrated, adhesion formation increased with the number of doses administrated (proportion: $p < 0.02$; total: $p < 0.01$; extent: $p < 0.01$; type: $p < 0.01$; tenacity: $p < 0.02$; Fig. 2, right) (GLM procedure, eight groups, two variables, i.e. amount and number of doses of r-PA).

In experiment 2 (Fig. 3, Table 1), adhesion formation was reduced with the administration of 1 μg r-PA compared with in the control group (proportion: $p < 0.04$,

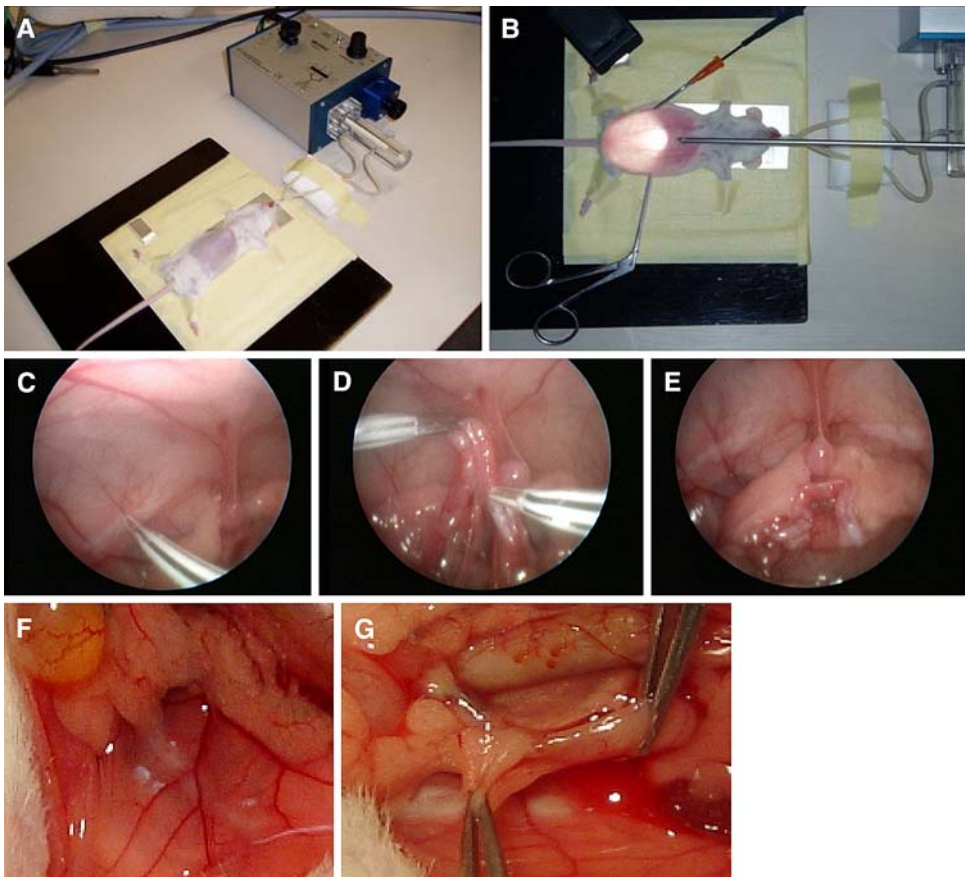
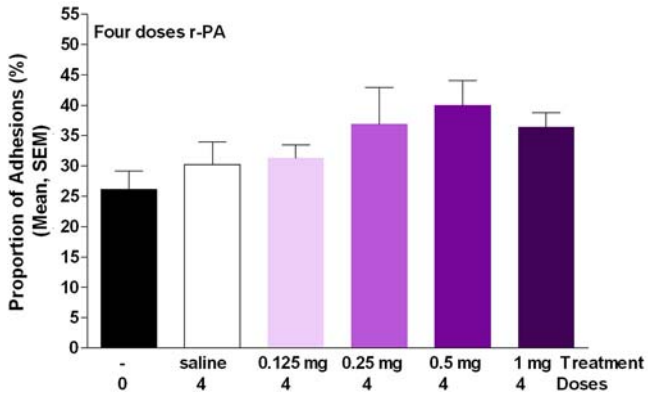


Fig. 1 Laparoscopic mouse model: representative pictures of the model, lesions and postoperative adhesions. Mice were connected to a mechanical ventilator (**A**, **B**); the endoscope and two trocars for placing the grasper and the bipolar coagulator were introduced into the abdomen (**B**). Ten-millimetre lesions were created under laparoscopic vision on the wall left (**C**, **E**) and right (**E**) and on the uterine horns (**D**, **E**). After 7 days, adhesions were evaluated under microscopic vision. Adhesions to the walls (**F**) and to the uterine horns (**G**) were qualitatively and quantitatively scored after 1 week



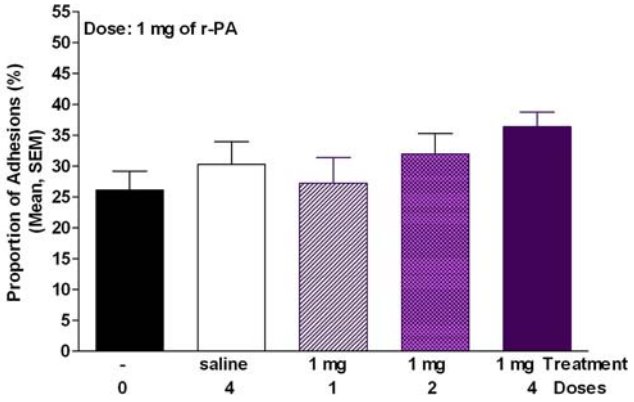


Fig. 2 Effect of different amounts and number of doses of r-PA on pneumoperitoneum-enhanced adhesions. Pneumoperitoneum was maintained for 60 min and humidified pure CO_2 at 20 cmH_2O insufflation pressure was used. Adhesions were induced during laparoscopy by performing a bipolar lesion. The effects of various amounts and doses of Reteplase (r-PA) on adhesion formation were evaluated. Adhesions were scored after 7 days during laparotomy. The quantitative scoring system (proportions) is presented. *Statistics*: GLM procedure, eight groups, two variables, i.e. amount and number of doses of r-PA. Significance for the amount was $p = 0.05$ (proportion) and for the number of doses was $p < 0.02$ (proportion)

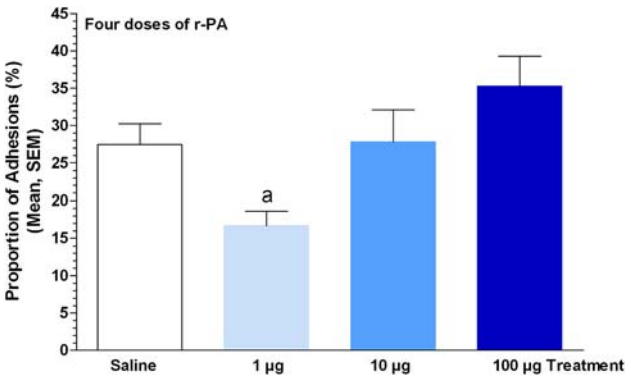


Fig. 3 Effect of the administration of lower doses of r-PA on pneumoperitoneum-enhanced adhesions. Pneumoperitoneum was maintained for 60 min using pure CO_2. The gas was humidified and the insufflation pressure was 20 cmH_2O. Adhesions were induced during laparoscopy by performing a bipolar lesion. Four doses of 1, 10 and 100 µg r-PA were administrated. A control group was administrated four times saline. Adhesions were scored after 7 days during laparotomy. The quantitative scoring system (proportions) is presented. *Statistics*: $^{a}p < 0.05$, compared with the control, to 10 µg and to 100 µg of t-PA (Wilcoxon test)

Wilcoxon). There was no effect of 10 and 100 µg compared with the control group (not significant, NS, each comparison). The administration of 1 µg r-PA also reduced adhesion formation compared with animals treated with 10 µg (proportion: $p = 0.05$) or 100 µg (proportion: $p < 0.01$, extent: $p < 0.02$).

In experiment 3, the effect of PAI neutralizing antibodies was evaluated. No effect was observed with the PAI-1 neutralizing antibodies compared with the non-neutralizing antibodies for either the quantitative (proportions, control group: $22.8 \pm 4.3\%$ treated group: $28.1 \pm 5.0\%$) or qualitative (Table 1) scoring systems.

Discussion

Until some years ago, streptokinase and urokinase were used in thrombolytic therapy. Subsequently, second-generation plasminogen activators (recombinant human tissue plaminogen activator [rt-PA] or Alteplase) and third-generation plasminogen activators (mutants of rt-PA, e.g. recombinant human PA [r-PA] or Reteplase) were developed [5]. These PAs have several advantages over streptokinase and urokinase, i.e. they are not antigenic, they do no cause immunogenic reactions and they have little or no general side effects. These fibrinolytic agents have already been tested for the prevention of adhesions. In these experiments, different numbers of doses, concentrations, vehicles and animal models have been used, and adhesions were induced during laparotomy [5]. However, nothing is known about the effect of these fibrinolytic agents during laparoscopy. Specifically, Reteplase and PAI-1 antibodies have never been tested during either laparotomy or laparoscopy for their anti-adhesive properties. Therefore, the aim of the present study was to evaluate the effect of both Reteplase and PAI-1 antibodies upon adhesion formation in a laparoscopic mouse model.

From our results, we can confirm that r-PA is able to prevent adhesion to some extent, i.e. four i.p. doses of 1 µg r-PA reduced adhesion formation during laparoscopy. These experiments also show the importance of using the correct dose. As explained in the "Materials and Methods" section, we initially decided to use a higher concentration found in the literature in a rat model, 5 mg rt-PA/rat (or 25 mg rt-PA/kg b.w.) [17] and to double this dosage to ensure that sufficient PA would be available. Therefore, the doses 0.125, 0.25, 0.5 and 1 mg r-PA per mouse were used in experiment 1 (1 mg r-PA/mouse = 50 mg r-PA/kg

Table 1 Summary of experimental results

Experiment	Number of mice	Number of doses	Concentration r-PA or PAI-1 antibodies	Qualitative scoring (mean ± SE)			
				Extent	Type	Tenacity	Total
1 (r-PA)	8	0	–	1.3 ± 0.1[a]	1.0 ± 0.1[a]	1.1 ± 0.1[a]	3.4 ± 0.3[a]
	8	4	500 µl saline	1.4 ± 0.2[a]	1.0 ± 0.1[a]	1.1 ± 0.1[a]	3.5 ± 0.4[a]
	8	4	0.125 mg/500 µl	1.5 ± 0.1[a]	1.2 ± 0.1[a]	1.3 ± 0.1[a]	4.0 ± 1.1[a]
	8	4	0.250 mg/500 µl	1.8 ± 0.2[a]	1.4 ± 0.1[a]	1.4 ± 0.1[a]	4.6 ± 0.5[a]
	8	4	0.500 mg/500 µl	1.8 ± 0.2[a]	1.6 ± 0.1[a]	1.6 ± 0.1[a]	5.0 ± 0.4[a]
	8	4	1 mg/500 µl	1.8 ± 0.1[a]	1.6 ± 0.1[a]	1.6 ± 0.1[a]	5.0 ± 0.2[a]
	8	2	1 mg/500 µl	1.5 ± 0.1[a]	1.2 ± 0.1[a]	1.3 ± 0.2[a]	4.0 ± 0.4[a]
	8	1	1 mg/500 µl	1.3 ± 0.2[a]	1.1 ± 0.1[a]	1.0 ± 0.1[a]	3.4 ± 0.2[a]
2 (r-PA)	8	4	500 µl saline	1.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	3.3 ± 0.4
	8	4	1 µg/500 µl	0.9 ± 0.1[b]	1.1 ± 0.1	1.2 ± 0.1	3.2 ± 0.4
	8	4	10 µg/500 µl	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	3.6 ± 0.4
	8	4	100 µg/500 µl	1.6 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	4.2 ± 0.4
3 (PAI-1 antibodies)	8	3	30 µg/500 µl non-neutralizing Ab	1.2 ± 0.2	1.1 ± 0.1	1.2 ± 0.2	3.5 ± 0.5
	8	3	30 µg/500 µl neutralizing Ab	1.3 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	3.3 ± 0.4

Pneumoperitoneum was maintained for 60 min and humidified pure CO_2 at 20 cmH_2O insufflation pressure was used. Adhesions were induced during laparoscopy by performing a bipolar lesion. The effects of various amounts and doses of Reteplase (r-PA) were evaluated upon adhesion formation in experiments 1 and 2. The effects of PAI-1 antibodies on adhesion formation were evaluated in experiment 3. Adhesions were scored after 7 days during laparotomy. The qualitative scoring system is presented. [a] $p < 0.05$. GLM procedure, eight groups, two variables, i.e. amount and number of doses of r-PA. Adhesion formation increased with the dose of r-PA when four times r-PA was administrated, and also with the number of doses administrated when the dose of 1 mg was administrated. [b] $p < 0.05$ versus 100 µg r-PA (Wilcoxon test)

b.w.). However, these doses showed an increase in adhesion formation. From experiment 1, we can conclude that the doses were too high for our experimental model since adhesion formation increased significantly with both the number of doses and the concentration of r-PA used. This increase in adhesion formation can most likely be explained by the "plasminogen steal" induced by a high concentration of r-PA, which means that a high concentration of PA is associated with a depletion of plasminogen and, therefore, no plasmin will be formed and no fibrin degradation will occur [22]. Consistent with that, it was shown in an in vitro model that the speed of clot lysis increases with increasing t-PA concentrations up to 2 µg/ml and decreases above 2 µg/ml, giving a bell-shaped curve of fibrinolysis [23]. Moreover, on the suggestion of Dr. Hellebrekers who had made similar observations in an in vivo mouse model, we decided to reduce the doses of Reteplase in experiment 2, the results of which showed that 1 µg produced a reduction of 40% in adhesion formation, confirming the "plasminogen stealing" hypothesis.

In experiment 3, PAI-1 antibodies were tested in our model. Surprisingly, no effect of these antibodies was observed. Although the efficiency of PAI-1 inhibition in vivo by monoclonal antibodies has been demonstrated in a number of studies [24, 25], they were all tested in thrombosis models. Specifically, the antibody used in this experiment, MA-33H1F7, exhibited an effect in reducing thrombus formation in rat models [26, 27]. However, this is the first time that these antibodies were tried in a laparoscopic mouse model and it may be that the absence of effect could be explained because the dose was not optimized. In addition, the antibodies were injected i.v. in the thrombosis models, in contrast to the i.p. administration in this study, which may also explain the absence of an effect.

It was demonstrated that pneumoperitoneum can have an influence on the fibrinolytic system. First, in vitro experiments have showed that mesothelial cells increase synthesis and release of PAI-1 when exposed to CO_2 [28]. Second, mesothelial cells seem to respond to acidification by an increased release and production of PAI-1 in vitro [29]. In contrast, mesothelial cells in vitro exposed to CO_2 and helium showed enhanced PA activity associated with a decrease in PAI-1 concentrations compared with the control (CO_2 with oxygen), from which it can be concluded that these changes may participate in the observed reduction in adhesions after laparoscopic surgery relative to open surgery [30]. It was shown in in vivo experiments that pneumoperitoneum enhanced adhesions in PAI-1, uPA and tPA wild-type mice, and that, compared with those wild-type mice, pneumoperitoneum did not enhance adhesions in knockout mice for each one of those factors [3]. In addition, PAI-1 concentration increased after 60 min of

pneumoperitoneum, whereas tPA concentration did not change in wild-type mice. These two observations indicate that PAI-1 upregulation by CO_2 pneumoperitoneum is a mechanism of pneumoperitoneum-enhanced adhesion formation.

CO_2 pneumoperitoneum was postulated to be a cofactor in adhesion formation since adhesions increase with duration of pneumoperitoneum and with insufflation pressure, and since the addition of 3% oxygen to both CO_2 and helium pneumoperitoneum reduces adhesions [2]. Moreover, it was demonstrated that avoiding desiccation by using humidified gas and reducing body temperature decreased adhesion formation [8, 9]. Therefore, we postulate that both *conditioning of the pneumoperitoneum* (i.e. using humidified CO_2 insufflation gas to prevent desiccation with the addition of 3% oxygen to prevent hypoxia, and lowering body temperature) and also the *application of products which decrease adhesions* (i.e. anti-inflammatory agents, calcium-channel blockers, barriers and surfactants [10, 11], as well as r-PA, as demonstrated in this study) may be a way to reduce adhesions. This is the third part of our screening experiments in which different agents are being evaluated for their anti-adhesion properties during laparoscopy. We hope in the near future to be able to define the best combination of treatments to reduce adhesions to the minimum.

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