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Role of the plasminogen system in basal adhesion formation and carbon dioxide pneumoperitoneum–enhanced adhesion formation after laparoscopic surgery in transgenic mice

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Objective: To evaluate the role of plasminogen activator inhibitor-1 (PAI-1), urokinase plasminogen activator (uPA), and tissue-type plasminogen activator (tPA) in adhesion formation after laparoscopic surgery.

Design: Prospective, randomized study.

Setting: Academic research center.

Animal(s): Seventy female wild-type and transgenic knockout mice for PAI-1 (PAI-$1^{-/-}$), uPA (u$PA^{-/-}$) or tPA (t$PA^{-/-}$).

Intervention(s): Standardized lesions to induce peritoneal adhesions were performed during laparoscopy. To evaluate basal adhesions and pneumoperitoneum-enhanced adhesions, the pneumoperitoneum was maintained for 10 minutes or 60 minutes, respectively. Peritoneal biopsy samples were obtained during and after 60 minutes of carbon dioxide pneumoperitoneum.

Main Outcome Measure(s): Adhesions were blindly scored after 7 days. Concentrations of PAI-1 and tPA were measured by using enzyme-linked immunosorbent assay.

Result(s): In PAI-1, uPA, and tPA wild-type mice, pneumoperitoneum enhanced adhesions. Compared with wild-type mice, basal adhesions were fewer in PAI-$1^{-/-}$ mice and more in u$PA^{-/-}$ and t$PA^{-/-}$ mice. Pneumoperitoneum did not enhance adhesions in these transgenic mice. PAI-1 concentration increased after 60 minutes of pneumoperitoneum whereas tPA concentration did not change.

Conclusion(s): Impaired fibrinolysis increases basal adhesions. The absence of pneumoperitoneum-enhanced adhesions in PAI-$1^{-/-}$, u$PA^{-/-}$, and t$PA^{-/-}$ mice and the increase in PAI-1 expression indicate that PAI-1 up-regulation by carbon dioxide pneumoperitoneum is a mechanism of pneumoperitoneum-enhanced adhesion formation. (Fertil Steril® 2003;80:184–92. ©2003 by American Society for Reproductive Medicine.)

Key Words: Adhesion formation, laparoscopy, CO_2 pneumoperitoneum, plasminogen activator inhibitor-1, urokinase-type plasminogen activator, and tissue-type plasminogen activator, transgenic mice

Peritoneal injury initiates an inflammatory reaction that determines fibrin deposition on the injured surface and migration, proliferation, and differentiation of various cell types, such as inflammatory and immune cells, mesothelial cells, and fibroblasts. These cells release molecules that modulate the subsequent evolution of the peritoneal lesion to normal healing or to adhesion formation.

After complete fibrinolysis, few or no adhesions form, whereas insufficient fibrinolysis will provide a scaffold for migrating cells (fibroblasts) and capillaries. The latter process results in extracellular matrix deposition and angiogenesis, leading eventually to adhesion formation (1–3). This information is based on a series of studies demonstrating more adhesions with decreased fibrinolysis (4) and fewer adhesions with increased fibrinolysis, such as after the administration of plasminogen activator inhibitor-1 (PAI-1) antibodies (5) or of recombinant tissue-type plasminogen activator (tPA) (6–12).

Besides its role in fibrin degradation, the plasminogen system has a direct role in other processes of tissue repair, such as activation of proenzymes of the matrix metalloprotease family (13), extracellular matrix degradation (14), activation of urokinase-type plasminogen activator (uPA) (15), liberation and activation of growth factors (16, 17), angiogenesis (18), and cellular migration (19). It is unclear whether the reported effects on adhesion formation involve only fibrinolysis or other processes as well.

The plasminogen system is a complex system comprising the serine protease plasmin, zymogen plasminogen, plasminogen activators (tPA and uPA), plasminogen activator inhibitors (PAI-1, PAI-2, PAI-3, and protease nexin 1), and plasmin inhibitors (α_2-macroglobulin, α_2-antiplasmin, and α_1-antitrypsin) (20–23). Plasminogen is abundant in almost all tissues and is converted to plasmin by tPA and uPA. Both uPA and tPA are equally efficient in degradation of fibrin clots in blood, whereas tPA seems to have a higher efficacy in tissue (24–27).

The activity of tPA and uPA is modulated mainly by the glycoproteins PAI-1 and PAI-2 through formation of inactive complexes. Plasminogen activator inhibitor-1 has a stronger inhibitory action than PAI-2. The other PAIs and the plasmin inhibitors are considerably less active (28–31).

The overall fibrinolytic activity in the peritoneum is crucial in the pathogenesis of adhesion formation. Whereas tPA, uPA, PAI-1, and PAI-2 have been widely studied, PAI-3, protease nexin 1, and plasmin inhibitors have not. Peritoneal fibrinolytic activity decreases after different types of peritoneal trauma, such as suturing, retractors, foreign bodies, and infection (2, 32, 33). Peritoneal fibrinolytic activity is modulated by various proinflammatory cytokines released after a peritoneal injury; such cytokines include tumor necrosis factor-α, interleukins 1 and 6, and transforming growth factor-β, which decrease the fibrinolytic activity of human mesothelial cells in vitro (34–37).

Postoperative adhesion formation in vivo has been investigated mainly after open surgery. We recently demonstrated in rabbits and mice that the pneumoperitoneum is a cofactor in adhesion formation after laparoscopic surgery. We suggested that mesothelial hypoxia is the driving mechanism for this pneumoperitoneum-enhanced adhesion formation, since adhesions increase with the duration of pneumoperitoneum and insufflation pressure and decrease with the addition of oxygen to both carbon dioxide and helium pneumoperitoneum (38–41). This finding led to categorization of adhesions as basal (occurring after a peritoneal lesion only) or pneumoperitoneum enhanced (occurring after a peritoneal lesion with the additional effect of the pneumoperitoneum).

The importance of trauma with tissue necrosis and the inflammatory reaction and repair mechanism, involving local cellular hypoxia, has been recognized in adhesion formation (42–46). Furthermore, hypoxia modulates the expression of several molecules involved in different stages of adhesion formation, such as PAI-1, tPA, transforming growth factor-β, matrix metalloproteinases, and tissue inhibitors of metalloproteinases (47–56).

We sought to evaluate the role of PAI-1, uPA, and tPA in basal and in pneumoperitoneum-enhanced adhesion formation after surgery by using a laparoscopic mouse model. The choice of PAI-1, uPA, and tPA was dictated by the availability of knockout mice for genes encoding for these factors.

MATERIALS AND METHODS

Animals

The study was performed in 70 female 10- to 12-week old mice that weighed 30 to 40 g.

For the first experiment, we used twenty 87.5% C57Bl/6J − 12.5% 129SvJ wild-type and transgenic mice that were deficient for the gene encoding for PAI-1 ($PAI\text{-}1^{+/+}$ and $PAI\text{-}1^{-/-}$). For the second experiment, we used thirty 75% C57Bl/6J − 25% 129SvJ wild-type and transgenic mice that were deficient for the genes encoding for uPA ($uPA^{+/+}$ and $uPA^{-/-}$) and tPA ($tPA^{+/+}$ and $tPA^{-/-}$). All wild-type and transgenic mice were obtained from the Center for Transgene Technology and Gene Therapy of the Katholieke Universiteit Leuven. The uPA, tPA, and PAI-1 knockout mice were generated as described elsewhere (57–59). For the third experiment, 20 Naval Medical Research Institute mice were used.

The animals were kept under standard laboratory conditions (temperature 20°C to 22°C, relative humidity 50% to 60%, 14 hours light and 10 hours dark) at the animal facilities of the Katholieke Universiteit Leuven. They were fed a standard laboratory diet (Muracon G; Carsil Quality, Turnhout, Belgium) and had constant free access to food and water. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia

After anesthesia with pentobarbital (Nembutal; Sanofi Sante Animale, Brussels, Belgium), 0.07 mg/g, was administered i.m., the abdomen was shaved and the animal was secured to the table in the supine position. Endotracheal intubation was performed with a 22-gauge catheter (Insyte-W, Vialon; Becton Dickinson, Madrid, Spain) by transillumination of the vocal cords. The catheter was connected to a mechanical ventilator (Rodent Ventilator; Harvard Apparatus, Holliston, MA), and the animal was ventilated with room air (tidal volume 500 μL, 85 strokes/min).

Laparoscopy

Laparoscopy was performed as described in detail elsewhere (41). In brief, a 3.5-mm midline incision was performed caudal to the xyphoides appendix, and a 2-mm endoscope with a 3.3-mm external sheath (Karl Storz, Tüt-

tlingen, Germany) was introduced into the abdominal cavity. The endoscope was connected to a video camera (Karl Storz) and a light source (Karl Storz) and secured in a holder. Because the mouse abdominal wall is very thin, gas leakage occurred, causing flow to vary. Therefore, the incision was suture gas tight around the endoscope by using 6/0 polypropylene suture (Prolene; Ethicon, Johnson and Johnson International, Brussels, Belgium).

For the pneumoperitoneum, the gas was insufflated through the main port with the Thermoflator Plus (Karl Storz), using heated (37°C) (Optitherm; Karl Storz) and humidified (Aquapor; Dräger, Lübeck, Germany) 100% carbon dioxide as the insufflation gas. An insufflation pressure of 17 mm Hg and a flow rate of 1.5 L/min together with a water valve and an elastic balloon were used to ascertain a continuous insufflation pressure of 20 cm H_2O (about 15 mm Hg).

The water valve and the balloon are necessary to adapt the flow rate to a mouse and to dampen the pressure changes during insufflation. Any excess of carbon dioxide freely escapes from the water valve, whereas pressure is maintained accurately in the water valve and changes in pressure are minimized.

Because the peritoneum has a large surface and high exchange capacity, in theory some oxygen could diffuse from the circulation to the abdominal cavity. To ascertain continuously a constant 100% carbon dioxide concentration in the abdominal cavity, the gas was continuously replaced. This was achieved by inserting a 26-gauge needle (BD Plastipak; Becton Dickinson) through the abdominal wall, providing a continuous flow through the abdominal cavity of 10 mL/min at 20 cm H_2O. The pneumoperitoneum was maintained for different periods according to the experimental design.

Induction of Intraperitoneal Adhesions

Induction of adhesions was performed as described previously (41). After carbon dioxide pneumoperitoneum was established, two 14-gauge catheters (Insyte-W, Vialon; Becton Dickinson) were inserted under laparoscopic vision in the right and left flank for the working instruments. The uterus was grasped in the midline by using a 1.5-mm grasper, and standardized 10-mm × 1.6-mm lesions were made in the antimesenteric border of both right and left uterine horns by monopolar or bipolar coagulation (10 W, standard coagulation mode, for 10 seconds) (Autocon 350; Karl Storz). Identical lesions were made in right and left pelvic sidewalls. The type of lesion in each side was randomly determined. Monopolar coagulation was performed with a homemade 1.6-mm ball electrode, whereas bipolar coagulation was performed with a 1.6-mm probe (Bicap; Circon Corp., Santa Barbara, CA).

To evaluate postoperative basal adhesion formation and pneumoperitoneum-enhanced adhesion formation, the pneumoperitoneum was maintained for the minimum time needed to induce the peritoneal lesions (10 minutes) or for a longer period (60 minutes), respectively. The secondary ports were removed after the peritoneal lesions were completed, and the incisions were closed. The first incision was closed at the end of the surgery. All incisions were closed in a single layer with 6/0 polypropylene suture (Prolene; Ethicon, Johnson and Johnson International).

Scoring of Adhesions

A xyphopubic midline incision and a bilateral subcostal incision were made, and the abdominal cavity was explored by laparotomy 7 days after induction of adhesions, as described elsewhere (41). After the evaluation of port sites and viscera, the pelvic fat tissue was carefully removed and adhesions were blindly scored under microscopic vision by using a qualitative and a quantitative scoring system.

In the qualitative scoring system, which was modified from that of Leach et al. (60), the following characteristics were assessed: extent (0, no adhesions; 1, 1% to 25%; 2, 26% to 50%; 3, 51% to 75%; 4, 76% to 100% of the injured surface involved), type (0, no adhesions; 1, filmy; 2, dense; 3, capillaries present), tenacity (0, no adhesions; 1, falls apart; 2, requires traction; 3, requires sharp dissection), and total (extent + type + tenacity). The quantitative scoring system was described by Holmdahl et al. (61). This system has the advantage of precluding subjective interpretation. It measures the proportion of the lesions covered by adhesions by using the following formula: adhesions (%) = (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, with lesions inflicted by monopolar or bipolar coagulation), which were individually scored.

Tissue Sampling, Protein Extraction, and Assays for PAI-1, tPA, and Total Protein

The abdomen was opened at different times before or after exposure to pneumoperitoneum as described above, and biopsy samples were obtained from the pelvic sidewall within the first 4 minutes. The samples were rinsed with ice-cold phosphate-buffered saline, frozen immediately in liquid nitrogen, and stored at −80°C. Tissues were homogenized in 500 μL of phosphate-buffered saline containing 1% Triton X-100 (diluted), 0.1% sodium dodecylsulfate, 0.5% sodium deoxicholate, 0.2% sodium azide, and a cocktail of protease inhibitors (Complete; Roche Diagnostics GmbH, Mannhein, Germany).

After centrifugation (8500 × g at 4°C for 10 minutes), the supernatants were assayed for total protein, PAI-1, and tPA concentration. Tissue protein concentration was measured by using a detergent-compatible formulation based on bicinchoninic acid for the colorimetric detection and quantification of total protein. Bovine serum albumin was used as a standard

(BCA Protein Assay Kit; Pierce, Rockford, United Kingdom). Concentrations of PAI-1 and tPA were measured as described elsewhere (62, 63) by using a homemade ELISA. All samples were assayed at four serial dilutions, and results (pg of PAI-1/mg of protein or pg of tPA/mg of protein) are expressed as the mean (±SE).

Experimental Design

All experiments were performed using block randomization by day. Therefore, one block of mice, comprising one animal of each group, was operated during the same day. Within a block, animals were operated in random order.

In the first experiment (n = 20), peritoneal adhesions were induced as described, and basal adhesions and pneumoperitoneum-enhanced adhesions were assessed in PAI-1 wild-type mice (n = 5 and n = 5, respectively) and PAI-1 knockout mice (n = 5 and n = 5, respectively).

In the second experiment (n = 30), peritoneal adhesions were induced as described, and basal adhesions and pneumoperitoneum-enhanced adhesions were assessed in uPA/tPA wild-type mice (n = 5 and n = 5, respectively), uPA knockout mice (n = 5 and n = 5, respectively), and tPA knockout mice (n = 5 and n = 5, respectively).

In the third experiment (n = 20), we evaluated the effect of 60 minutes of exposure to carbon dioxide pneumoperitoneum, with no other peritoneal lesion, on the time course of expression of PAI-1 and tPA in the abdominal wall. Samples were collected before (n = 5) and immediately (n = 5) or 3 (n = 5) or 6 hours (n = 5) after 60 minutes of pneumoperitoneum.

Statistical Analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC), using a nonparametric test (Kruskal–Wallis) to compare individual groups and Spearman correlation to evaluate association. All data are presented as the mean (±SE). *P* values <.05 were considered statistically significant.

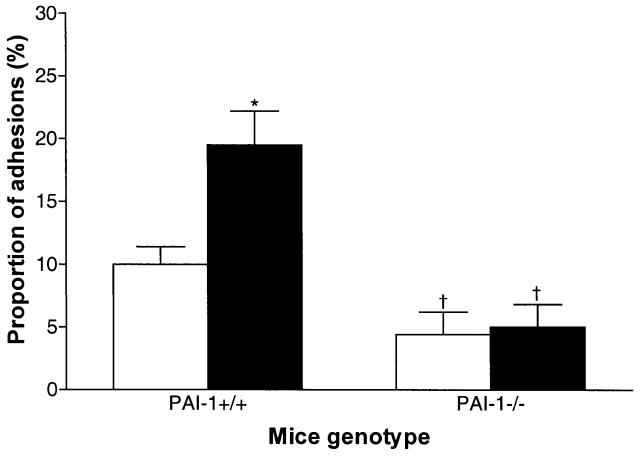
RESULTS

All animals survived the surgical procedures and, in the adhesion formation experiments, all were available for adhesion scoring after 7 days. Adhesions formed between the injured visceral site and the pelvic fat or between the injured parietal site and the pelvic fat. No adhesions were observed at the site of the laparoscopic ports or at other sites.

Monopolar lesions systematically induced more adhesions than bipolar lesions. The respective proportion of adhesions among monopolar and bipolar lesions were as follows: in PAI-1 wild-type mice, 16% ± 3% and 4% ± 2% for basal adhesions and 25% ± 4% and 14% ± 3% for pneumoperitoneum-enhanced adhesions; in PAI-$1^{-/-}$ mice, 6% ± 4% and 3% ± 3% for basal adhesions and 8% ± 3% and 2% ± 2% for pneumoperitoneum-enhanced adhesions; in uPA/tPA wild-type mice, 8% ± 5% and 4% ± 3% for basal adhesions and 18% ± 5% and 15% ± 3% for pneumoperitoneum-enhanced adhesions; in uPA$^{-/-}$ mice, 12% ± 4% and 12% ± 6% for basal adhesions and 18% ± 2% and 8% ± 3% for pneumoperitoneum-enhanced adhesions; and in tPA$^{-/-}$ mice, 29% ± 2% and 21% ± 7% for basal adhesions and 24% ± 7% and 19% ± 3% for pneumoperitoneum-enhanced adhesions.

FIGURE 1

Proportion of basal adhesions (□) and pneumoperitoneum-enhanced adhesions (■) in wild-type and knockout mice for plasminogen activator inhibitor-1 (PAI-$1^{+/+}$ and PAI-$1^{-/-}$). Data are means (±SE). *$P \leq .05$, Kruskal–Wallis analysis, for pneumoperitoneum-enhanced versus basal adhesions; †$P \leq .05$, Kruskal–Wallis analysis, for knockout versus wild-type mice.



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Similar data were obtained in terms of scores for extent, type, tenacity, and total adhesions (data not shown). To maximize statistical significance, only the means of both nonpolar and bipolar lesions are used for further analysis.

In PAI-1 wild-type mice, pneumoperitoneum enhanced adhesion formation (proportion, *P*=.02; extent, *P*=.01; type, *P*=.01; tenacity, *P*=.01; total, *P*=.01). Compared with PAI-1 wild-type mice, basal adhesions were lower in PAI-$1^{-/-}$ mice (proportion, *P*=.03; extent, *P*=.02; type, *P*=.01; tenacity, *P*=.02; total, *P*=.01). In PAI-$1^{-/-}$ mice, pneumoperitoneum did not enhance adhesions. Therefore, compared with PAI-1 wild-type mice, pneumoperitoneum-enhanced adhesions were even lower in PAI-$1^{-/-}$ mice (proportion, *P*=.01; extent, *P*=.01; type, *P*=.01; tenacity, *P*=.01; total, *P*=.01) (Fig. 1, Table 1). Similar effects were observed when monopolar and bipolar lesions were analyzed individually.

In uPA/tPA wild-type mice, pneumoperitoneum enhanced adhesion formation (proportion, *P*=.03; extent, *P*=

TABLE 1

Basal adhesions and pneumoperitoneum-enhanced adhesions in wild-type and knockout mice for plasminogen activator inhibitor-1.

Genotype	Type of adhesion	Scores			
		Extent	Type	Tenacity	Total
PAI-1$^{+/+}$ (n = 10)	Basal	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	2.2 ± 0.2
	Pneumoperitoneum-enhanced	1.3 ± 0.1[a]	1.6 ± 0.1[a]	1.7 ± 0.1[a]	4.5 ± 0.2[a]
PAI-1$^{-/-}$ (n = 10)	Basal	0.2 ± 0.1[b]	0.2 ± 0.1[b]	0.3 ± 0.1[b]	0.7 ± 0.3[b]
	Pneumoperitoneum-enhanced	0.3 ± 0.1[b]	0.3 ± 0.1[b]	0.4 ± 0.2[b]	1.0 ± 0.3[b]

Note: Data are means (±SE). PAI-1 = plasminogen activator inhibitor-1.

[a] $P<.05$ for pneumoperitoneum-enhanced vs. basal adhesions.

[b] $P<.05$ for knockout vs. wild-type mice.

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.02; type, P not significant; tenacity, $P=.02$; total, $P=.03$). Compared with uPA/tPA wild-type mice, basal adhesions were higher in both uPA$^{-/-}$ mice (P not significant) and tPA$^{-/-}$ mice (proportion, $P=.02$; extent, $P=.02$; type, $P=0.03$; tenacity, $P=.03$; total, $P=.02$). In both uPA$^{-/-}$ and tPA$^{-/-}$ mice, pneumoperitoneum did not enhance adhesions. Compared with uPA/tPA wild-type mice, pneumoperitoneum-enhanced adhesions were similar in both uPA$^{-/-}$ and tPA$^{-/-}$ mice. The tPA$^{-/-}$ mice developed more basal adhesions (extent, $P=.04$) and more pneumoperitoneum-enhanced adhesions (type, $P=.05$; tenacity, $P=.03$) than did uPA$^{-/-}$ mice (Fig. 2, Table 2). Similar effects were observed when monopolar and bipolar lesions were analyzed individually.

The protein concentration of PAI-1 in the abdominal wall increased (Spearman correlation) for at least 6 hours after 60 minutes of carbon dioxide pneumoperitoneum exposure ($P<.001$). By Kruskal–Wallis analysis, the increase in PAI-1 concentration was significant after 1 hour ($P=.02$), 3 hours ($P=.02$), and 6 hours ($P=.01$) (Fig. 3). The protein concentration of tPA did not change significantly over time after 60 minutes of exposure to carbon dioxide pneumoperitoneum (P not significant, Spearman correlation; P not significant, Kruskal–Wallis analysis compared with control group) (Fig. 3).

DISCUSSION

We used a laparoscopic mouse model to evaluate formation of basal and pneumoperitoneum-enhanced adhesions. Basal adhesions results not only from a peritoneal lesion inflicted by electrocautery but also from the effect of as little as 10 minutes of carbon dioxide pneumoperitoneum. Formation of basal adhesions independent of an additional effect of carbon dioxide pneumoperitoneum would require the shortest duration of pneumoperitoneum possible, the minimum insufflation pressure, and 3% oxygen added to the carbon dioxide pneumoperitoneum, since adhesion formation de-

TABLE 2

Basal adhesions and pneumoperitoneum-enhanced adhesions in wild-type and knockout mice for urokinase and tissue-type plasminogen activator.

Genotype	Type of adhesion	Scores			
		Extent	Type	Tenacity	Total
uPA$^{+/+}$/tPA$^{+/+}$(n = 10)	Basal	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	1.3 ± 0.4
	Pneumoperitoneum-enhanced	0.9 ± 0.1[a]	0.9 ± 0.1	1.1 ± 0.1[a]	2.9 ± 0.2[a]
uPA$^{-/-}$ (n = 10)	Basal	0.6 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	2.2 ± 0.4
	Pneumoperitoneum-enhanced	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	2.3 ± 0.3
tPA$^{-/-}$ (n = 10)	Basal	1.3 ± 0.2[b]	1.9 ± 0.4[b]	1.8 ± 0.4[b]	4.9 ± 0.9[b]
	Pneumoperitoneum-enhanced	1.2 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	4.4 ± 0.7

Note: Data are means (±SE). tPA = tissue-type plasminogen activator; uPA = urokinase plasminogen activator.

[a] $P<.05$ for pneumoperitoneum-enhanced vs. basal adhesions.

[b] $P<.05$ for knockout vs. wild-type mice.

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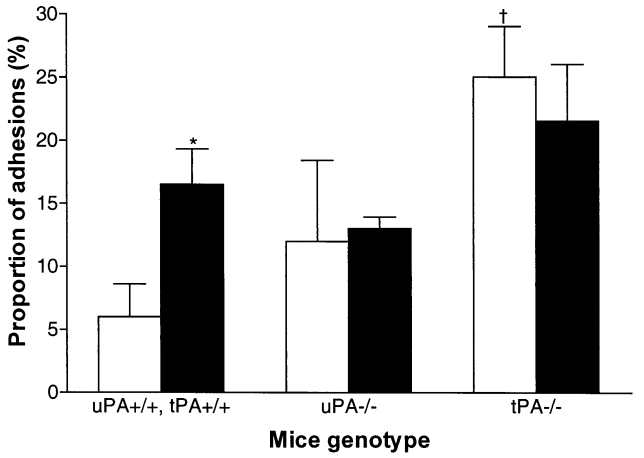
FIGURE 2

Proportion of basal adhesions (□) and pneumoperitoneum-enhanced adhesions (■) in wild-type and knockout mice for urokinase plasminogen activator and tissue-type plasminogen activator (uPA$^{+/+}$/tPA$^{+/+}$, uPA$^{-/-}$, and tPA$^{-/-}$). Data are means (±SE). *$P \leq .05$, Kruskal–Wallis analysis, for pneumoperitoneum-enhanced versus basal adhesions; †$P \leq .05$, Kruskal–Wallis analysis, for knockout versus wild-type mice.



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creases with shorter duration of pneumoperitoneum, lower pressure, and with the addition of oxygen (38–41).

To evaluate basal adhesions, pneumoperitoneum was maintained for the minimum time required to induce the lesions (standardized at 10 minutes). However, we used 100% carbon dioxide at 20 cm H_2O because a lower pressure and the addition of oxygen, although theoretically better, would introduce additional variables. Using both controls was not feasible because of the limited availability of transgenic animals.

To our knowledge, this is the first report on adhesion formation in PAI-1, uPA, and tPA knockout mice using a laparoscopic model. The effects of these components of the plasminogen system on basal adhesion formation were as expected and confirmed the findings of previous studies (2–12). Compared with wild-type mice, PAI-1 knockout mice developed fewer adhesions, whereas both uPA and tPA knockout mice developed more adhesions. This effect was expected since the lack of uPA and tPA reduces plasmin activation and fibrin degradation, thus leading to adhesion formation, whereas the lack of PAI-1 reduces the inactivation of uPA and tPA, increasing plasmin levels and fibrin degradation, thus reducing adhesion formation.

The smaller increase in adhesion formation in uPA than in tPA knockout mice is also consistent with the effects of recombinant uPA and tPA in animal models. Whereas tPA administration clearly reduces adhesion formation, the results of uPA administration are controversial (6–12, 64). These differences between uPA and tPA could be explained by the fact that uPA activity requires the specific binding of plasminogen to fibrin, whereas tPA activity is greatly enhanced by fibrin, since its binding to its specific receptor exposes a strong plasminogen-binding site on the surface of the fibrin molecule (26, 65–67).

This study also confirmed our previous finding that the pneumoperitoneum is a cofactor in adhesion formation (38–41). Pneumoperitoneum-enhanced adhesions were observed in all wild-type mice.

The absence of pneumoperitoneum-enhanced adhesions in PAI-1, uPA, and tPA knockout mice may be explained by postulating that pneumoperitoneum-enhanced adhesion formation observed in wild-type mice was due at least in part to up-regulation of PAI-1. In PAI-1 knockout mice, up-regulation of PAI-1 and, thus, pneumoperitoneum-enhanced adhesions is impossible. Mice lacking uPA or tPA already have increased basal adhesions. Up-regulation of PAI-1 would not inhibit the nonexistent uPA and tPA activity and would thus not further increase adhesion formation.

Up-regulation of PAI-1 in carbon dioxide pneumoperitoneum was confirmed by the ELISA of the abdominal wall. This finding is consistent with the reported up-regulation of PAI-1 in hypoxia (53–55), since carbon dioxide pneumoperitoneum-enhanced adhesion formation is probably mediated by mesothelial hypoxia (41).

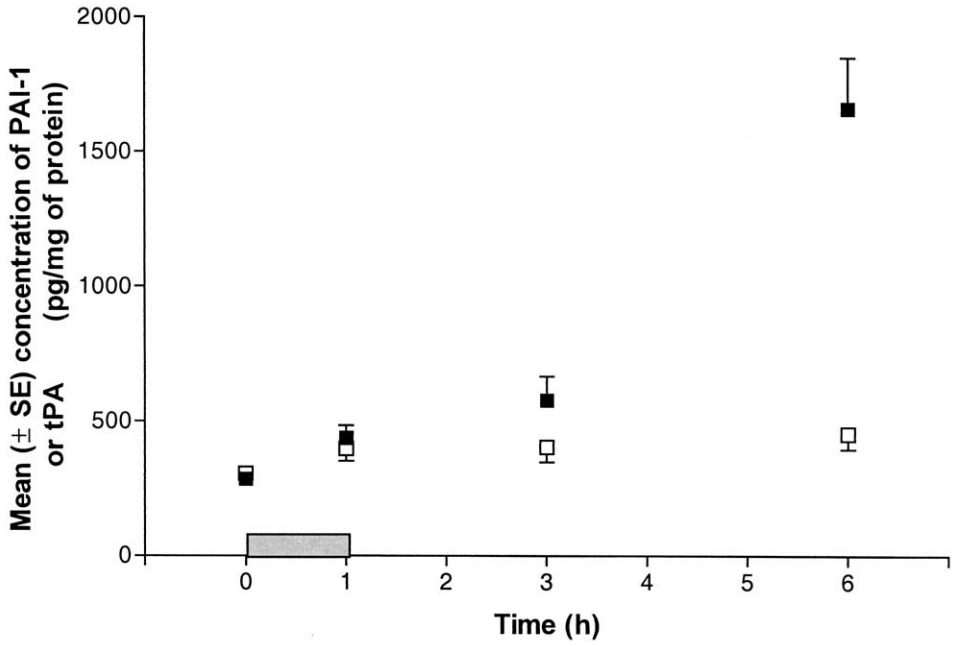
It is unclear whether pneumoperitoneum-induced mesothelial damage causes an inflammatory reaction, which could up-regulate PAI-1. Indeed, PAI-1 is regulated by many factors, such as thrombin, endotoxin, interleukin-1, tumor necrosis factor-α, transforming growth formation-β, trauma, and infection; a common link among these factors is the inflammatory reaction (68–70).

Tissue injury locally up-regulates PAI-1 and down-regulates tPA (33, 71–73), probably in response to local hypoxia or inflammation (or both). The carbon dioxide pneumoperitoneum up-regulates PAI-1 and down-regulates tPA (74), probably in response to hypoxia or inflammation in the entire peritoneal mesothelium. It therefore remains unclear whether the mechanisms of PAI-1 up-regulation after tissue injury (hypoxia and inflammation) or after pneumoperitoneum (hypoxia followed by inflammation) are identical. More detailed investigations are required in the normal and in the damaged peritoneum to elucidate the effects of the duration of pneumoperitoneum, insufflation pressure, and addition of oxygen.

Adhesion formation varied with the strain of mice used. The 87.5% C57Bl/6J − 12.5% 129SvJ mice developed more adhesions than did 75% C57Bl/6J − 25% 129SvJ mice. These strain differences were seen for both basal adhesions and pneumoperitoneum-enhanced adhesions. However,

FIGURE 3

Concentrations of plasminogen activator inhibitor-1 (*PAI-1*) (■) and tissue-type plasminogen activator (*tPA*) (□) in the abdominal wall before and after 60 minutes of carbon dioxide pneumoperitoneum (▨), but no other peritoneal lesion, in wild-type mice. Data are means (±SE). ■$P<.001$; □*P* not significant (Spearman correlation).



Molinas. Plasminogen system, laparoscopy, and adhesions. Fertil Steril 2003.

these data should be interpreted with caution because they are derived from two different experiments.

Taking all our available data together, adhesion formation seems to be most pronounced in Naval Medical Research Institute and Swiss mice and least in 129SvJ mice. The C57Bl/6J mice had an intermediate rate of adhesion formation. These findings were observed in experiments with 100% Naval Medical Research Institute (41, 75), 100% Swiss, 87.5% Swiss – 12.5% 129SvJ, 75% Swiss – 25% 129SvJ, 50% Swiss – 50% 129SvJ, and 100% C57Bl/6J mice (Molinas CR, et al., unpublished data). Again, however, these data should be interpreted with caution because they are combined from different experiments. To ascertain and evaluate these finding, we are planning further studies.

Differences among strains may yield useful information concerning the pathophysiology of adhesion formation. These observations are not surprising, since strain differences have been reported for fibrosis and healing responses in such situations as hepatic fibrosis (76), lung fibrosis (77), colorectal fibrosis (78), ear wound healing (79), myocardial healing (80), and bone regeneration (81).

Monopolar lesions systematically induced more adhesions than did bipolar lesions. This was not surprising, since monopolar lesions produced macroscopically larger lesions. The surgical trauma of both monopolar and bipolar coagulations is well documented and depends on the power setting and duration of the procedure (82). The exact relationship between surgical trauma and adhesion formation, however, has never been explored.

Pneumoperitoneum clearly enhanced both monopolar and bipolar lesions in wild-type mice. The data do not allow us to determine whether pneumoperitoneum simply adds an adhesion factor—for example, 10 or so additional points on the scoring system—or whether it amplifies basal adhesions.

In conclusion, our data confirm the role of PAI-1, uPA, and tPA in formation of basal adhesions and the role of the pneumoperitoneum as a cofactor in adhesion formation. Our findings in knockout mice and on ELISA indicate that PAI-1 up-regulation is a mechanism for pneumoperitoneum-enhanced adhesion formation. This is consistent with the concept that carbon dioxide pneumoperitoneum causes mesothelial hypoxia and with up-regulation of PAI-1 through hypoxia in injured areas of peritoneum.

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