Impact of intraperitoneal pressure and duration of surgery on levels of tissue plasminogen activator and plasminogen activator inhibitor-1 mRNA in peritoneal tissues during laparoscopic surgery†

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BACKGROUND: Our objective was to evaluate the impact of intraperitoneal pressure (IPP) and duration of a CO₂ pneumoperitoneum on the peritoneal fibrinolytic system during laparoscopic surgery.

METHODS: Human study: Patients undergoing laparoscopic surgery were divided into two groups: low (8 mmHg, n = 32) or standard (12 mmHg, n = 36) IPP. Normal peritoneum was collected from the parietal wall at the beginning of surgery and every 60 min thereafter. Mouse study: Mice were divided into three groups: low (2 mmHg) or high (8 mmHg) IPP or laparotomy. Peritoneal tissue was collected at 0, 4, 8, 24, 48 and 72 h, and 5 and 7 days after surgery. Real-time RT–PCR was performed in humans and mice to measure the levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) mRNA in peritoneal tissues.

RESULTS: Human study: The tPA/PAI-1 mRNA ratio was significantly decreased in the 12 mmHg group at 1 h [P < 0.0001 versus matched initial peritoneal biopsies (MI)]. The tPA/PAI-1 mRNA ratio decreased in both groups at 2 h (P < 0.01 versus MI). Mouse study: The tPA/PAI-1 ratio was decreased at 0 h, and the difference was significant at 4 h in both the laparotomy (P < 0.001 versus controls, 0 h, 5 and 7 days) and high-IPP (P < 0.0001 versus 0, 48 and 72 h, 5 and 7 days) groups. No changes in tPA/PAI-1 ratio were observed in the low-IPP group.

CONCLUSIONS: A low IPP and shorter duration of surgery appear to minimally impact the fibrinolytic system during a CO₂ pneumoperitoneum.

Key words: adhesions / CO₂ pneumoperitoneum / intraperitoneal pressure / laparoscopy

Introduction

Laparoscopic surgery is generally considered to be less adhesiogenic than open surgery. However, post-operative adhesion formation remains a major clinical problem (Ray et al., 1998; Ellis et al., 1999). Although the underlying mechanisms of post-operative adhesion formation remain to be elucidated, it is well known that impairment of the fibrinolytic system is crucial to the process (Holmdahl, 1997; diZerega and Campeau, 2001). Decreased tissue plasminogen activator (tPA) activity leads to hypofibrinolysis. tPA activity depends on the ratio of tPA to its inhibitor, plasminogen activator inhibitor-1 (PAI-1) (tPA/PAI-1 ratio) (Ivarsson et al., 2003; Saed and Diamond, 2003; Gago et al., 2006).

Many previous clinical studies have demonstrated impairment of tPA activity as well as expression levels of tPA and PAI-1 in peritoneal...
fluid and peritoneal tissues during and after surgery (Batzoﬁn et al., 1985; Thompson et al., 1989; Scott-Coombes et al., 1995a,b; Edelstam et al., 1998; Holmdahl et al., 1998; Ivarsson et al., 1998; Bergström et al., 2002, Neudecker et al., 2002a, 2005; Hellebrekers et al., 2005, 2009; Brokeman et al., 2006, 2009a,b; Tarhan et al., 2008). However, to date, only a few studies of the fibrinolytic system in peritoneal tissues during laparoscopic surgeries have been performed (Bergström et al., 2002; Neudecker et al., 2002a; Brokeman et al., 2006, 2009a,b).

Today, in the clinical setting, 12–15 mmHg of intraperitoneal pressure (IPP) is generally applied during laparoscopic surgery. A pneumoperitoneum is required to perform laparoscopic surgery; however, animal experiments, including those performed in our laboratory, have suggested that a high IPP might adversely affect the surgical peritoneal environment (Wittich et al., 2000; Bourdel et al., 2007; Matsuzaki et al., 2009, 2010a,b). Laparoscopic surgery technology has evolved markedly over the past two decades, and continues to advance. However, much less attention has been focused on how altering the laparoscopic surgical environment might improve clinical outcomes.

Further studies are required to investigate the impact of IPP on the peritoneal fibrinolytic system during laparoscopic surgery. In addition, to develop novel strategies for treatment and/or prevention of postoperative adhesion formation, it is necessary to first understand the time course during which the peritoneal environment impairs the fibrinolytic system during laparotomy or laparoscopy.

In the present study, we investigated the impact of IPP and duration of surgery on the peritoneal tPA/PAI-1 ratio during laparoscopic surgery. In addition, we performed a time course study to investigate the impact of changes in the surgical peritoneal environment induced by a CO2 pneumoperitoneum at high or low IPP, as well as during laparotomy, on the peritoneal tPA/PAI-1 ratio during the first 7 days after surgery in a mouse model.

Materials and Methods

Patients

Patients undergoing laparoscopic hysterectomy with or without pronomotokation for benign gynecological disease were recruited. Patients with a previous history of pelvic surgery, endometriosis and/or infection were excluded from the present study. Insufﬁation of CO2 gas was performed using a Storz electronic endoflator (Karl Storz Endoscopy & GmbH). When the IPP reached 15 mmHg, four trocars were inserted, immediately after which the IPP was decreased to 12 or 8 mmHg and then maintained at these levels throughout surgery. The duration between insufﬁation of CO2 gas and insertion of the four trocars was <5 min.

In our clinical setting, the minimum IPP required to perform major laparoscopic surgical procedures is 8 mmHg (Neudecker et al., 2002b). In IPP of 12 mmHg was applied to patients who underwent surgery performed by M.C., whereas 8 mmHg was applied to patients who underwent surgery performed by R.B. and K.J. Macroscopically normal peritoneum was collected from the anterior parietal wall at the beginning of surgery and every 60 min thereafter. Because the vagina is opened at the end of the total hysterectomy procedure, all the samples in the present study were collected before opening the vagina for evaluation of the impact of a CO2 pneumoperitoneum at a constant IPP. The area from which the peritoneal biopsy was acquired was intact and located at a constant distance from the port through which the CO2 gas was insufﬁated. In view of the fragility of the mesothelial layer, the peritoneal biopsies were performed meticulously to minimize the possibility of trauma to the specimen. In addition, a study demonstrated the inﬂuence of the sampling method on the peritoneal fibrinolytic activity (Neudecker et al., 2002c). The full thickness of the peritoneum was excised using only a pair of scissors, and peritoneal tissues were then collected by atraumatic forceps in all cases.

None of the patients had intra- or post-operative complications. In addition, no cases were converted to laparotomy. Finally, peritoneal samples were collected from a total of 36 patients (age range: 42–69 years; median: 48.5 years) during CO2 pneumoperitoneum at 12 mmHg and 32 patients (age range: 42–63 years; median: 48.0 years) at 8 mmHg. As this is a pilot study, a power-calculation was not performed. Peritoneal samples were collected at 0 and 1 h from a total of 20 patients at 12 mHg and 17 patients at 8 mmHg, and at 0, 1 and 2 h from a total of 16 patients at 12 mmHg and 15 patients at 8 mmHg.

Peritoneal samples were immediately collected in RNAlater (Ambion, Cambridge, UK) and stored at −20°C until further analysis. All tissue samples were obtained with full and informed patient consent. The research protocol was approved by the Consultative Committee for Protection of Persons in Biomedical Research of the Auvergne region.

Animals

Animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, Public Health Service, 1985), and institutional review board approval was obtained for the current study. Studies were conducted in adult (8-week-old, 18–20 g) female C57BL/6 mice (Iffa-Credo, Lyon, France). The mice were maintained in a light- and temperature-controlled environment (14-h light, 10-h dark cycle; 22–25°C) and allowed a 2-week period of acclimation to the vivarium before any procedures were performed. After completion of the experiment, all mice were euthanized with an anesthetic overdose.

Anesthesia and videendoscopy-assisted endotracheal intubation

Anesthesia, videendoscopy-assisted endotracheal intubation and mechanical ventilation were performed as described previously (Bourdel et al., 2007; Matsuzaki et al., 2007, 2009, 2010a; Azuar et al., 2009).

Mice were ventilated with a tidal volume of 200 μl (Mini Vent type 845, Harvard Apparatus GmbH, March-Hugstetten, Germany) at 250 strokes (laparoscopy) or 220 strokes (laparotomy, anesthesia alone) per minute.

Surgical procedures

Laparotomy or CO2 insufﬁation for 60 min was performed as described previously (Bourdel et al., 2007; Matsuzaki et al., 2007, 2009, 2010a; Azuar et al., 2009).

Homeothermia

After the induction of anesthesia, all procedures were performed on a thermostatically regulated, feedback-controlled heating pad (Homeothermic Blanket Control Unit, Harvard Apparatus GmbH) (Bourdel et al., 2007; Matsuzaki et al., 2007, 2009, 2010a; Azuar et al., 2009). After intubation, a temperature probe was inserted into the rectum. A rectal temperature of 37°C was maintained during the surgical procedure.

Experimental design

On Day 0, C57Bl/6 mice were divided into three groups: CO2 pneumoperitoneum at a low (2 mmHg) or high (8 mmHg) IPP, or laparotomy. Groups were further subdivided into eight groups of five animals each, and
laparotomy was performed to collect peritoneal tissue at 0, 4, 8, 24, 48 and 72 h, and 5 and 7 days after surgery. Peritoneal tissues were collected from the left parietal peritoneum, 1.5 cm lateral to the midline to avoid contamination from the injured midline at the laparotomy or laparoscopic trocar sites.

In addition, a total of five animals that underwent anesthesia alone for 60 min served as controls, and laparotomy was performed to collect peritoneal tissue at 0 h after anesthesia alone. Once collected, the samples were immediately placed in RNAlater (Ambion) and stored at −20°C until RNA extraction.

**RNA extraction**

Total RNA was extracted using the Qiagen RNeasy Mini Kit according to the manufacturer’s instructions and stored at −80°C until use. To eliminate potential genomic DNA contamination, RNA samples were treated with DNaseI (15 U; DNaseI, Qiagen, Courtaboeuf, France) at room temperature for 15 min.

**Examination of RNA yield and integrity**

RNA yield and integrity were analyzed using the RNA 6000 Pico kit and the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA; Matsuzaki et al., 2010b). The RNA 6000 Pico kit allows determination of the integrity of very small amounts of RNA as well as estimation of the quantity of the isolated RNA, which has a linear range of 200–5000 pg/μl. The RIN (RNA integrity number) value was >8.0 in all of the samples included in the present analysis using real-time RT–PCR (Fleige et al., 2006; Schroeder et al., 2006).

**Quantitative real-time RT–PCR**

Quantitative real-time RT–PCR with a Light Cycler was performed as described previously (Matsuzaki et al., 2007, 2009, 2010a,b). Total RNA (100 ng) was subjected to a reverse transcriptase reaction using Superscript II Reverse Transcriptase (Invitrogen, Cergy Pontoise, France). Quantitative real-time PCR was performed in a LightCycler System using the FastStart DNA Master SYBR Green I kit as recommended by the manufacturer (Roche, Mannheim, Germany). In a total volume of 20 μl, each reaction contained 2 μl SYBR green I reaction mix (consisting of Taq DNA-polymerase reaction buffer, dNTP mix, SYBR green I, MgCl₂ and Taq DNA polymerase), 0.3–0.5 μM of each primer, 4 mM MgCl₂, 2 μl cDNA and standard or nuclease-free water as a negative control. Primer sets are shown in Table I. Quantification of the targets in the samples was performed using a relative quantification method with external standards. The target concentration is expressed relative to the concentration of a reference housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase. After each run, a melting curve analysis was performed to verify the specificity of PCR. The procedure was repeated three times independently to ensure the reproducibility of the results. All the samples with a Ct (cycle threshold) coefficient of variation value higher than 5% were retested.

**Statistical analysis**

The Statview 4.5 program (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analysis. The Wilcoxon signed-rank test was performed to compare the differences in paired peritoneal samples within the same patients. Comparisons between different groups were made using the one-way analysis of variance following Scheffe’s method or the Mann–Whitney U-test. Statistical significance was defined as P < 0.05.

**Results**

**Human study**

**tPA mRNA level**

No significant difference in tPA mRNA levels in the 8 and 12 mmHg groups was observed after 1 or 2 h compared with that of initial biopsies within the same patients (Fig. 1A). However, tPA mRNA levels in the 12 mmHg group were significantly increased after 1 h compared with those of initial biopsies within the same patients (Fig. 1B). Furthermore, tPA mRNA levels in the 12 mmHg group were significantly higher than those in the 8 mmHg group at 1 h of CO₂ pneumoperitoneum (Fig. 1B, Table III). No significant difference in tPA mRNA levels within the same patients. Comparisons between different groups were made using the one-way analysis of variance following Scheffe’s method or the Mann–Whitney U-test. Statistical significance was defined as P < 0.05.

**PAI-1 mRNA level**

In the 8 mmHg group, no significant difference in PAI-1 mRNA levels was observed after 1 h versus at initial biopsy within the same patients (Fig. 1B). However, PAI-1 mRNA levels in the 12 mmHg group were significantly increased after 1 h compared with those of initial biopsies within the same patients (Fig. 1B). Furthermore, PAI-1 mRNA levels in the 12 mmHg group were significantly higher than those in the 8 mmHg group at 1 h of CO₂ pneumoperitoneum (Fig. 1B, Table III). At 2 h of CO₂ pneumoperitoneum, PAI-1 mRNA levels were significantly increased in both groups compared with those of initial biopsies within the same patients (Fig. 1B). However, PAI-1 mRNA levels in the 12 mmHg group were significantly higher than those in the 8 mmHg group at 2 h of CO₂ pneumoperitoneum (Fig. 1B, Table III).

**tPA/PAI-1 mRNA ratio**

In the 8 mmHg group, no significant difference in the tPA/PAI-1 mRNA ratio was observed after 1 h compared with that of initial biopsies within the same patients (Fig. 1C). However, the tPA/PAI-1 mRNA ratio in the 12 mmHg group was significantly decreased after 1 h compared with that of initial biopsies within the same patients (Fig. 1C). The tPA/PAI-1 mRNA ratio in the 12 mmHg group was significantly lower than that in the 8 mmHg group at 1 h of CO₂

**Table I. Sequences of the primers used for mRNA quantification by real-time RT–PCR.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primers</th>
<th>Antisense primers</th>
<th>Bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human tPA</td>
<td>5′-CGCCTGTGAAGCAATCATGGA-3′</td>
<td>5′-GCCATGACTGTATGTGCTGTA-3′</td>
<td>184</td>
</tr>
<tr>
<td>Human PAI-1</td>
<td>5′-AAGACTCTCTTCCCCAGCTC-3′</td>
<td>5′-GCCATGACTGTATGTGCTGTA-3′</td>
<td>123</td>
</tr>
<tr>
<td>Human GAPDH</td>
<td>5′-TGCACACCAAACGCTTGA-3′</td>
<td>5′-CTCTCGTCTACCGCTTCA-3′</td>
<td>176</td>
</tr>
<tr>
<td>Mouse tPA</td>
<td>5′-CTACAGAGGACGACTGAGCATG-3′</td>
<td>5′-ATACAGGGCGCTGCTGACAGT-3′</td>
<td>179</td>
</tr>
<tr>
<td>Mouse PAI-1</td>
<td>5′-GCCACCCCTCGAGGTCTCA-3′</td>
<td>5′-TCTGATGAGGATGCTGAACTGAT-3′</td>
<td>91</td>
</tr>
<tr>
<td>Mouse GAPDH</td>
<td>5′-CCTGCACCAACCACTGCTCA-3′</td>
<td>5′-TCTGAGGCCCTCCGCAA-3′</td>
<td>76</td>
</tr>
</tbody>
</table>

tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
pneumoperitoneum (Fig. 1C). At 2 h of CO₂ pneumoperitoneum, the tPA/PAI-1 mRNA ratio was significantly decreased in both groups compared with those of initial biopsies within the same patients (Fig. 1C). However, the tPA/PAI-1 mRNA ratio in the 12 mmHg group was significantly lower than that in the 8 mmHg group at 2 h of CO₂ pneumoperitoneum (Fig. 1C).

**Figure 1**  Human study: tPA mRNA levels (A), PAI-1 mRNA levels (B) and tPA/PAI-1 mRNA ratio (C) in peritoneal tissues at 1 or 2 h of CO₂ pneumoperitoneum during laparoscopic surgery. mRNA levels were determined using RT–PCR. The values indicate the fold change of tPA mRNA levels (A), PAI-1 mRNA levels (B) and tPA/PAI-1 mRNA ratio (C) in peritoneal tissues at 1 or 2 h relative to the matched initial peritoneal biopsies within the same patients. Results are presented as the mean ± SEM. Bars indicate SEM. 8-1 h: CO₂ pneumoperitoneum at 8 mmHg at 1 h (n = 32). 12-1 h: CO₂ pneumoperitoneum at 12 mmHg at 1 h (n = 36). 8-2 h: CO₂ pneumoperitoneum at 8 mmHg at 2 h (n = 15). 12-2 h: CO₂ pneumoperitoneum at 12 mmHg at 2 h (n = 16). a: P < 0.0001 versus matched initial peritoneal biopsies within the same patients. b: P < 0.01 versus CO₂ pneumoperitoneum at 8 mmHg at 1 h. c: P < 0.002 versus matched initial peritoneal biopsies within the same patients. d: P < 0.0001 versus matched initial peritoneal biopsies within the same patients. e: P < 0.02 versus CO₂ pneumoperitoneum at 8 mmHg at 2 h. f: P < 0.0001 versus matched initial peritoneal biopsies within the same patients. g: P < 0.01 versus CO₂ pneumoperitoneum at 8 mmHg at 1 h. h: P < 0.01 versus matched initial peritoneal biopsies within the same patients. i: P < 0.01 versus matched initial peritoneal biopsies within the same patients. j: P < 0.01 versus CO₂ pneumoperitoneum at 8 mmHg at 2 h.

**Table II** Results of quantitative real-time RT–PCR for tPA mRNA levels in human peritoneal tissues.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>0 h (initial biopsy)</th>
<th>1 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mmHg IPP</td>
<td>1.80 (0.13–11.4) (n = 36)</td>
<td>1.50 (0.10–8.10) (n = 36)</td>
<td>1.50 (0.18–4.80) (n = 16)</td>
<td></td>
</tr>
<tr>
<td>8 mmHg IPP</td>
<td>1.50 (0.34–14.8) (n = 32)</td>
<td>1.48 (0.243–7.70) (n = 32)</td>
<td>1.90 (0.787–5.74) (n = 15)</td>
<td></td>
</tr>
</tbody>
</table>

Levels of tPA mRNA are given relative to those of the reference gene, GAPDH. All data are expressed as median (range).

**Table III** Results of quantitative real-time RT–PCR for PAI-1 mRNA levels in human peritoneal tissues.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>0 h (initial biopsy)</th>
<th>1 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mmHg IPP</td>
<td>0.21 (0.13–1.18) (n = 36)</td>
<td>0.49 (0.02–2.10) (n = 36)</td>
<td>1.70 (0.71–4.00) (n = 16)</td>
<td></td>
</tr>
<tr>
<td>8 mmHg IPP</td>
<td>0.35 (0.12–1.20) (n = 32)</td>
<td>0.21 (0.02–1.68) (n = 32)</td>
<td>1.27 (0.21–2.70) (n = 15)</td>
<td></td>
</tr>
</tbody>
</table>

Levels of PAI-1 mRNA are given relative to those of the reference gene, GAPDH. All data are expressed as median (range).

**Mouse study**

**tPA mRNA level**

In the laparotomy group, tPA mRNA was significantly increased compared with that of the control group from 8 to 24 h after surgery (Fig. 2A). In the high-IPP group, tPA mRNA was also increased.
compared with that in the control group beginning at 0 h after surgery, and the difference became and remained significant from 4 to 72 h after surgery (Fig. 2B). However, no significant differences in tPA mRNA were observed between the low IPP and control groups during the first 7 days after surgery (Fig. 2C).

**PAI-1 mRNA level**

In the laparotomy group, PAI-1 mRNA level was increased compared with that in the control group beginning at 0 h after surgery, and the difference became and remained significant from 8 to 48 h after surgery (Fig. 3A). In the high-IPP group, PAI-1 mRNA was also increased compared with that in the control group beginning at 0 h after surgery, and the difference became and remained significant from 4 to 24 h after surgery (Fig. 3B). However, no significant differences in PAI-1 mRNA level were observed between the low IPP and control groups during the first 7 days after surgery (Fig. 3C).

**tPA/PAI-1 mRNA ratio**

In the laparotomy group, the tPA/PAI-1 ratio was decreased compared with that in the control group beginning at 0 h after surgery, and this difference became and remained significant from 4 to 72 h after surgery (Fig. 4A). In the high-IPP group, the tPA/PAI-1 ratio was also decreased compared with that in the control group beginning at 0 h after surgery, and the difference became and remained significant from 4 to 24 h after surgery (Fig. 4B).

The tPA/PAI-1 ratio in the low-IPP group tended to be lower than that in the control group up to 4 h after surgery. However, no significant differences in the tPA/PAI-1 ratio were observed between the low IPP and control groups from 4 h to post operative day (POD) 7 (Fig. 4C).

**Discussion**

The results of the present clinical study suggest that a low IPP and a shorter duration (1 h) of surgery might have a minimal impact on the peritoneal fibrinolytic system during a CO₂ pneumoperitoneum. In addition, the results of the present time course study in the mouse model suggest that the surgical peritoneal environment of a CO₂ pneumoperitoneum at a low IPP appears to have a minimum impact on the peritoneal fibrinolytic system up to 7 days after surgery. The effect of CO₂ pneumoperitoneum duration on the fibrinolytic system was not evaluated in the present animal system, because our present mouse model has been optimized for a 1-h procedure. Further studies are required to fully investigate this time course in mice. In addition, the present study investigated only the impact of CO₂ gas on the peritoneal fibrinolytic system. A previous animal experiment using pig models demonstrated that both helium and CO₂ gas at 15 mmHg did not induce a significant decrease of tPA activity in the parietal peritoneum, whereas CO₂ gas induced a larger decrease in tPA activity in cecum and ileum samples compared with helium during laparoscopy (Nagelschmidt et al., 2001). Further animal experiments are required to determine whether helium
pneumoperitoneum at a low IPP is associated with fewer adverse effects on the fibrinolytic system in both parietal and visceral peritoneum compared with CO₂ pneumoperitoneum at a low IPP.

The underlying molecular mechanisms that cause a low IPP to induce fewer adverse effects on the peritoneal fibrinolytic system remain to be clarified; however, one potential explanation may be that a standard IPP of 12 mmHg, particularly when the surgical duration is long, might induce more peritoneal tissue trauma (Holmdahl et al., 1998; Suematsu et al., 2001). In the present study, we used the cool and dry CO₂ gas, which could cause structural, morphologic and biochemical injury to the peritoneal tissues (Volz et al., 1996; Neuhaus and Watson, 2004; Erikoglu et al., 2005; Rosário et al., 2006; Sammour et al., 2009). A low IPP and shorter duration of surgery might induce less peritoneal tissue trauma by minimizing the total volume of cool and dry CO₂ gas flow.

To date, several studies have suggested that surgical trauma could increase PAI-1 expression in peritoneal tissues (Ivarsson et al., 1998; Falk et al., 2001; Bergström et al., 2002; Reed et al., 2004). However, the results of the impact of surgical trauma on tPA expression in peritoneal tissues are conflicting (Scott-Coombes et al., 1995a; Bakkum et al., 1996; Holmdahl et al., 1998; Ivarsson et al., 1998; Hellebrekers et al., 2000a,b; Reijnen et al., 2002; Reed et al., 2004; Brokelman et al., 2009b). Some studies have demonstrated an immediate decrease in tPA levels (Scott-Coombes et al., 1995a; Holmdahl et al., 1998, Ivarsson et al., 1998; Brokelman et al., 2009b), whereas other studies demonstrated no significant change or increased tPA levels during and/or after surgery (Bakkum et al., 1996; Hellebrekers et al., 2000a,b; Reijnen et al., 2002; Reed et al., 2004). In the present clinical study, no significant difference in tPA mRNA level was detected in either IPP group at 1 or 2 h compared with that of initial biopsy. In addition, the present mouse time course study demonstrated no significant decrease in tPA mRNA during the first 7 days after surgery. In contrast, we detected a significant increase in tPA mRNA within 24 h after surgery in both the laparotomy and high-IPP groups. Although the present study measured tPA mRNA in non-traumatized peritoneum in both clinical and mouse settings, the findings are in agreement with previous rodent time course studies, which demonstrated increased tPA antigen, tPA activity and/or tPA mRNA expression in traumatized peritoneum after laparotomy (Bakkum et al., 1996; Hellebrekers et al., 2000a; Reijnen et al., 2002; Reed et al., 2004). In addition, the present clinical study demonstrated that PAI-1 mRNA level was significantly increased in the 12 mmHg group, whereas no significant change in PAI-1 mRNA was detected in the 8 mmHg group at 1 h. PAI-1 mRNA was significantly increased in both groups at 2 h. The present mouse time course study demonstrated that PAI-1 mRNA was immediately increased after laparotomy and CO₂ pneumoperitoneum at a high IPP. It therefore appears that the decreased tPA/PAI-1 ratio detected in the present study was primarily attributable to the increased PAI-1 mRNA levels observed in both the clinical and mouse studies. Normal tPA mRNA and increased PAI-1

Figure 3 Mouse study: PAI-1 mRNA levels (determined using RT–PCR) in peritoneal tissues during the first 7 days after laparotomy (A), CO₂ pneumoperitoneum at high IPP (8 mmHg) (B) and CO₂ pneumoperitoneum low IPP (2 mmHg) (C). Levels of PAI-1 mRNA are presented relative to the expression levels of the reference gene, GAPDH. Results are presented as the mean ± SEM. Bars indicate SEM. C: controls (anesthesia alone). a: P < 0.0001 versus controls, 0 h, 48 h, 72 h, 5d and 7d. b: P < 0.001 versus controls, 0 h, 4 h, 72 h, 5d and 7d. c: P < 0.0001 versus controls, 0 h, 48 h, 72 h, 5d and 7d. d: P < 0.01 versus 5d and 7d.
mRNA levels might result in decreased fibrinolytic activity during and immediately after surgery (Ivarsson et al., 2003; Saed and Diamond, 2003; Gago et al., 2006). Although tPA mRNA was increased thereafter, fibrinolytic activity may remain decreased as a result of a marked increase of PAI-1 mRNA. However, it is not possible to compare the present results with those of previous studies owing to the different study designs, study populations, animal models and detection methods for tPA and PAI-1 levels. Further studies to evaluate tPA and PAI-1 mRNA as well as protein expression and fibrinolytic activity in both traumatized and non-traumatized peritoneum in both human and animal models could provide further insights on the impact of surgical trauma on the peritoneal fibrinolytic system.

Currently, an IPP of 12–15 mmHg is generally applied in the clinical setting and because of the hemodynamic changes associated with an increased IPP, higher IPPs have not been recommended (Neudecker et al., 2002b). However, much less attention has been paid to whether lower IPPs might result in a better clinical outcome. To date, most studies that investigated the impact of a low IPP during a CO$_2$ pneumoperitoneum have been focused on post-operative pain (Sarli et al., 2000; Barczyński and Herman, 2003; Sandhu et al., 2009). A recent Cochrane review concluded that a low-pressure pneumoperitoneum remains to be established (Gurusamy et al., 2009). In the present study, we did not observe any intra- or post-operative complications. In addition, no cases were converted to laparotomy. However, exposure of the surgical field under a low IPP might not be adequate for surgeons with little or no experience. Thus, the use of a low IPP should be recommended only for experienced surgeons (Revaz Botchorishvili, personal communication).

In the present study, we included only patients who had a macroscopically normal peritoneum. In addition, the present study was not randomized, and different teams of surgeons operated on the 8 versus 12 mmHg groups. Further randomized controlled studies with a larger sample size that includes patients with pathological peritoneal tissues (i.e. adhesion, endometriosis) are required to confirm the present findings and to fully assess safety.

In the present study, the ratio of tPA/PAI-1 in mice was decreasing at 0 h, and this difference was significant at 4 h in both the laparotomy and high-IPP groups. Although no significant changes were detected in the low-IPP group, the ratio of tPA/PAI-1 tended to be lower at 4 h. These findings suggest that the critical time for the prevention of post-operative adhesion formation by increasing peritoneal fibrinolytic

**Figure 4** Mouse study: tPA/PAI-1 mRNA ratio in peritoneal tissues during the first 7 days after laparotomy (A), CO$_2$ pneumoperitoneum at high IPP (8 mmHg) (B) and CO$_2$ pneumoperitoneum at low IPP (2 mmHg) (C). Results are presented as the mean ± SEM. Bars indicate SEM. C: controls (anesthesia alone). C: n = 5, 4 h: n = 5, 8 h: n = 5, 24 h: n = 5, 48 h: n = 5, 72 h: n = 5, 5d: n = 5, 7d: n = 5. a: P < 0.001 versus controls, 0 h, 5d and 7d. b: P < 0.0001 versus controls, 0 h, 48 h, 72 h, 5d and 7d. c: P < 0.02 versus controls.
activity might be during surgery and up to 4 h after surgery. In addition, in agreement with the present findings, Hellebrekers et al. (2000a,b) suggested that therapeutic agents to promote fibrinolytic activity to prevent adhesion formation should be used immediately after or during surgery. A previous animal experiment demonstrated that a neurokinin 1 receptor (NK-1R) antagonist significantly reduced intra-abdominal adhesions when administered during or 1 h after laparotomy, resulting in moderately reduced adhesions when administered at 5 h, and had no effect at 12 or 24 h (Reed et al., 2004). A NK-1R antagonist might decrease post-operative adhesion formation through increased expression and activity of tPA (Reed et al., 2004). These findings may support our present speculation and that by Hellebrekers et al. (2000a,b).

In conclusion, the results of the present study suggest that a low IPP (8 mmHg) may be better than the standard IPP (12 mmHg) to minimize the impact on the peritoneal fibrinolytic system during a CO2 pneumoperitoneum. In addition, the results of the present mouse study suggest that the critical time for the prevention of post-operative adhesion formation by increasing peritoneal fibrinolytic activity might be during surgery and up to 4 h after surgery.

Authors’ roles

S.M. was involved in concept and design, sample collection, animal experiments, RT–PCR analysis, acquisition of data, analysis and interpretation of data; drafting the article; and revising the manuscript. R.B. played a role in concept and design, sample collection, interpretation of data and revising the manuscript. E.M. prepared the sample and conducted RT–PCR analysis. M.C. played a role in concept and design, sample collection, interpretation of data; critical comments on the paper; and revising the manuscript. G.M. was involved in concept and design, sample collection. All authors read and approved the final version of the paper.

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