Effects of supplemental perioperative oxygen on post-operative abdominal wound adhesions in a mouse laparotomy model with controlled respiratory support*

Sachiko Matsuzaki1,2,5, Michel Canis1,2, Jean-Etienne Bazin1,3, Claude Darcha4, Jean-Luc Pouly1,2 and Gérard Mage1,2

1Université d’Auvergne—Clermont I, Faculté de Médecine, Centre d’Endoscopie et des Nouvelles Techniques Interventionnelles (CENTI), Clermont-Ferrand, France; 2CHU Clermont-Ferrand, Polyclinique-Hôtel-Dieu, Gynécologie Obstétrique et Médecine de la Reproduction, Boulevard Léon Malfreyt, 63058 Clermont-Ferrand Cédex, France; 3CHU Clermont-Ferrand, Hôtel Dieu, Service d’Anesthésie Réanimation, Clermont-Ferrand, France; and 4CHU Clermont-Ferrand, Hôtel-Dieu, Service d’Anatomie et cytologie pathologiques, Clermont-Ferrand, France

5Correspondence address. Tel: +33-4-73-75-01-38; Fax: +33-4-73-93-17-06. E-mail: sachikoma@aol.com

BACKGROUND: Post-operative adhesion formation is a major clinical problem. Tissue oxygenation is one of the most important determinants in adhesion formation. The objective of this study was to investigate whether supplemental perioperative oxygen could reduce post-operative adhesion formation through increasing the peritoneal tissue oxygen tension (PitO2) in a mouse model. METHODS: Adult C57BJ6 mice were randomly assigned to two groups: Group 1 (n = 20), Fraction of Inspired Oxygen (FiO2): 0.21; Group 2 (n = 20), FiO2: 0.80. On day 0, over the course of the 90 min procedure including the 60 min of laparotomy, PitO2 was continuously monitored. On day 7, a second laparotomy was performed to assess abdominal wound adhesions. Real-time RT–PCR was performed to measure expression levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) mRNA in peritoneal tissues. RESULTS: The PitO2 levels in Group 2 were significantly higher compared to Group 1 (P < 0.001) and controls (P < 0.003). There was no significant difference in the incidence of abdominal wound adhesions; however, the severity of adhesions was significantly reduced in Group 2 compared to Group 1 (P < 0.03). A significantly higher tPA/PAI-1 mRNA ratio was detected in Group 2 and the controls compared to Group 1 (P < 0.02 and P < 0.002, respectively). CONCLUSIONS: Supplemental perioperative oxygen may help to reduce post-operative adhesion formation.

Keywords: post-operative adhesions; peri-operative oxygen; plasminogen activator inhibitor-1; tissue plasminogen activator; abdominal wound

Introduction

Post-operative adhesion formation is a major clinical problem since it leads to complications such as intestinal obstruction (49–74% incidence), chronic pelvic pain (20–50% incidence), and infertility (15–20% incidence) (Hershlag et al., 1991; Monk et al., 1994). Post-operative adhesion formation has been reported to occur in 55–100% of patients after surgery (Trimbos-Kemper et al., 1985). Additionally, in the USA, the total cost in 1994 for adhesiolysis hospitalizations was $1.44 billion (Ray et al., 1998), making adhesions a significant economic problem.

The exact cause of adhesion formation after major abdominal procedures is not known; however, trauma, ischemia, hemorrhage, and infection are proposed contributors (diZerega and Campeau, 2001). Adequate delivery of oxygen to tissues is vital in normal wound healing; thus, tissue oxygenation is one of the most important determinants in wound adhesion formation (Gottrup, 2004).

An impaired fibrinolytic system is crucial for the formation of adhesions (Holmdahl, 1997; diZerega and Campeau, 2001). Tissue plasminogen activator (tPA) function depends on the ratio of tPA to its inhibitor, plasminogen activator inhibitor-1 (PAI-1). A recent study demonstrated that the tPA to PAI-1 mRNA ratio is markedly decreased in peritoneal fibroblasts following hypoxia treatment in vivo (Saed and Diamond, 2003). Tissue hypoxia in wounds is common (Crowther et al., 2001), thus a decreased tPA/PAI-1 ratio might precipitate postoperative adhesion formation (Saed and Diamond, 2003).
Recently, we demonstrated that the peritoneal tissue oxygen tension (PitO$_2$) levels in non-injured peritoneum during a CO$_2$ pneumoperitoneum at a low intra-peritoneal pressure were elevated approximately 2-fold over the levels during laparotomy (Bourdel et al., 2007). In general, laparoscopic surgery is considered to be less adhesiogenic than laparotomy (Gutt et al., 2004). This may be because a higher PitO$_2$ level during laparoscopy minimizes hypoxia. Supplemental perioperative oxygen increases arterial oxygenation, thereby raising tissue oxygen tension (Greif et al., 2000). Thus, we hypothesized that supplemental perioperative oxygen, by preventing wound hypoxia, could reduce post-operative adhesion formation.

The present study uses our established mouse surgical model with controlled respiratory support (Bourdel et al., 2007). We investigated the effects of supplemental perioperative oxygen on the incidence and severity of abdominal wound adhesions. Additionally, we measured the mRNA expression levels of tPA and PAI-1 in the injured peritoneum.

**Materials and Methods**

**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 study. Experiments were conducted in adult (eight week old, 18–20 g) female C57BL6 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 two-week period of acclimation to the vivarium before any procedure was performed. After completion of the experiment, all mice were euthanized with an anesthetic overdose.

**Anesthesia and videoendoscopy-assisted endotracheal intubation**

All mice were anesthetized in a container with 3–5% vaporized isoflurane with either air ([fraction of inspired oxygen] FiO$_2$: 0.21) or air plus oxygen (FiO$_2$: 0.80). A rigid 2 mm endoscope (Karl Storz Endoscopy & GmbH, Tuttingen, Germany) was used to visualize the epiglottis, larynx and vocal cords with the image displayed on a monitor as previously described (Bourdel et al., 2007). An endotracheal tube (24 gauge i.v. catheter, Insyte, Becton Dickinson, Le pont de clai, France) was inserted parallel to the scope and thereby raising tissue oxygen tension (Greif et al., 2000). Thus, we hypothesized that supplemental perioperative oxygen, by preventing wound hypoxia, could reduce post-operative adhesion formation.

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**Experimental design**

A total of 40 mice were randomized into the following two groups of 20 animals each: (i) FiO$_2$: 0.21, (ii) FiO$_2$: 0.80. Because this is a pilot study, a power-calculation was not performed. On day 0, mice were anesthetized with vaporized isoflurane either with air (FiO$_2$: 0.21) or air plus oxygen (FiO$_2$: 0.80). After 10 min of anesthesia, a 3 cm abdominal incision was made and the peritoneal cavity exposed for 60 min. Then, the abdominal incision was closed and anesthesia was maintained for an additional 20 min. Over the course of the 90-min procedure, the PitO$_2$ was continuously monitored. As controls, an additional five mice, not subjected to laparotomy, received 90 min anesthesia alone with FiO$_2$: 0.21.

On day 7, a laparotomy was performed to assess the incidence, extent, and type of adhesions. Entry into the abdominal cavity was performed through bilateral lateral incisions to avoid disrupting any midline abdominal adhesions. Samples of injured peritoneal tissue from the abdominal wound were collected and divided into two portions. One was fixed in 10% formalin-acetic acid and embedded in paraffin for histopathological examination. The other was immediately placed in RNA later (Ambion, Cambridgeshire, UK) and stored at −20°C until RNA extraction.

**Assessment of post-operative adhesion formation**

Evaluation of the incidence, extent, and type of adhesions in the midline was performed through laparotomy. Because our preliminary experiments showed no interobserver variance, only one observer, who was blinded to the treatment group, assessed post-operative adhesion formation. The extent and type of adhesion was scored according to Vrijland et al. (2002). The extent was assessed by estimating the overall length of the midline incision covered by adhesive tissues and scored as (0 = no adhesions, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%). The type of adhesion was scored as follows: (1 = filmy; 2 = stronger adhesion, blunt dissection possible, partly sharp dissection; 3 = strong adhesion, lysis possible by sharp dissection only; 4 = very strong adhesion, lysis possible by sharp dissection only, damage of organs likely). Severity of an adhesion was calculated by multiplying its extent and type.
Quantitative real-time RT–PCR

Total RNA was extracted using the Qiagen RNeasy Mini Kit according to the manufacturer’s instructions and stored at −80°C until use. Quantitative real-time RT–PCR with a Light Cycler was performed as previously described (Matsuzaki et al., 2004, Matsuzaki et al., 2005). Total RNA (100 ng) was subjected to an RT reaction using Superscript II Reverse Transcriptase (Invitrogen). Quantitative real-time PCR was performed in a Light Cycler 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 (for tPA) or 0.5 µM (for PAI-1) of primer, 4 mM MgCl₂ and 2 µl complementary DNA, and standard or nuclease-free water as a negative control. Primer sets are shown in Table 1. Quantification of the targets in the unknown samples was performed using a relative quantification method with external standards. The target concentration is expressed relative to a reference housekeeping gene, Glyceraldehyde-3-phosphate dehydrogenase. After each run, a melting curve analysis was performed to verify the specificity of the PCR reaction. The procedure was repeated three times independently to ensure the reproducibility of the results. All of the samples with a 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. Comparisons were made using the Mann–Whitney test, Kruskal–Wallis test or Fisher exact test. Statistical significance was defined as P < 0.05.

Results

PitO₂

The PitO₂ levels in Group 2 (95.4 ± 14.2 mmHg, mean ± SEM) were significantly higher compared to those of Group 1 (38.1 ± 10.3 mmHg, P < 0.001) and controls (35.8 ± 3.2, P < 0.003).

Incidence and severity of abdominal wound adhesions

Adhesions to the midline incision site were detected in a total of 10 mice (50%) in Group 1 and 4 (20%) in Group 2 (Table 2). There was no significant difference in the incidence of abdominal wound adhesions; however, the severity of adhesions was significantly reduced in Group 2 compared to Group 1 (P < 0.03, Table 1). No animals developed adhesions elsewhere in the abdominal cavity.

Expression levels of tPA and PAI-1 mRNA

There was no significant difference in the tPA and PAI-1 mRNA expression levels among Group 1, Group 2 and the controls. However, the tPA/PAI-1 mRNA ratio was significantly higher in Group 2 and the controls compared to Group 1 (P < 0.02, P < 0.002, respectively, Fig. 1). There was no significant difference in the tPA/PAI-1 mRNA ratio between Group 2 and the controls (Fig. 1).

Discussion

In the present study, we detected no significant difference in the incidence of abdominal wound adhesions, although the incidence tended to be lower in mice receiving supplemental perioperative oxygen. However, supplemental perioperative oxygen did reduce significantly the severity of adhesions. In addition, we detected a significantly higher ratio of tPA/PAI-1 mRNA in the mice receiving supplemental oxygen. Although the peritoneal tPA/PAI-1 ratio is known to be markedly decreased by hypoxia treatment in vitro (Saed and Diamond, 2003), the present study provides evidence that the peritoneal tPA/PAI-1 ratio might be modulated by the PitO₂ levels in vivo. Further studies are necessary to determine whether protein levels are similarly altered. These findings suggest that supplemental perioperative oxygen may, in part, reduce post-operative adhesion formation through increasing tissue oxygen tension.

The mechanism of post-operative adhesion formation is quite complex and likely involves other factors besides tissue hypoxia (diZerega and Campeau, 2001). The group of Diamond et al. recently proposed a two-step model for the development of post-operative adhesion formation (Alpay et al., 2006). Initiation is triggered by hypoxia and progression by inflammation (Alpay et al., 2006). Pro-inflammatory cytokines, including interleukins-1 and 6 and tumor necrosis factor, mediate the release of PAI-1 by mesothelial cells (Whawell and Thompson, 1995). This might affect the deposition of fibrin within the peritoneal cavity. Experiments in animals suggest that anti-inflammatory drugs are effective for the prevention of post-operative adhesion formation (Guvenal et al., 2001; Greene et al., 2005). Further studies are needed to investigate whether a combination of supplemental perioperative oxygen and anti-inflammatory drugs is more effective than either alone. In addition, post-surgical peritoneal repair occurs in 5–8 days (diZerega and Campeau, 2001). A recent study demonstrated that intermittent oxygen exposure for 7 days can satisfy the need for oxygen in ischemic tissue in a mouse wound model (Hopf et al., 2005). It is necessary to investigate whether supplemental oxygen administered during days 5–8 post-operatively is more effective for the prevention of post-operative adhesion formation.

Results from animal experiments on oxygen supplementation are mixed. One group demonstrated a reduction in adhesion formation (Mynbaev et al., 2002; Elkelani et al., 2006). The present study demonstrated that intermittent oxygen exposure for 7 days can satisfy the need for oxygen in ischemic tissue in a mouse wound model (Hopf et al., 2005). It is necessary to investigate whether supplemental oxygen administered during days 5–8 post-operatively is more effective for the prevention of post-operative adhesion formation.

Table 1: Sequences of the primers used for mRNA quantitation by real-time RT–PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primers</th>
<th>Antisense primers</th>
<th>Bp</th>
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<tr>
<td>TPA</td>
<td>5′-CTACAGAGCGCCTGCAGAGAT-3′</td>
<td>5′-AATACAGGGCTCTGACACGT-3′</td>
<td>179</td>
</tr>
<tr>
<td>PAI-1</td>
<td>5′-GGACACCCTCCCAGTCTTA-3′</td>
<td>5′-TCTGATGAGTTCAGCATCCAAGAT-3′</td>
<td>91</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-CCTGCACCACCACTGCTTA-3′</td>
<td>5′-TCATGACGCCCTCCACAA-3′</td>
<td>76</td>
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2004) and another showed no benefit (Demirbarg et al., 2005) when 3% oxygen was added to the CO₂ pneumoperitoneum. Both of these groups used methods that were different from that of the present study. However, these studies did not evaluate PitO₂ levels. Thus, it is unclear whether the addition of 3% oxygen to the CO₂ pneumoperitoneum could increase the PitO₂. Additionally, adding oxygen directly to the CO₂ pneumoperitoneum requires special equipment and is impossible to perform with laparotomy. A simpler way to increase tissue oxygenation in the clinical setting is both laparoscopy and laparotomy, without the use of special equipment, is to increase the inspiratory oxygen concentration. This method also permits oxygenation to be increased post-operatively. Prolonged administration of oxygen at concentrations near 100% results in pulmonary toxicity; however, short-term exposure in the perioperative period is non-toxic (Kabon and Kurz, 2006). Perioperative concentrations of oxygen not exceeding 80% do not provoke atelectasis or other pulmonary dysfunction (Greif et al., 2000; Kabon and Kurz, 2006).

In the present study, we focused on the impact of PitO₂ on adhesion formation in an abdominal wound. The small size of our animal model limits where measurements may be taken. For example, placing the catheter within the bowel wall could cause significant injury to the animal. However, a recent pig experiment did demonstrate that increasing supplemental oxygen resulted in an increase in intestinal intramural oxygenation (Ratnaraj et al., 2004). Further animal experiments are needed to investigate whether supplemental perioperative oxygen could prevent or minimize post-operative intra-abdominal adhesion formation involving the bowels or other abdominal organs, before clinical studies.

In conclusion, the present study demonstrates that in a mouse model, supplemental perioperative oxygen may help to reduce post-operative adhesion formation, through increasing tissue oxygen tension.

Acknowledgements

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References


Figure 1: Results of tissue plasminogen activator/plasminogen activator inhibitor-1 (tPA/PAI-1) mRNA ratio in injured peritoneal tissues in the mouse. Results are presented as the mean ± SEM. Bars indicate SEM. Group 1 (n = 20): FiO₂: 0.21, Group 2 (n = 20): FiO₂: 0.80. Control (n = 5).*P < 0.02 versus Group 2, P < 0.002 versus Control

Table 2: Incidence and severity of abdominal wound adhesions

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (FiO₂: 0.21) (n = 20)</th>
<th>Group 2 (FiO₂: 0.80) (n = 20)</th>
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<tbody>
<tr>
<td>Incidence</td>
<td>50%</td>
<td>20%</td>
</tr>
<tr>
<td>Severity*</td>
<td>2.87 ± 0.74</td>
<td>0.25 ± 0.55*</td>
</tr>
</tbody>
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*Data are mean ± SEM. *P < 0.03 versus Group 1: determined by the Mann–Whitney test.


