Peritoneal transforming growth factor beta 1 expression during laparoscopic surgery
Peritoneal transforming growth factor beta-1 expression during laparoscopic surgery

Abstract

Background
Transforming growth factor beta 1 (TGF-beta1) is a growth factor involved in various biological processes, including peritoneal wound healing and dissemination of malignancies. Laparoscopic surgery is evolving rapidly and indications are growing. The peritoneal TGF-beta1 expression during laparoscopic surgery is unknown.

Methods
Fifty patients scheduled for laparoscopic cholecystectomy were randomised in five groups, operated with various pressures, light intensities and dissection devices. Peritoneal biopsies were taken at the start and end of surgery. Tissue concentrations of total and active TGF-beta1 were measured using ELISA techniques.

Results
There was no significant difference in either total or active TGF-beta1 concentration in peritoneal biopsies taken at the start of surgery compared to samples taken at the end of the procedure. Patients operated with the ultrasonic scalpel had significant lower levels of both active (p<0.005) and total (p<0.01) TGF-beta1 at the end of surgery compared to patients operated with electrocautery. Patients operated with a high light intensity have significant lower levels of total TGF-beta1 levels (p<0.005) with an unchanged active part compared to patients operated with low light intensity.

Conclusion
The choice of dissection device and the light intensity used in laparoscopic surgery affect peritoneal TGF-beta1 concentrations, indicating that peritoneal biology can be affected by laparoscopic surgery. Because TGF-beta1 is involved in various biological processes in the peritoneal cavity, this observation may have important clinical consequences.

Introduction
Transforming growth factor beta 1 (TGF-beta1) is a naturally occurring growth factor and is involved in various biological processes including peritoneal wound healing and dissemination of malignancies. Peritoneal wound healing and subsequent adhesion formation, are regulated by a complex mechanism of molecular processes. Alterations in the local concentrations of cytokines, growth factors, and proteases all may contribute to the process of peritoneal healing. TGF-beta1 appears to be a major stimulator of peritoneal adhesion formation, mainly by increasing the peritoneal production of Plasminogen Activator Inhibitor-1 (PAI-1), which is the main inhibitor of fibrinolysis and a key factor in adhesiogenesis. Moreover, TGF-beta1 is a major stimulator of extracellular matrix deposition by inducing the production of collagen, fibronectin and integrins. Increased TGF-beta1 concentrations have been found in peritoneal fluid of patients with adhesions and in adhesion tissue itself. Moreover, postoperative peritoneal administration of TGF-beta1 increased adhesion formation in mice while its inactivation is reported to reduce the incidence of adhesions. TGF-beta1 regulates chemotaxis, mitogenesis and angiogenesis and thereby involved in dissemination processes. TGF-beta1 secretion and the activation of TGF-beta1 signaling pathways have been associated with increased aggressiveness of several types of tumors including pancreas, colon, stomach, endometrium, prostate, breast, brain, and bone. Literature on the relation between laparoscopic surgery and peritoneal dissemination and port-site metastasis is controversial. Endoscopic surgery has developed rapidly in the last decades. It minimizes the surgical trauma, thereby reducing recovery time and the incidence of postoperative complications. Few studies have suggested that this strategy might also reduce the incidence of peritoneal adhesion formation. The effect of laparoscopy on peritoneal TGF-beta1 expression is unknown. Endoscopic surgery induces new entities in the abdominal cavity including an intense illumination of the peritoneal cavity and increased intra-abdominal pressure. Moreover, the use of new dissection devices, including the ultrasonic scalpel, is progressively advocated. The current study was conducted to evaluate the hypothesis that peritoneal biology, and specifically the peritoneal TGF-beta1 expression, could be affected by laparoscopic surgical techniques. The effects of illumination, intra-abdominal pressure and the choice of dissection devices were studied in patients undergoing a laparoscopic cholecystectomy.
**Patients and Methods**

**Design of study**

Fifty patients, with a diagnosis of symptomatic gallbladder stone disease and scheduled for elective laparoscopic cholecystectomy were randomised in five groups (Table 1). In order to evaluate the effect of the intra-abdominal pressure, three groups, of 10 patients each, were operated with intra-abdominal pressures of 10, 13 and 16 mm Hg (Group A, B and C respectively). All of them were operated with the same light intensity and using electrocautery. The effect of light intensity was studied by comparing a group operated with a high light intensity with a group operated with a low light intensity (Groups D and B, respectively), using an intra-abdominal pressure of 13 mm Hg and electrocautery. The influence of the dissection device was assessed by comparing two groups operated with either electrocautery or the ultrasonic scalpel, with equal light intensities and intra-abdominal pressures (Groups B and E, respectively).

Randomisation by envelope was done just before the operation. Institutional Review Board approval was obtained and written informed consent was given before enrolment.

**Operative procedure**

A uniform technique of videolaparoscopic cholecystectomy was applied, including the use of 4 trocar ports in the ‘American’ position and using a 0° optic scope. The gallbladder hilum and the Calot triangle were dissected and metal clips for the cystic duct and artery were used. Biopsies of the parietal peritoneum were taken with forceps and scissors immediately after CO₂ insufflation and after 45 minutes of surgery, without using electrocautery or ultrasonic scalpel. When the procedure was finished prior to 45 minutes the 2nd biopsy was taken just before desufflation.

**Tissue sampling and processing**

The peritoneum was carefully dissected taking care not to include the underlying muscle. The tissue specimens were snap frozen in liquid nitrogen and stored at −70°C until further processing. Before homogenizing, a sample of thawing peritoneal tissue was cut off before being blotted and weighed. Each biopsy was rinsed with phosphate buffered saline (PBS) with 0.5M sodium chloride (pH 7.4), cut into small pieces and placed into ice-cold homogenization buffer (PBS with 0.01% Triton X-100 (Sigma, St.Louis, MO, USA) in a final concentration of 40 mg tissue/ml buffer. The tissue was homogenized for 60 s on ice using a Polytron homogenizer (Ultra Troux IK A T-25, Janke & Kunkel, Staufen, Germany), centrifuged at 10,000 g for 4 min at 4°C, and the supernatant was stored at −70°C until further analysis. Tissue processing and assays were done in batches.

**Biochemical assays**

Concentrations of active and total TGF-b₁ were measured using commercially available (Promega, Madison, WI, USA) enzyme linked immunosorbent assays (ELISA). Both the active and total form of TGF-beta₁ were measured since TGF-beta₁ is inactive when produced and it has to be activated to become an active cytokine. The active and total amounts of TGF-b₁ were performed in separate steps, first the active fraction of TGF-b₁ were assayed directly in the ELISA plate and secondly, the total amount of TGF-b₁ were assayed by acidifying the samples with 1 mol/L HCl to pH 3, following by a 15 min. incubation at 22°C, resulting in an activation of TGF-b₁. To neutralize samples, 1 mol/L NaOH were supplemented before addition to the ELISA plate, according to the instructions from the manufacturer. The lower detection limit for the TGF-b₁ assay were 32 pg/mL. The intra-assay variation were 3.3-4.5% (CV%) and the inter assay variation were 7.6-19.1%.

**Statistics**

Values are given as mean and standard deviation. Analysis of differences between groups was performed using the Kruskal-Wallis test and the Mann-Whitney U test. All tests were two tailed.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pressure</th>
<th>Light intensity</th>
<th>Dissection device</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>50%</td>
<td>Electrocautery</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>50%</td>
<td>Electrocautery</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>50%</td>
<td>Electrocautery</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>80%</td>
<td>Electrocautery</td>
</tr>
<tr>
<td>E</td>
<td>13</td>
<td>50%</td>
<td>Ultrasonic scalpel</td>
</tr>
</tbody>
</table>

*Table 1* Randomisation into five groups of the patients scheduled for laparoscopic cholecystectomy
**Results**

**Clinical results**
There was no difference in sex (m 22%, f 78%) and age (51 ± 16 years) between groups. The overall incidence of previous laparotomies was 30%, without differences between groups. Moreover, there was no difference in the occurrence of intra-peritoneal adhesions and the incidence of bile leakage between groups. Histological studies of the removed gall bladders demonstrated no significant differences in the incidence of chronic inflammation between groups.

The timing of the second biopsy was equal in all groups (38 ± 9.2 min).

**Biochemical results**

**Light intensity**
Patients operated with a high light intensity had significant lower levels of total TGF-beta1 levels (p<0.005) at the end of surgery, compared to biopsies from patients operated with a low light intensity. The active TGF-beta1 concentrations were similar in both groups. There were no differences between groups at the start of the procedure. (figures 1a and 1b)

**Intra-abdominal pressure**
There was no difference in the measured total and active TGF-beta1 levels in specimens from patients operated with an intra-abdominal pressure of 10, 13 and 16 mmHg. (figures 2a and 2b)

**Dissection device**
Peritoneal biopsies of patients where the dissection was done using an ultrasonic scalpel had significant lower levels of both total (p<0.005) and active (p<0.01) TGF-beta1 at the end of surgery, compared to patients operated with electrocautery. There were no differences at the start of the procedure between groups. (figures 3a and 3b)

There was no difference among the groups in either total or active TGF-beta1 concentration, as shown by comparison between peritoneal biopsies taken at the beginning of the procedure and samples taken at the end of surgery. When the surgical variation arms were eliminated and the overall tissue levels before and after the intervention were compared, no significant differences were found in peritoneal levels between total and active TGF-beta1. There was no difference in peritoneal TGF-beta1 expression between patients with intra-abdominal adhesions and those without adhesions.

*Figure 1a* Total transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with low and high light intensity (B and D). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). *p* < 0.005.

*Figure 1b* Active transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with low and high light intensity (B and D). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).
Discussion

In the current study we have demonstrated that peritoneal biology can be affected by laparoscopic surgery and that various surgical techniques may have different effects. A high light intensity and the use of an ultrasonic scalpel reduced the peritoneal expression of total TGF-beta1, while the ultrasonic scalpel also decreased the active part of TGF-beta1. Within clinical applicable variances, the used intra-abdominal pressure is not of influence on the peritoneal TGF-beta1 expression during short-term laparoscopy.

The use of an ultrasonic scalpel in laparoscopic surgery is progressively advocated for several reasons. Coagulation of vascular structures is easy and safe with the ultrasonic scalpel and there is no effect on pacemaker function, which is a major drawback of electrocautery. Most importantly, it has been described to reduce the incidence of gallbladder injury and iatrogenic bowel perforation and the operating time was significantly shorter. We have demonstrated a decreased peritoneal expression of both active and total TGF-beta1 when the patient was operated with the ultrasonic scalpel.

Since TGF-beta1 is a main stimulator of adhesion formation, the use of an ultrasonic scalpel might affect the peritoneal healing process by decreasing PAI-1 expression. In a previous study, however, we did not find any effect of the ultrasonic scalpel on tPA, uPA and PAI-1 levels expressed in the peritoneum of patients undergoing laparoscopic cholecystectomy. Schemmel et al. found no differences in peritoneal adhesion formation between traditional incision, electrosurgery and the ultrasonic scalpel in a rabbit uterine horn model. Similar observations were described by Tulandi et al. in a rat uterine horn model. At this writing, clinical data are lacking.

The decreased TGF-beta1 expression using the ultrasonic scalpel might have other important clinical consequences. TGF-beta1 is involved in various biological processes, including chemotaxis, mitogenesis, angiogenesis, all important in oncologic processes. An increasing part of laparoscopic procedures is performed for oncologic pathology such as laparoscopic colectomy, nephrectomy and hysterectomy. Experimental studies have demonstrated that laparoscopy is associated with less intra-peritoneal tumor growth compared to laparotomy, while insufflation of CO2 may promote peritoneal tumor growth compared to gasless laparoscopy. Lecuru et al., however, did not find any deleterious effect of CO2 insufflation on ovarian tumor growth when compared to gasless laparoscopy or midline laparotomy in a rat model. Only few clinical data exist to allow assessment whether these experimental concerns may be translated into clinical problems. Velanovich found no effect of laparoscopy on the occurrence of trocar-site disease or peritoneal disease progression of pancreatic cancer. The observation that the ultrasonic scalpel decreases TGF-beta1 levels might suggest that this could be the dissection device of choice in this kind of operations. Our results warrant further studies focusing on this topic.

The effects of light on peritoneal biology are merely unknown. High temperatures have been described at the end of fiber optic bundle of light cables and endoscopes with both halogen and xenon light sources. This heat generation is largely due to the radiated power in the visible light spectrum. Increased local temperature might affect mesothelial cell function, including the production and release of TGF-beta1. Surprisingly, the use of high light intensity also decreased the levels of total TGF-beta1, without affecting its active part. One might have expected that a high light intensity would lead to an increased damage of the peritoneum and thus an increase in TFG-beta1. The opposite, however, was true. Laparoscopy is accompanied by insufflation of the peritoneal cavity with CO2, leading to cooling of the peritoneum, and peritoneal injury. The high light intensity might have decreased the temperature shift in the peritoneal cavity due to a higher energy transmission. Additionally, specific frequencies of the light might change the biological behavior of mesothelial cells. The relation between light, and its specific frequencies, and mesothelial cell biology should be subjected to further experimental studies.

In the current study we could not demonstrate an effect of the used intra-abdominal pressure on the peritoneal TGF-beta1 expression during short-term laparoscopy. The differences in intra-abdominal pressures in the current study, however, might have been too small to detect any differences due to the fact that we were limited to clinical applicable variances in intra-abdominal pressure. Molinas et al. have demonstrated the effect of pressure on peritoneal wound healing processes. They have demonstrated that the incidence of adhesions increases with the pressure of insufflation in an experimental study. Further experimental studies are indicated to elucidate this subject.

Overall measurements have shown no significant difference in TGF-beta1 expression in specimens taken at the start of surgery compared to biopsies taken at the end of surgery. The latter, however, might have been too short to detect any differences, at least at the protein level. The majority of all endoscopic procedures remain short-term procedures (<1 hour) including diagnostic laparoscopy, appendectomy and cholecystectomy, indicating the clinical relevance of the current study. Additional studies on prolonged endoscopic procedures are required to further elucidate the effect of endoscopic surgery on peritoneal TGF-beta1 expression. The timing of the first biopsy is another point of interest. In the current study the first biopsy was taken as soon as possible after initiating the pneumoperitoneum. At that time point however, the peritoneal layer might already have been damaged by insufflation, which results in increased abdominal pressure, intense illumination and cooling of the peritoneal cavity. This hypothesis is supported by the observa-
tion of Bergstrom et al. who have described increased peritoneal PAI-1 levels immediately after initiating a laparoscopic cholecystectomy compared to conventional cholecystectomy. Additional experimental studies are needed to clarify this subject.

In conclusion, this current study suggests that peritoneal biology can be modulated by laparoscopic surgery and that various surgical techniques may have different effects. The use of an ultrasonic scalpel and the use of higher light intensity decrease peritoneal TGF-beta1 levels. The involvement of TGF-beta in oncologic and peritoneal repair processes urges the need for further clinical trials.

Reference List
