Peritoneal biology during laparoscopic surgery

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11 november 2010

Paranimfen
Roy Brokelman
Hans ter Haar

Layout and cover design
FYN Werk, Fleur Bominaar, www.fynwerk.nl

Printed by
Gildeprint Drukkerijen, www.gildeprint.nl

ISBN: 9789039354179

Peritoneal biology during laparoscopic surgery

Peritoneale biologie bij laparoscopische chirurgie
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 11 november 2010 des ochtends te 10.30 uur

door

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geboren op 20 augustus 1971
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Introduction and outline of the thesis

Laparoscopic surgery

The first laparoscopic procedure dates back to 1901, when a laparoscopic gynecological procedure was performed in Russia.[1] Initially, laparoscopy was mainly used to diagnose and treat conditions related to the female reproductive organs. In the following decades laparoscopy developed rapidly and nowadays it has a central role in the diagnosis, staging and treatment of many diseases.

Laparoscopic surgery has many advantages, when compared to conventional surgery. Various studies have indicated that laparoscopic surgery is associated to reduced peroperative bloodloss, which is possibly related to an increased visibility due to the magnification effect of the camera.[2] The minimal invasive character of laparoscopic surgery has also a major impact on postoperative pain. Studies have demonstrated that laparoscopically treated patients have less postoperatieve pain during the first 48 postoperative hours, and consequently a shorter reconvalescence and reduced hospitalisation.[3] Obviously, laparoscopic surgery is associated with better cosmetic outcome since incisions are smaller. Smaller incisions will also decrease the incidence of cicatricial hernia.[4] Various studies have demonstrated that a reduced surgical trauma may also decrease the incidence of postoperative adhesion formation.[5-9] Although adhesions may have a potential benefit, they are responsible for various clinical problems. Adhesions are the main cause of intestinal obstruction in the developed world and account for approximately 70% of readmissions for small bowel obstruction.[10] They are responsible for 15% to 20% of cases of secondary infertility in women and are associated with chronic abdominal and pelvic pain.[11,12] Moreover, adhesions increase the duration of surgery during repeat laparotomy and increase the incidence of complications. The incidence of inadvertent enterotomy due to adhesions is 20% and this increases the incidence of postoperative complications, admissions to intensive care and prolonged hospital stay.[13] Adhesion-related hospital readmissions and re-operations are a major economic burden to our health system.[14-16]

The peritoneum

The peritoneal organ consists of a monolayer of cells originating from the mesenchyme, supported by a basement membrane and an underlying sheet of connective tissue, consisting of glycoproteins, glycosaminoglycans and proteoglycans. Mesothelial cells may either be squamous or cuboidal. The latter are mainly found in proximity to parenchymal organs and are also observed within an injured or stimulated mesothelium. The nucleus is prominent with rough endoplasmic reticulum, Golgi apparatus and numerous vesicles, indicating a dynamic biosynthetic ability and active transmembrane transport. The peritoneum may facilitates the physiological function of the intra-abdominal organs due to peritoneal fluid

production. The peritoneal fluid circulates in continuity with the pleural fluid, the lymphatic system and vascular system. Diffusion and resorption of fluid freely occurs while transudation and exudation facilitates molecules to enter the peritoneal cavity.

Peritoneal healing

Peritoneal healing and subsequent adhesion formation is a complex biological process. When the peritoneum is perturbed, mesothelial cells balloon and detach from the peritoneal surface. This creates gaps and denuded areas in the peritoneal lining providing a means for plasma proteins including fibrinogen to ooze and deposit in the peritoneal cavity. In analogy with dermal healing, a peritoneal defect is considered healed when mesothelization is completed. Rapid mesothelization of damaged surfaces is therefore favorable in peritoneal healing. The origin of the mesothelial cell population contributing to mesothelization is still a matter of dispute. It has been suggested that mesothelial cells migrate from the edges, differentiate from underlying mesenchymal cells, or that they might origin from an intracavitary population of "free-floating", non-attached, mesothelial cells that implant and form islands of mesothelium. A disruption of the peritoneal lining is likely to be a major cause of the inclination of surfaces to adhere. Restoration of the mesothelial lining including mesothelial repopulation of denuded areas therefore seems to be of importance to reduce post-surgical adhesion development. Bertram et al. described that adhesion formation can be reduced by intraperitoneal transplantation of mesothelial cells, most likely by nidation of 'free floating' cells.[17] Similar results were found by Lucas et al., who found that the intraperitoneal injection of mesenchymal stem cells, immediately after surgery, reduced postsurgical adhesion formation in the rat.[18]

Abdominal surgery inflicts an inflammatory reaction in the peritoneum, characterised by cellular infiltration and the production of various mediators. Increased vascular permeability promotes the extravasation of fluids, proteins and polymorphonuclear granulocytes (PMN's) and various chemo-attractans, cytokines and growth factors are produced, including IL-8, MCP-1, TNF-α, IL-1β, IL-6, TGF-β, IGF-1 and PDGF. Transforming growth factor-beta 1 (TGF-beta1) is a naturally occurring growth factor and is involved in various biological processes including wound healing and dissemination of malignancies.[19] TGF-beta is an important inducer of extracellular matrix deposition by stimulating the production of collagen, fibronectin and integrins and it regulates chemotaxis, mitogenisis and angiogenesis.[20,21,22] TGF-beta appears to be a major stimulator of peritoneal adhesion formation.[23] Increased TGF-beta concentrations have been found in peritoneal fluid of patients with adhesions and in adhesion tissue itself.[24] TGF-beta increases the

peritoneal production of plasminogen activator inhibitor-1 (PAI-1), which is the main inhibitor of fibrinolysis. The peritoneal plasmin system is considered to be a key factor in peritoneal healing processes.[25]

The plasmin system

Under physiological circumstances, intra-abdominal fibrin deposits are degraded through the action of the highly effective plasmin, which is converted from plasminogen. Plasminogen is present in high concentrations and only small amounts of plasminogen activators are needed for the production of large amounts of plasmin.[26] Plasmin stimulates the conversion of fibrin into fibrin degrading products. Plasmin is inhibited by several protease inhibitors, such as alpha-2-macroglobulin, alpha-1-antitrypsin and alpha-2-antiplasmin. The most important activators of plasminogen are tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). tPA has a high affinity for fibrin and is responsible for 95% of plasminogen activating activity in the peritoneal cavity.[27] It is produced by a variety of cell types, including endothelial cells, mesothelial cells and macrophages. uPA is a less effective plasminogen activator, when compared to tPA, because its affinity for fibrin is much lower. Both proteins, however, are equally effective in the degradation of fibrin. Beside activation of plasminogen, uPA may play an important role in tissue remodeling.[28] The activity of plasminogen activators is hampered by plasminogen activating inhibitors-type 1 and 2 (PAI-1 and PAI-2). PAI-1 is the main inhibitor of plasminogen activators and is produced by different cells including endothelial cells, mesothelial cells, macrophages and fibroblast. In a free form, plasminogen activators are rapidly inactivated by PAI-1, by forming inactive one-on-one complexes. PAI-2 is less effective in counteracting the plasminogen activators, but may play a different role in peritoneal tissue repair.[29]

Abdominal surgery inflicts a disturbance in the equilibrium between coagulation and fibrinolysis, in favor of the coagulation system, resulting in the persistence of fibrin clots, which are the nidus for postsurgical adhesion formation.[27,30-36] A reduced peritoneal fibrinolysis may be caused by both decreased levels of activators and increased levels of inhibitors. Peritoneal surgery is accompanied by a decreased tPA concentrations and increased PAI levels in peritoneal tissue, most likely leading to a decreased fibrinolytic activity in the peritoneal cavity.[27,30,32,34-36] Moreover, decreased fibrinolytic activity is also found in the peritoneal fluid, which is caused by increased PAI levels in the peritoneal fluid.[31,33,37] Probably, PAI also plays an important role in the emergence of adhesions, since high PAI concentrations are found in adhesions and in peritoneal tissue of patients who have extensive adhesions.[32]

Outline of the thesis

The minimal invasive character of laparoscopic surgery may reduce surgical trauma to the peritoneum. Laparoscopic procedures, however, introduce novel entities in the abdominal cavity, which all may affect peritoneal integrity and biology. The studies, described in this theses were conducted to study the effects of laparoscopic surgery on the peritoneal fibrinolytic response. Moreover, the possible effects of illumination, increased intra-abdominal pressure and the choice of dissection device were studied as were the consequences of intra-peritoneal temperature shifts. Additionally, the hypothesis that peritoneal TGF-beta1 expression could be affected by laparoscopic surgical techniques was tested. Finally, the peritoneal fibrinolytic response to both conventional and laparoscopic colonic surgery was clarified.

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Peritoneal changes due to laparoscopic surgery

CHAPTER 2

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Surgical Endoscopy 2010 June. Epub ahead of print.

ABSTRACT

Introduction
Laparoscopic surgery has been incorporated in common surgical practice. The peritoneum is an organ with various biological functions that may be affected by laparoscopic and open techniques in different ways. Clinically, these alterations may be important in issues as peritoneal metastasis and adhesion formation.

Methods
A literature search identified articles focusing on the key issues laparoscopy, peritoneum, inflammation, morphology, immunology and fibrinolysis using the Pubmed and Cochrane databases.

Results
Laparoscopic surgery induces alterations in the peritoneal integrity and causes local acidosis, probably due to peritoneal hypoxia. The local immune system and inflammation are modulated by a pneumoperitoneum. Additionally, the peritoneal plasmin system is inhibited leading to peritoneal hypofibrinolysis.

Conclusion
Similar to open surgery, laparoscopic surgery affects both the integrity and biology of the peritoneum. These observations may have implications for various clinical conditions.

Introduction

In the last decades laparoscopic techniques have been integrated in common surgical practices. The rationale for the acceptance of these techniques is evident. Laparoscopic procedures have been associated with a decreased hospital stay and an earlier return to normal activities and work.[1,2] The avoidance of a median laparotomy reduces the incidence of cicatricial hernia and, by minimizing surgical trauma, likely decreases the incidence of postoperative adhesions.[3] Moreover, endoscopic surgery is associated with superior cosmesis.

The abdominal cavity is lined by the peritoneum, which comprises a single layer of mesothelial cells, supported by a basement membrane and an underlying sheet of connective tissue. The peritoneal organ has multiple biological functions, including the regulation of inflammation, fibrinolysis, angiogenesis, and tissue remodeling processes.[4-7] Surgical trauma results in mesothelial damage and elicits an inflammatory response. Mesothelial cells balloon and detach from the basal membrane, thereby creating denuded areas. The inflammatory reaction is accompanied by the production and release of a broad spectrum of biologically active proteins and the exudation of protein-rich fluid. The peritoneal fibrinolytic response is rapidly disturbed.[8,9]

The minimal invasive character of laparoscopic surgery may reduce surgical trauma to the peritoneum. Laparoscopic procedures, however, introduce novel entities in the abdominal cavity, such as increased abdominal pressure, insufflation gases and temperature shifts, which all may affect peritoneal integrity and biology. The current study was performed to review the literature concerning the biological repercussions of laparoscopic surgery to the peritoneal organ.

Methods

A search was conducted to identify reports in which peritoneal changes due to laparoscopy were described, using the MEDLINE and Cochrane databases. The following MeSH search terms were used: 'laparoscopy', 'peritoneum', 'inflammation', 'morphology', 'immunology' and 'fibrinolysis'. These terms were applied in various combinations in addition to the use of the 'related articles' function. A total of 113 articles were selected and assessed. Manual cross-referencing was performed and relevant references from selected papers were reviewed. The articles were restricted to those written in the English language.

Morphologic alterations

The peritoneal surface consists of a mesothelium composed of cubic, flat or intermediate cell types delimited by a basal lamina. Cubic mesothelial cells are predominantly present in the serosa of parenchymal organs, flat mesothelial cells in the intestinal, omental and parietal peritoneum and an intermediate mesothelial cell type is found in the gastric peritoneum.[10] The submesothelial connective tissue layer is composed of collagen fiber bundles, fibroblasts and free cells, such as macrophages, granulocytes and mast cells, and contains blood and lymphatic vessels.

During conventional surgical procedures mesothelial cells balloon and detach from the basal membrane thereby creating denuded areas. Various experimental studies have shown that the peritoneal integrity may also be disturbed by laparoscopic surgery. Suematsu et al. found in an experimental study in mice that bulging up of the mesothelial cells was present immediately after initiation of a pneumoperitoneum and fully resolved after 72 hours.[11] These data were confirmed by another experimental study, in which mesothelial cells were bulging up 2 hours after the initiation of a pneumoperitoneum.[12] The intercellular clefts thereby increased in size and the underlying basal lamina became visible and were subsequently infiltrated by peritoneal macrophages and lymphocytes. A disruption of the peritoneal lining is likely to be a major cause of the inclination of surfaces to adhere. Restoration of the mesothelial lining, including mesothelial repopulation of denuded areas, seems to be of importance to reduce post-inflammatory and post-surgical adhesion development. Bertram et al. have described that adhesion formation may be reduced by intraperitoneal transplantation of mesothelial cells, most likely by nidation of 'free floating' cells.[13] Similar results were found after intraperitoneal injection of mesenchymal stem cells, immediately after surgery.[14]

Inflammatory cells have been found in the peritoneum already 2 hours after initiation of a pneumoperitoneum, while after 24 hours a chronic infiltrate and reactive mesothelial cells with congestion was observed.[15] Suematsu et al. found that peritoneal changes during laparoscopy may be affected by the choice of insufflation gas, the amount of pressure, and the duration of insufflation. Intercellular clefts were found after both helium and CO_2 pneumoperitoneum, but not after insufflations with normal air.[11] This was confirmed by Rosario et al. who described that CO_2 pneumoperitoneum is more harmful to the mesothelial ultra-structure than air insufflation.[16] In addition, Hazebroek et al. also described a retraction and bulging of mesothelial cells and exposure of the basal lamina after insufflation with CO_2. In their study the effect was independent of the temperature and humidifying of the CO_2.[17] In contrast to that study, Erikoglu et al. found, in an electron and light microscopic study, that heated and humidified CO_2 resulted in less peritoneal alterations compared to cold and dry CO_2.[18]

Few studies have described the peritoneal changes during laparoscopic surgery in humans. Liu et al. have described the peritoneal morphology in 40 patients undergoing either conventional or laparoscopic surgery. In similarity with the experimental studies, a pneumoperitoneum induced bulging of mesothelial cells that was evident immediately at initiation. After 30 minutes of surgery intercellular spaces could be found and at 1 hour the underlying basement membrane could be seen and lost its continuity. After 2 hours lymphocytes and macrophages were found in the intercellular clefts. These observations are in contrast with the observations during conventional surgery, wherein up to the time of 60 minutes no marked changes of the mesothelial cells where found. After 120 minutes of conventional surgery intercellular spaces became significant.[19]

Hypoxia and acidosis in the peritoneum

Besides morphologic alterations, laparoscopic surgery may also induce metabolic changes in the peritoneum. The creation of a pneumoperitoneum may affect local peritoneal oxygen levels, which may induce metabolic changes including acidosis. In 2003, Wildbrett et al.have shown that a pneumoperitoneum,[20] created with either CO_2 or helium decreased the partial oxygen pressure in the rat abdominal wall, as measured with an implanted micro catheter. In contrast, insufflation with a non-hypoxic gas mixture, consisting of 80% CO_2 and 20% oxygen, did not affect the oxygen pressure. Based on this study and additional in-vitro experiments, they concluded that insufflation with either CO_2 or helium affects the intra- and extracellular parameters regulating essential cell functions such as oxidative phosphorylation to produce ATP, cell proliferation, and the onset of apoptosis. The role of local oxygen pressure was further studied by Bourdel et al. in a mouse laparoscopic model with controlled respiratory support. They found that the peritoneal tissue-oxygen tension levels in non-injured peritoneum during a low-pressure CO_2 pneumoperitoneum were higher than during a laparotomy in ventilated mice. After CO_2 insufflation, the peritoneal tissue-oxygen tension immediately increased and remained at a higher level. A similar effect was not found when normal air was used as insufflation gas.[21]

In two experimental studies, Molinas el al. have shown that peritoneal hypoxia is relevant in the occurrence of postsurgical adhesions, possibly by reducing capillary flow in the superficial peritoneal layers during pneumoperitoneum. In a murine model, they found that the incidence of adhesions increased with duration of pneumoperitoneum and with the insufflation pressure. Interestingly, the incidence decreased when oxygen was added to the insufflation gas. The adhesion reducing effect of oxygen was maximal when 2%-3% was added.[22,23]

This theory was confirmed by Elkelani et al. in a study in mice, wherein adhesion formation decreased with the addition of 3% oxygen to the CO_2 pneumoperitoneum. The addition of higher oxygen concentrations, however, was deleterious. Adhesions always increased with time and duration of the pneumoperitoneum.[24] In another experiment the role of hypoxia inducible factors (HIF) 1alpha and 2alpha in CO_2 pneumoperitoneum-enhanced adhesion formation was studied.[25] It was observed that CO_2 pneumoperitoneum enhanced adhesion formation and that this effect is mediated, at least in part, by an up-regulation of HIF-1alpha and HIF-2alpha. Additionally, Binda et al. have recently shown that the reactive oxygen species scavengers superoxide dismutase, catalase, melatonin, and ascorbic acid may decrease adhesion formation. Moreover, they have shown that the hypoxia-inducible factor (HIF) inhibitors may reduce adhesion formation.[26]

Peritoneal hypoxia may induce metabolic changes, inducing a metabolic acidosis. Recently, it was shown that insufflation of CO_2 into the peritoneal cavity results in a decreased peritoneal pH. An immediate local drop in pH to 6.6 occurred in the peritoneum following CO_2 insufflation. During the pneumoperitoneum the pH further declined, stabilizing at 6.4. The pH was completely restored during the recovery period. This peritoneal effect also appeared to affect the systemic acid-base balance, probably due to trans-peritoneal absorption.

Using helium as insufflation gas the opposite effect was found. Tissue pH slightly increased to 7.5 during insufflation, followed by a continuous decrease during pneumoperitoneum and recovery, reaching 7.2. This indicates that other factors than high intra-abdominal pressure are probably involved.[27] These data confirm the study of Hanly et al., who also found that abdominal insufflation with CO_2 causes a peritoneal acidosis, which is independent of the systemic pH. In their study insufflation with helium did not affect the peritoneal pH.[28] Additionally, Wong et al., also found that CO_2 pneumoperitoneum results in severe peritoneal acidosis. In their study this effect was unaltered by heating or humidification. They hypothesized that the alteration in peritoneal pH may conceivably be responsible for providing an environment favorable for tumor-cell implantation during laparoscopy.[29]

The induction of a peritoneal acidosis by laparoscopy may change the peritoneal immunoprotection. Acidification of the peritoneal cavity by abdominal insufflation increased serum IL-10 and decreased serum TNF-alpha levels in response to systemic lipopolysaccharide challenge. The degree of peritoneal acidification correlated with the degree of inflammatory response reduction.[30] Thus, pneumoperitoneum-mediated peritoneal cell acidification may attenuate the inflammatory response after laparoscopic surgery.

Peritoneal immunology

After conventional surgery polymorphonuclear leucocytes infiltrate the damaged peritoneal areas, soon followed by macrophages. Degranulation of peritoneal mast cells increases vascular permeability, which results in an inflammatory response and the release of active components. These active components include complement factors and opsins. In addition cytokines and growth factors, such as transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF-α), are secreted by polymorphs. Macrophages also release monokines like interleukin-1 (IL-1), IL-6 and arachadonic acid metabolites.[31] The peritoneal macrophage function activates the immune system due to the release of acute-phase proteins, ultimately leading to the activation of repair mechanisms. In contrast to the peritoneal reaction to a laparotomy, little is known about the local immune response to endoscopic procedures.

Clinical and experimental studies have demonstrated that laparoscopic surgery may preserve the systemic immune system better than open procedures.[32-35] Both systemic C- reactive protein levels and IL-6 levels are reduced in patients undergoing laparoscopy compared to those that underwent a laparotomy.[36,37] An animal study comparing conventional versus laparoscopic-assisted colonic resection showed reduced levels of systemic IL-6 in the laparoscopic group. Laparoscopic surgery is characterized by a decreased acute-phase pro-inflammatory response of TNF-α and IL-1 with subsequent attenuation of late-phase immunosuppression seen with laparotomy.[38-40] These systemic observations, however, may not reflect the local immune response of the peritoneum to surgical trauma.

As a first line of defense, peritoneal macrophages and polymorphonuclear neutrophil granulocytes are of primary importance in protecting the body. Experimental animal studies have shown both air and carbon dioxide may affect the function of these cells.[41] Air exposure triggered a higher transmigration rate of polymorphonuclear neutrophils from the blood compartment into the peritoneal cavity and decreased polymorphonuclear neutrophil apoptosis, as compared with CO_2. In another study it was shown that peritoneal macrophages show a decreased basal TNF-alpha release when exposed to CO_2.[42] Interestingly, these cells showed an increased TNF-alpha release after a second immune stimulation (E. coli), suggesting a greater competency of interaction in an immune defense reaction after CO_2 exposure.

West et al. reported an inhibition of IL-1 production by peritoneal macrophages in vitro after 15 min of CO_2 exposure,[43] whereas after 30 minutes the TNF-α production was also decreased. These findings were supported by additional in vivo experiments comparing a capno-pneumoperitoneum to a pneumoperitoneum with helium showing less TNF-α and IL-1 secretion when CO_2 was used.[44] As the tumor scavenging action of peritoneal macrophages is mediated by inflammatory

cytokines such as TNF-α, these observations might affect tumor implantation.[45] Additionally, Neuhaus et al. showed in an experimental model that the choice of insufflation gas may affect the incidence of port sites metastases, possibly be related to local defense mechanisms.[46]

Various factors may contribute to the changed local immune response during laparoscopic surgery. As described above, pneumoperitoneum causes an acidification of the peritoneal surface, which may affect the immune response. This, however, may not entirely explain why IL-1 is inhibited as early as 15 minutes after CO_2 exposure, while TNF-α was inhibited after a minimum of 30 minutes.[47] This might implicate that IL-1 production is inhibited via a transcriptional control mechanism, whereas intracellular acidosis may contribute to the inhibition of TNF-α. A decreased IL-1 mRNA and normal levels of TNF-α mRNA supported this assumption.

A second factor may be the abdominal temperature. CO_2 insufflation has been associated with a reduced body and abdominal temperature. During laparoscopy, the intra-abdominal temperature has been demonstrated to decrease to as low as 27.7° Celsius.[48] Animal studies have shown significant changes in body core temperature during laparoscopy when using dry cold gas compared to the use of humidified and heated gas.[49] Hypothermia may have affected peritoneal macrophage functions. Patients undergoing a laparoscopic cholecystectomy with a pneumoperitoneum at room-temperature were shown to have higher levels of cytokines in the peritoneal fluid compared to those that were operated with pneumoperitoneum at body temperature.[50] TNF-α and IL-1 were significantly increased, while IL-6 was marginally elevated.[51]

Growth factors and adhesion molecules

The activation of peritoneal inflammation and modulation of the immune response following laparoscopic surgery will eventually regulate peritoneal healing processes. Besides the previously described cytokines, growth factors and adhesion molecules may also play important roles in peritoneal healing. Peritoneal mesothelial cells produce multiple cellular mediators, such as transforming growth factor beta (TGF-β), and may also regulate the response of other cells.[52] Wheras TGF-beta stimulates fibroblasts to produce many proteins including collagen, fibronectin, and integrins, it decreases the production of proteins such as collagenase and heparinase whose function is to degrade the extracellular matrix. Mesothelial cells express various adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1).[53]

Other proteins present in the peritoneum are connective tissue growth factor (CTGF), heparin binding epidermal growth factor (HB-EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet derives growth

factor (PDGF). Studies focusing on laparoscopic surgery in relation to these proteins are lacking. An exception is TGF-beta, which has been studied in patients undergoing a laparoscopic cholecystectomy.[54] In that study it was shown that short-term laparoscopy does not affect the peritoneal levels of TGF-beta. The use of an ultrasonic scalpel, frequently used in laparoscopic surgery, decreased the levels of TGF-beta compared to electrocautery. The intensity of light used to illuminate the peritoneal cavity also influenced the local concentrations of TGF-beta.
A range of studies support the theory that TGF-β is a major stimulator of peritoneal adhesion formation, mainly by increasing the production of plasminogen activator inhibitor (PAI-1). Furthermore TGF-β is a stimulator of extracellular matrix deposition, resulting in a net accumulation of connective tissue.[55-57] The relation between TGF-beta and adhesion formation has been established by various studies demonstrating elevated levels of TGF-beta in areas of adhesion formation in humans.[58,59] Additionally, Freeman et al. have shown in an experimental rat study that peritoneal adhesions have increased levels of TGF-beta1 and beta3 mRNA transcripts compared with both uninjured and normally healed peritoneum.[60]
Sendt et al. studied the expression of various indicators of the inflammatory response,[61] including IL-1, IL-6, ICAM-1, and antibodies against macrophage inhibiting factor-related proteins 8 and 14 (MRP 8 and 14) in patients undergoing open end laparoscopic cholecystectomy. They found no difference in any of the measured parameters, except IL6, between the open and laparoscopic groups. ICAM-1 was also significantly increased in the laparoscopic group. They concluded that minimally invasive surgery does not necessarily mean minimal peritoneal damage.
The role of vascular endothelial growth factor (VEGF) during laparoscopy was studied by Molinas et al. in an experimental mouse model.[62] After 60 minutes of CO_2 pneumoperitoneum an increase incidence of adhesion were observed. Antibodies against VEGF-1 receptors significantly reduced adhesion formation suggesting that VEGF plays an important role in complex design of peritoneal healing following laparoscopic surgery.

Peritoneal fibrinolysis

There is substantial evidence that the peritoneal plasmin system plays a crucial role in peritoneal healing and subsequent adhesion formation. Peritoneum mesothelial cells produce both activators and inhibitors of the plasmin system. Tissue type plasminogen activator (tPA) is the major plasminogen activator in the peritoneal organ,[63] followed by urokinase-type plasminogen activator (uPA). Their activity is limited by plasminogen activating inhibitors, principally type 1 (PAI-1). Conventional abdominal surgery is accompanied by a rapid decline in the peritoneal fibrinolytic activity. The decreased peritoneal fibrinolysis may be caused by

both an increase of its inhibitors,[64] and a quick release of tPA from the visceral peritoneum during surgery.[65]

In an in vitro model, Ziprin et al. assessed the effect of a pneumoperitoneum on the fibrinolytic activity of mesothelial cells.[66] Human peritoneal cells were incubated in CO_2, helium and standard culture conditions. They found an enhanced plasminogen activator activity from cells exposed to both CO_2 and helium in the absence of oxygen, because of a reduction in PAI-1 concentrations. No changes in tPA levels were observed. Changes in insufflation pressures did not affect plasminogen activator activity.

Bergström at al found that flowing CO_2 increases PAI-1 expression by mesothelial cell in vitro.[67] In that study, one group of primarily cultured human peritoneal mesothelial cells were exposed to CO_2 flowing through the box without elevated pressure and another to CO_2 at pressure of 14 mmHG. The mesothelial cells exposed to flowing CO_2 released more PAI-1 than those exposed to pressurized CO_2 and controls. Cells exposed to flowing CO_2 had an increased PAI-1 mRNA expression. PAI-1 up-regulation has been described to be a mechanism of pneumoperitoneum-enhanced adhesion formation in mice.[68]

In another experimental study, Nagelschmidt et al. found that a CO_2 pneumoperitoneum significantly decreased peritoneal tPA activity in pigs.[69] These experimental studies have indicated that, in similarity with open surgery, laparoscopic surgery might lead to hypofibrinolysis by both an up-regulation of inhibitors and a down-regulation of activators of the plasmin system.

Tarhan et al. have studied the peritoneal fluid after both open and laparoscopic cholecystectomy.[70] They found higher tPA levels after the open procedure, possibly related to more intense tissue handling. The role of fluid concentrations in peritoneal healing and its relation to the peritoneal levels of fibrinolytic enzymes, however, remain a matter of debate. In another study in patients undergoing a laparoscopic cholecystectomy there was no effect on the expression of peritoneal tPA, PAI-1 or uPA levels. Moreover, there was no difference in the biopsies taken from patients operated with various pressures and light intensities.[71] The short operation time may have contributed to these observations.

This hypothesis was confirmed in another study assessing the effect of prolonged laparoscopic surgery.[72] Prolonged laparoscopic surgery decreased peritoneal tPA antigen expression and its activity after 90 minutes of surgery. It was concluded that, in contrast to short-term laparoscopic surgery, prolonged laparoscopic surgery causes a decreased fibrinolytic activity in the peritoneum due to decreased tPA levels.

Another factor affecting the peritoneal fibrinolysis is the decreased abdominal temperature. In a study comparing patients operated with carbon dioxide at room

temperature compared to those operated with CO_2 at body temperature significantly increased PAI-1 levels were found in the first group. These observations suggest a possible adverse effect of cooling of the abdominal cavity on peritoneal fibrinolysis.[73]

Few human studies comparing open and laparoscopic studies have been performed at present. Bergström et al. found an initial rise in peritoneal PAI-1 concentration during laparoscopy, which suggests an adverse effect of carbon dioxide insufflation.[74] At the end of the cholecystectomy, however, there was no difference between groups. Neudecker found decreased tPA activity levels after both conventional and laparoscopic colonic resection.[75] In that study, unfortunately, all patients underwent an initial laparoscopy prior to randomisation, which might have affected their results. A similar study showed,[76] that after mobilization of the hemicolon, peritoneal levels of tPA antigen and activity were significantly higher in the laparoscopic group, due to a decrease in the conventional group. At the end of the procedure, the concentrations of tPA antigen and activity significantly decreased in the laparoscopic group to levels comparable with the conventional group. It was concluded that both conventional and laparoscopic surgery inflict a decrease in tPA antigen and its specific activity. The peritoneal hypofibrinolysis appears to initiate more rapidly during conventional surgery compared to laparoscopic surgery, which may reflect the minimal invasive character of the latter.

Discussion

The current review shows that laparoscopic surgery has a profound effect on both the peritoneal integrity and its biology. The immune system is altered and the plasmin system inhibited. Moreover, laparoscopy induces a peritoneal acidosis, probably due to a decreased local oxygen pressure. Many of the available data derived from experimental studies. It is unclear how these experimental data could be translated clinically. (figure 1)

The described changes may have been induced by various factors of laparoscopic surgery. Which effect is induced by which component of laparoscopic surgery is not obvious. First, the abdominal temperature may be important. A decreased temperature has been shown to inhibit the plasmin system and affect local cytokine concentrations. Second, the intense illumination of the peritoneum may affect the peritoneum, either directly or indirectly by causing local dissecation. Local TGF-beta levels were affected by the intensity of light. Other components are the intra-abdominal pressure, duration of procedure, choice of dissection devices, dissecation and the insufflation gas. Carbon dioxide, the most frequently used insufflation gas, has been shown to affect the peritoneal morphology and to

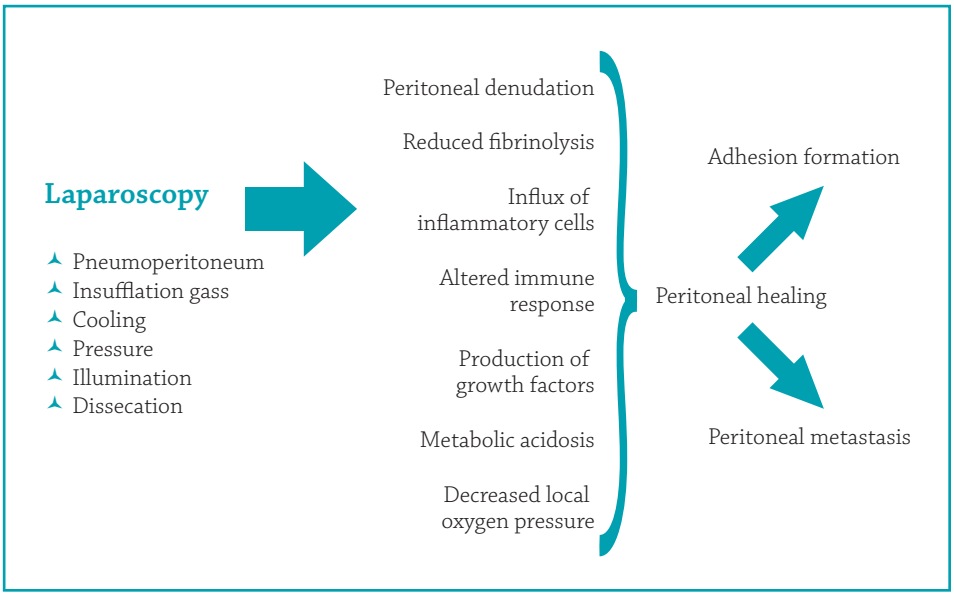

Figure 1 *Various aspects of laparoscopy and their possible peritoneal biological effects with subsequent clinical repercussions.*

cause local hypoxia leading to acidosis. Moreover it affects the local inflammatory response and disturbs peritoneal fibrinolysis. Other gases, such as helium or air, do not always induce similar effects. Addition studies are indicated to elucidate the individual effects of all described components.

The effects of laparoscopy on peritoneal integrity and biology may have repercussions for the occurrence of peritoneal metastasis. Despite the fact that dissemination to the peritoneum is not exceptional, mechanisms of action have not been fully elucidated. Only when the mesothelial cell layer has been breached, tumor cells may infiltrate and proliferate within the submesothelial connective tissue matrix. The early bulging of mesothelial cells during laparoscopic procedures may facilitate this phenomenon. Some experimental studies have suggested a prominent role of neutrophils in the occurrence of peritoneal metastasis. Others have proposed that tumor-conditioned media or exogenous inflammatory cytokines may induce mesothelial retraction and disaggregation to gain access to the submesothelial connective tissue.

Results from the current review show a remarkable effect of laparoscopic surgery on the peritoneal immunology and inflammation. Adhesion molecules may play an important role as well. Tumor cells have been shown to adhere rapidly to the mesothelial cell layer and play a role for the cell adhesion molecule CD44 and the

integrins 1, 2, 3 and 5 in mesothelial invasion.[77] Studies focusing on the effects of laparoscopic surgery on the expression of these adhesion molecules are therefore indicated.

Postsurgical adhesion formation remains a major concern, because it may cause severe and life-threatening complications such as small bowel obstruction. The economic burden of adhesions is high.[78] Regardless the observations that the incidence of adhesions after laparoscopic surgery may be lower compared to open surgery, efforts to reduce their occurrence still appear to be indicated.

There is considerable evidence that the plasmin system plays a key role in the pathophysiology of intra-abdominal adhesions. Clinical studies on the peritoneal fibrinolytic response to conventional surgery, using sequential biopsies taken during the procedure, have shown a progressive peritoneal hypofibrinolysis. During laparoscopic procedures, a prolonged period of surgery was needed before tPA levels decreased, a finding in contrast with the situation during open surgery, where a rapid decline of tPA levels has been shown in several studies. This might be due to a less intensive or different peritoneal trauma during laparoscopic surgery, compared with open surgery. These observations warrant further research focused on various components of laparoscopic surgery and peritoneal healing proces.

Laparoscopic procedures are regularly indicated for various infectious diseases, such as a perforated appendicitis or diverticulitis. A laparoscopic peritoneal lavage is frequently performed under these circumstances. Animal studies have suggested that the microbial adherence to the peritoneum is high, which may negatively affect the results of the lavage. Whether the effects of laparoscopic surgery on peritoneal biology affect this microbial adherence either positively or negatively, when compared to open lavage, remains to be investigated. This may be clinically important since lavage-resistant bacteria may be a source of persistent infection and might cause, through translocation, septic complications.

In conclusion, the results of the current review show that laparoscopic surgery affects the integrity of the peritoneum and its biological activity. Information about separate components of laparoscopic surgery and their specific effects remain to be elucidated.

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Peritoneal fibrinolytic response to various aspects of laparoscopic surgery

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Journal of Surgical Research 2006 Dec; 136(2):309-13

ABSTRACT

Background
Peritoneal fibrinolysis is important in peritoneal wound healing processes and adhesion formation. The peritoneal fibrinolytic response to laparoscopy is merely unknown. In the current study we investigate the effect of short-term laparoscopy on the peritoneal fibrinolytic response and the influence of intra-abdominal pressure, light intensity and choice of dissection device on this response.

Methods
50 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 beginning and the end of the procedure. Tissue concentrations of tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1) and the tPA activity were measured using ELISA techniques.

Results
There were no differences in tPA antigen, tPA activity, uPA antigen or PAI-1 antigen concentrations in biopsies taken at the beginning compared to samples taken at the end of the operation. Different intra-abdominal pressures, light intensities and the choice of dissection device did not affect any of the measured parameters.

Conclusion
Short-term laparoscopy does not affect the peritoneal fibrinolytic response. The used intra-abdominal pressure, light intensity and choice of dissection device do not affect peritoneal activity during short-term laparoscopy.

Introduction

Endoscopic surgery has developed rapidly in the last decades. Endoscopic surgery minimizes the surgical trauma, thereby reducing recovery time and the incidence of postoperative complications.[1] Few studies have suggested that this strategy might also reduce the incidence of peritoneal adhesion formation.[2,3]

Abdominal surgery elicits an inflammatory response, which is accompanied by the formation of fibrin in the peritoneal cavity. Under normal circumstances, fibrin is lysed rapidly by the fibrinolytic system. After conventional abdominal surgery, however, the equilibrium between coagulation and fibrinolysis is disturbed, in favor of the coagulation system.[4,5] Thus, fibrin will form deposits that are a matrix for ingrowth of fibrocollagenous tissue and may eventually develop into permanent fibrous adhesions.[6,7]

By producing both activators and inhibitors of fibrinolysis, the peritoneum is decisive in the genesis of adhesions. Tissue-type plasminogen activator (tPA) is the main peritoneal plasminogen activator.[4] A second, but less potent plasminogen activator is urokinase-type plasminogen activator (uPA), which also may play a role in tissue remodeling processes.[8] Their activity is restricted by plasminogen activating inhibitors, predominantly type 1 (PAI-1). High peritoneal PAI concentrations have been associated with adhesion formation.[9]

Laparoscopic surgery induces the activation of both coagulation and fibrinolytic pathways in plasma.[10] The effect of laparoscopy on the peritoneal fibrinolytic response is merely unknown. Endoscopic surgery induces new entities in the abdominal cavity including the intense illumination of the peritoneal cavity and a high intra-abdominal pressure. Moreover, the use of new dissection devices, including ultracision, is progressively advocated.[11]

The current study was conducted to investigate the effects of short-term laparoscopy on peritoneal biology, specifically the fibrinolytic response. Moreover, the possible effects of illumination, increased intra-abdominal pressure and the choice of dissection device were studied.

Patients and Methods

Fifty patients with a diagnosis of symptomatic gallbladder stone disease and scheduled for elective laparoscopic cholecystectomy were randomised in five groups (Table 1). Randomisation by envelope was done just before the operation. Institutional Review Board approval was obtained and written informed consent was given before enrolment.

Group	Pressure	Light intensity	Dissection device
A	10	50%	Electrocautery
B	13	50%	Electrocautery
C	16	50%	Electrocautery
D	13	80%	Electrocautery
E	13	50%	Ultracision

Table 1 *Randomisation of patients scheduled for laparoscopic cholecystectomy in five groups.*

Operative procedure

A uniform technique of video-laparoscopic 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. Two biopsies of the parietal peritoneum were taken with forceps and scissors in the right upper quadrant of the abdomen. The first was taken immediately after CO_2 insufflation and the second after 45 minutes of surgery, without using electrocautery or ultracision. When the procedure was finished prior to 45 minutes the 2nd biopsy was taken just before desufflation. Biopsies were snap frozen in liquid nitrogen and stored at –70°C until further processing.

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 homogenisation 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 homogeniser (Ultra Thurrax IKA 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

Determinations of plasminogen activators and inhibitors were done using commercially available ELISA-kits. Levels of tPA and PAI-1 were assessed using imulyse kits from Biopool (Umeå, Sweden) and uPA using a kit from Monozyme (Horsholm, Denmark)

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.

Results

Clinical results

There was no difference in sex (m: 22%, f: 78%) and age (51 ± 16 years) of the patients between groups. The overall incidence of previous laparotomies was 30%. There was no difference in the incidence of previous laparotomies between groups. Moreover, there was no difference in the occurrence of intraperitoneal adhesions and the incidence of bile leakage between groups. The timing of the second biopsy was equal in all groups (38 ± 9.2 min).

Biochemical results

There were no differences in tPa antigen, tPA activity, uPA antigen or PAI-1 antigen concentrations measured in biopsies taken at the beginning of laparoscopy compared to specimens taken at the end of the procedure. (figures 1-4)
There was no difference in any of the measured parameters between patients with and without a previous laparotomy, and between patients with and without intra peritoneal adhesions. The occurrence of bile leakage during the surgical procedure did not affect any of the measured fibrinolytic parameters.

Intra-abdominal pressure

There was no difference in measured tPa antigen, tPA activity, uPA antigen or PAI-1 antigen in specimens from patients operated with 10, 13 and 16 mmHg intra-abdominal pressure (groups A, B, and C, respectively). (figures 1-4)

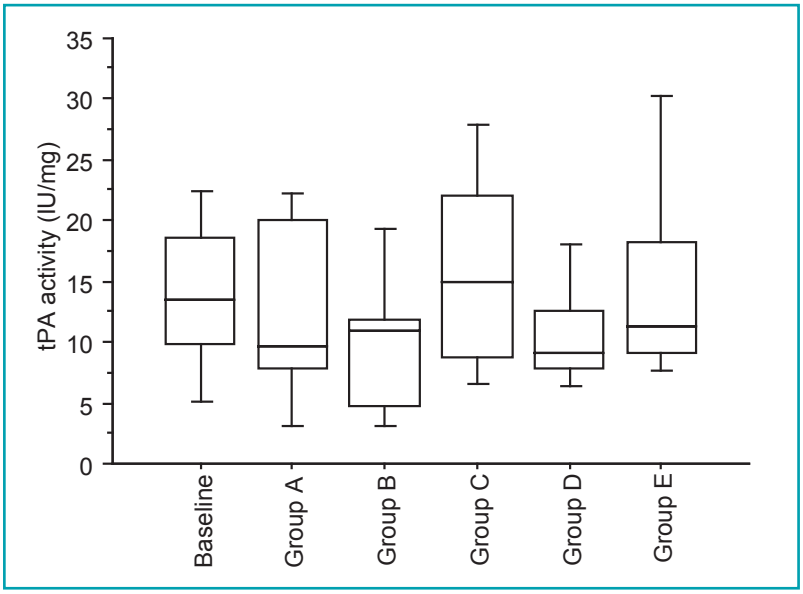

Figure 1 *Functional tissue-type plasminogen activator (tPA) concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

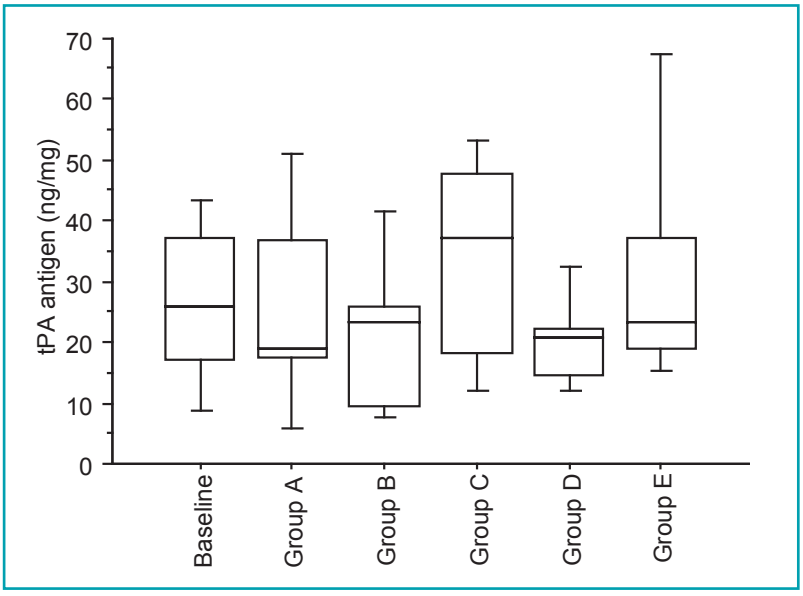

Figure 2 *Tissue-type plasminogen activator (tPA) concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

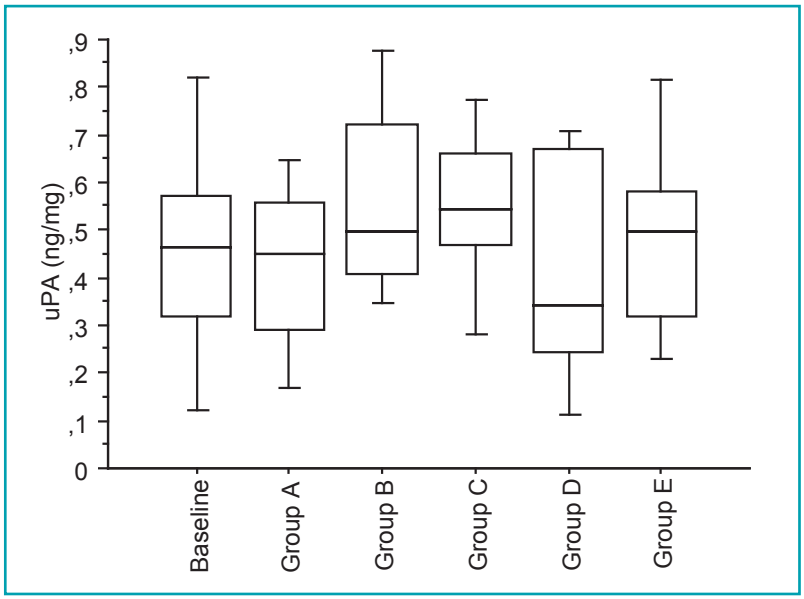

Figure 3 *Urokinase plasminogen activator (uPA) concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

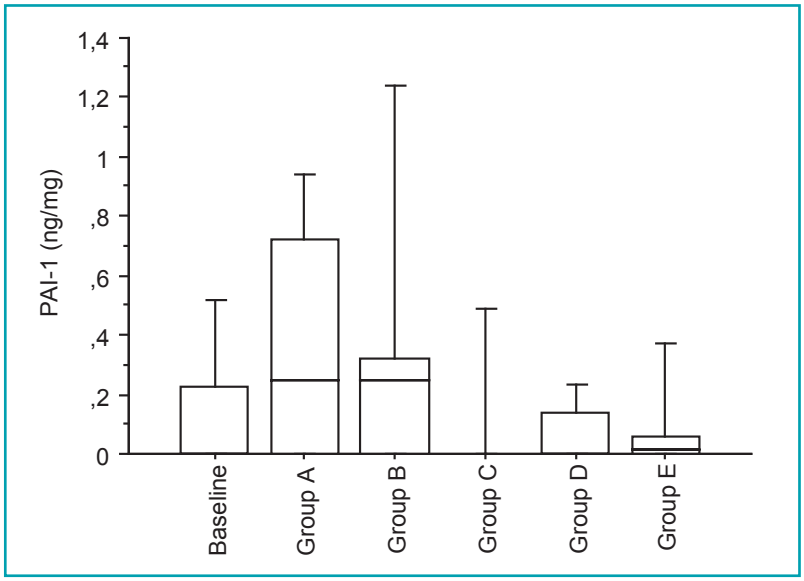

Figure 4 *Plasminogen activator inhibitor type 1 (PAI-1) concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

Light intensity

Peritoneal biopsies from patients operated with a high light intensity had similar levels of the fibrinolytic parameters compared to biopsies from patients operated with a low light intensity (groups B and D, respectively). (figures 1-4)

Dissection device

The choice of either electrocautery or ultracision as the used dissection device did not affect peritoneal levels of tPa antigen, tPA activity, uPA antigen or PAI-1 antigen after a laparoscopic cholecystectomy (groups B and E, respectively). (figures 1-4)

DISCUSSION

In the current study we demonstrate that short-term laparoscopic surgery does not affect the peritoneal fibrinolytic capacity. Within clinical applicable variances, the used intra-abdominal pressure and light intensity do not affect tPa antigen, tPA activity, uPA antigen, or PAI-1 antigen. Moreover, the choice of dissection device was not of influence on the measured parameters.

A reduced peritoneal fibrinolysis is a key factor in peritoneal wound healing and adhesiogenesis. Increased PAI-1 levels and decreased tPA concentrations result in the persistence of fibrin clots that eventually may evolve into permanent fibrous adhesions.[6,7] In the current study there was no effect of the laparoscopic procedure on the main fibrinolytical factors. This is in accord with the findings of Bergström et al. who have also found that tPA activity, tPA antigen and PAI-1 antigen do not change during laparoscopic cholecystectomy.[12] Open cholecystectomy, in contrast, induced increased peritoneal PAI-1 levels and decreased tPA levels. Neudecker et al. have described a decrease in tPA activity during laparoscopic assisted colectomy, with unaffected tPA antigen and PAI-1 antigen levels.[13] In that study, however, the second biopsy was taken after more than 2 h of surgery while in our study the second biopsy was taken already after 38 min. The latter might be too short to detect any differences, at least at protein level. This hypothesis is supported by experiments of Molinas et al. who have also found late changes in peritoneal fibrinolysis during laparoscopy in mice.[14] During conventional surgery, however, a decline in peritoneal tPA activity and tPA antigen has been described within 30 min of surgery.[4] The clinical relevance of the current study is obvious because the major part of all laparoscopic procedures, including appendectomy, diagnostic laparoscopy, and cholecystectomy, are procedures with a limited time frame.

One might discuss that the number of patients in each group was too low to detect any differences at all. However, based on previous studies of our group, groups of 10 patients each should be appropriate to detect any relevant differences in

peritoneal expression of fibrinolytical enzymes. Others have also demonstrated significant differences in human peritoneal fibrinolysis using similar numbers of patients.[15]

Laparoscopic surgery implicates the introduction of an increased intra-abdominal pressure and an intense illumination of the peritoneal cavity. Moreover, the use of ultracision is advocated over the use of electrocautery, predominantly used in conventional surgery. In the current study we could not detect an effect of the used intra-abdominal pressure or light intensity on fibrinolytic parameters. The differences in intra-abdominal pressures in the current study might have been too small to detect any differences because of 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.[16]

The effects of light on peritoneal biology are 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.[17] This heat generation is largely because of the radiated power in the visible light spectrum. Increased local temperature might affect mesothelial cell function, including fibrinolysis. Moreover, various frequencies of light and light sources might have different effects. Additional experimental studies are needed to clarify this subject.

Electrocautery induces thermal injury to treated tissue. Ultrasonic dissection is an alternative technique that may produce less thermal injury.[18] Additionally, ultracision has been described to produce less smoke plume than diathermy scissors.[19] Both the increased local temperature as toxins released in smoke might affect peritoneal fibrinolysis. The effects of smoke and temperature should be assessed in additional experiments.

Previously, we have described a lower peritoneal PAI-1 level immediately after initiating the laparoscopic procedure when compared to open cholecystectomy.[12] This suggests the peritoneal fibrinolytic system may have been influenced already before the first biopsy was taken. During this time the abdominal cavity is insufflated, resulting in increased abdominal pressure, illuminated and possibly cooled because of insufflation. Additional experimental studies are indicated to clarify this subject.

Results from the current study might support the hypothesis that laparoscopic surgery is less traumatic for the peritoneum compared to conventional surgery, which causes a progressive decrease in fibrinolytic activity. This concept is further supported by the findings of Suematsu et al. who described that the morphological peritoneal alterations after laparoscopy differ from those after laparotomy.[20]

Bulging up of the mesothelial cells was evident after pneumoperitoneum, whereas detachment of the mesothelial cells was present immediately after laparotomy. A reduced peritoneal trauma might explain the reduced adhesion formation found after laparoscopic surgery.[21]

In conclusion, we have demonstrated that short-term laparoscopy does not affect the peritoneal fibrinolytic capacity. Within clinical applicable variances, the used intra-abdominal pressure and light intensity and the choice of dissection device are not of influence on the peritoneal activity during short-term laparoscopy.

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Heating of carbon dioxide during insufflation alters the peritoneal fibrinolytic response to laparoscopic surgery

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Surgical Endoscopy 2008 May;22(5):1232-6

Abstract

Background
Laparoscopic surgery is evolving rapidly. It involves the creation of a pneumoperitoneum, mostly using carbon dioxide. Cooling of the peritoneum, due to insufflation, might traumatize the peritoneum and disturb peritoneal fibrinolysis, important in peritoneal healing processes. The current study was performed to elucidate the effects of the temperature of insufflation gas on the peritoneal fibrinolytic response to laparoscopic surgery.

Methods
Thirty patients scheduled for laparoscopic cholecystectomy were randomised in two groups. One group in which the pneumoperitoneum was created with carbon dioxide at room temperature, and one wherein carbon dioxide at body temperature was used. Peritoneal biopsies were taken at the start and end of surgery. Tissue concentrations of tPA antigen, tPA activity, uPA antigen and PAI-1 antigen were measured using ELISA techniques.

Results
Peritoneal PAI-1 antigen levels were significantly higher at the end of the procedure in patients operated with carbon dioxide at room temperature ($p<.05$). A slight, but not significant, decrease in tPA antigen and activity was observed in both groups during the procedure. Peritoneal concentrations of uPa antigen did not change during the procedure.

Conclusion
The temperature of carbon dioxide used for insufflation of the abdominal cavity affects peritoneal biology. Cooling of the peritoneum by unheated carbon dioxide causes increased peritoneal PAI-1 levels, important in peritoneal healing processes.

INTRODUCTION

Endoscopic surgery has gained popularity since it minimizes surgical trauma, thereby reducing recovery time and possibly the incidence of postoperative complications.[1] Various experimental studies have demonstrated that laparoscopic procedures induce fewer post-surgical adhesions compared to conventional surgery.[2,3] An increasing amount of surgical procedures are performed laparoscopic or laparoscopic-assisted. Laparoscopic surgery involves the creation of a pneumoperitoneum in order to create sufficient space in the abdominal cavity, leading to an increased intra-abdominal pressure. This might affect peritoneal biology. Carbon dioxide is the most commonly used gas for this purpose. The introduction of gases into the peritoneal cavity has been shown to decrease core and intra-abdominal temperature, possibly leading to increased postoperative pain.[4,5] During laparoscopy, the intra-abdominal temperature has been demonstrated to decrease to as low as 27.7° Celsius.[6] On the tissue level, carbon dioxide causes peritoneal acidosis, triggers the release of various cytokines and induces morphologic changes to the peritoneum.[7,8] In resemblance with conventional surgery, retraction and bulging of mesothelial cells and exposure of the basal lamina have been demonstrated during laparoscopic procedures.[9,10]

Gases, used for insufflation, are usually inflated at room temperature. Heating of the insufflated carbon dioxide to body temperature has been introduced in an attempt to minimize the decrease in core temperature. Clinical studies have failed to demonstrated an effect of heating on core temperature or postoperative pain sensation.[4,5] Studies on the effects of the temperature of insufflation gasses on peritoneal biology and wound healing processes, however, are lacking.

The peritoneal organ is an extensive serous membrane and has multiple biological functions. One of these is the regulation of intra-abdominal fibrinolysis, which is crucial in the peritoneal tissue repair processes.[11,12] The peritoneal plasmin system is crucial in peritoneal repair processes and subsequent adhesion formation.[10,11] Plasmin is highly effective in the degradation of fibrin into fibrin degradation products. Tissue-type plasminogen activator (tPA) is responsible for about 95% of the plasminogen activator activity in the peritoneum.[13] Plasminogen activation is hampered by plasminogen activating inhibitors. Plasminogen activator inhibitor type-1 (PAI-1) is the main inhibitor of tPA and is produced by a variety of cells, including endothelial cells, mesothelial cells, macrophages and fibroblasts. In free form, plasminogen activators are rapidly inactivated by PAI-1, by forming inactive one-to-one complexes.

During conventional surgery, the equilibrium between coagulation and fibrinolysis is rapidly disturbed, in favour of the coagulation system. Hence, fibrin clots

will form, which may eventually develop into permanent fibrous adhesions.[11,12] We have recently demonstrated that short-term laparoscopy does not affect the peritoneal fibrinolytic activity.[14] However, in that study carbon dioxide at room temperature was used in all groups. Cooling of the peritoneal cavity might have affected the results in that study.
The current study was performed to test the hypothesis that the temperature of insufflation gases affects peritoneal biology, specifically the peritoneal fibrinolytic response to laparoscopic surgery.

PATIENTS AND METHODS

Thirty consecutive patients scheduled for elective laparoscopic cholecystectomy for the diagnosis symptomatic gallbladder stone disease were randomised in two groups. Randomisation was done by envelope just prior to the operation. In the first group (n=15) the pneumoperitoneum was created with carbon dioxide at room temperature. In the second group (n=15), the carbon dioxide was heated to a temperature of 37° Celsius using a Thermoflator® (Karl Storz GmbH & Co, Tuttlingen, Germany).
Institutional Review Board approval was obtained and written informed consent was given before enrolment.

Operative procedure

In all patients, a uniform technique of video-laparoscopic 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. Two biopsies of the parietal peritoneum were taken with forceps and scissors not using electrocautery. The first sample was taken immediately after carbon dioxide insufflation and the second after 45 minutes of surgery. When the procedure was finished prior to 45 minutes the 2nd biopsy was taken just before deflation. Biopsies were snap frozen in liquid nitrogen and stored at –70°C until further processing.

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 homogenisation 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 Thurrax IKA T-25, Janke & Kunkel, Staufen, Germany), centrifuged at 10.000 g for 4 min at 4° Celcius, and the supernatant was stored at -70°C until further analysis. Tissue processing and assays were performed in batches.

Biochemical assays

Determinations of plasminogen activators and inhibitors were done using commercially available ELISA-kits. Levels of tPA and PAI-1 were assessed using imulyse kits from Biopool (Umeå, Sweden) and urokinase-type plasminogen activator (uPA) using a kit from Monozyme (Horsholm, Denmark)

Statistics

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

RESULTS

Clinical results

There were no differences in gender (m 27%, f 73%) or age (52 ± 15,7 years) between groups. The overall incidence of previous laparotomy was 23%, without difference between groups. Moreover, there was no difference in the occurrence of intra-peritoneal adhesions between groups. The timing of the second biopsy was equal in both groups (40.6 ± 9.7 min).

Biochemical results

PAI-1 antigen

The peritoneal concentration of PAI-1 antigen was 0.11 ± 0.20 ng/ml in patients where the pneumoperitoneum was created with carbon dioxide at room temperature. In the group operated with carbon dioxide at body temperature, the levels immediately after insufflation were 0.01 ± 0.05 ng/mL. (figure 1) This difference did not reach statistical significance. At the end of the procedure, however, peritoneal levels of PAI-1 antigen were significantly higher in patients operated with unheated carbon dioxide, when compared to patients operated with carbon dioxide at body temperature ($p<0.05$)

tPA antigen

At the start of the procedure, the overall peritoneal tPA antigen concentration was 24.8 ±14.7 ng/mL. There was a slight, but not statistically significant, decrease in

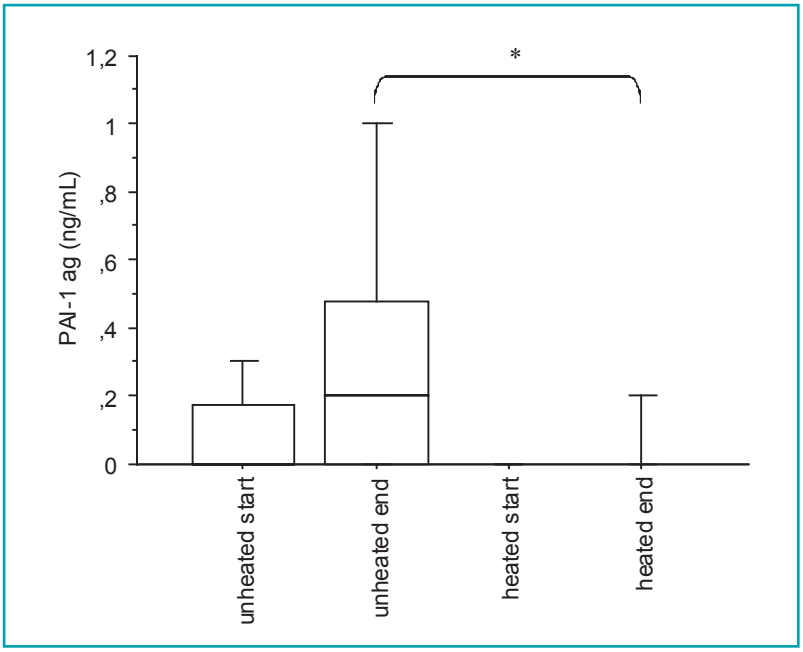

Figure 1 *Plasminogen activator inhibitor type-1 (PAI-1) antigen concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). * = p<0.05.*

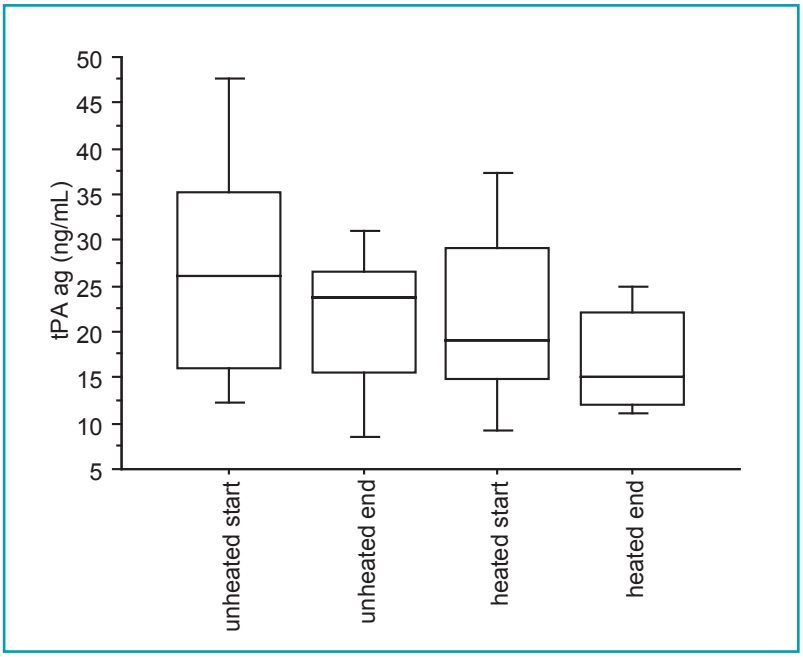

Figure 2 *Tissue-type plasminogen activator (tPA) antigen concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

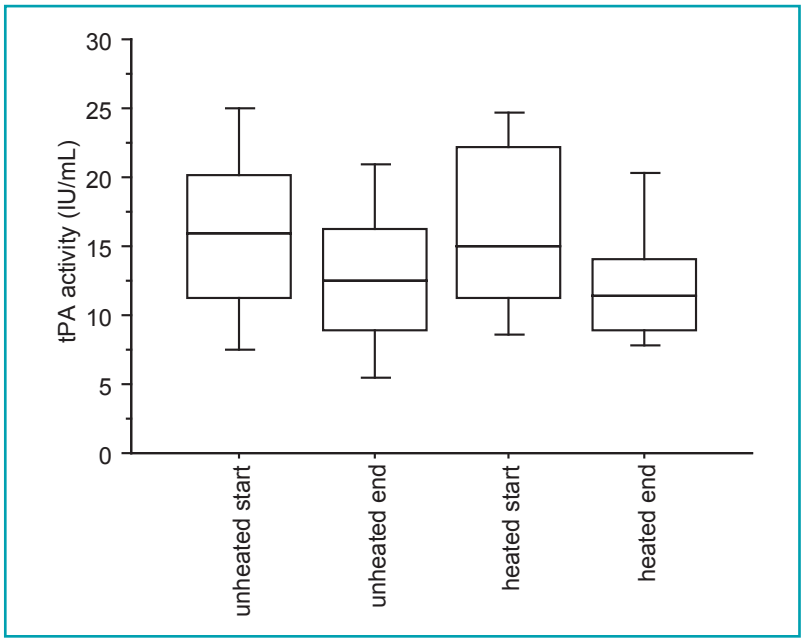

Figure 3 *Functional Tissue-type plasminogen activator (tPA) concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

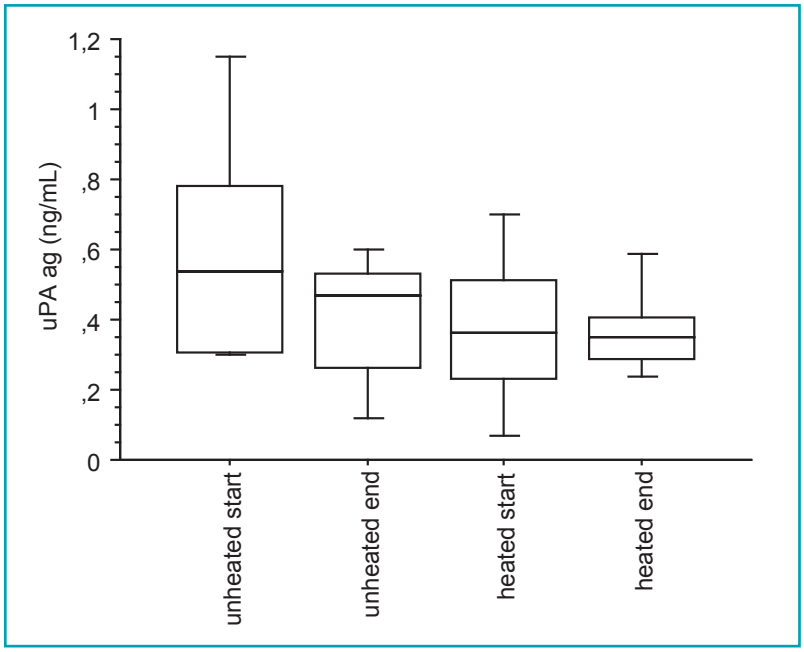

Figure 4 *Urokinase plasminogen activator (uPA) antigen concentration in peritoneal samples. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

peritoneal tPA antigen levels during the procedure in both groups. (figure 2) There were no significant differences between patients operated with carbon dioxide at room temperature and those operated with carbon dioxide at body temperature neither at start or completion of the procedure.

tPA activity

As for the antigen levels, the tPA activity showed a slight, but not statistical significant, decrease during the procedure in both groups. (figure 3) Again, there were no significant differences in the functional concentrations of tPA between patients operated with unheated carbon dioxide and the group treated with heated carbon dioxide.

uPA antigen

The mean overall uPA antigen concentration at the start of the procedure was 0.51 ± 0.20 ng/mL. Peritoneal levels of uPA did not change during the procedure and were not affected by the temperature of the carbon dioxide. (figure 4)

DISCUSSION

In the current study we have demonstrated that cooling of the peritoneal cavity by carbon dioxide insufflation affects peritoneal biology by increasing the inhibition of plasmin activation, which causes a decreased fibrinolytic activity. At the end of the procedure peritoneal PAI-1 levels were significantly lower in patients operated with carbon dioxide heated to body temperature compared to peritoneal concentrations from patients operated with carbon dioxide at room temperature.

Increased PAI levels have been associated with an increased post surgical adhesion formation. High PAI concentrations are found in adhesions and peritoneal tissue of patients with extensive adhesions.[15] In traumatized peritoneal tissue, both increased concentrations of PAI-1 and decreased levels of tPA result in a decreased fibrinolytic activity.[13,16-19] Accordingly, PAI-1 is considered to be an important factor in peritoneal tissue repair. Our observation that peritoneal PAI-1 levels are lower in patients operated with heated carbon dioxide might indicate that cooling of the peritoneum traumatizes the peritoneal layer, leading to decreased fibrinolytic activity. Whether the reduced hypofibrinolysis in patients operated with heated CO_2 will also lead to a further reduction in the formation of post-surgical adhesions remains to be investigated. Although the technique of heating is easy to implement in daily surgical practice and investments are low, it seems to be preliminary to advocate the use of heated CO_2 during all laparoscopic procedures.

Immediately after starting the procedure the mean levels of PAI were already approximately 10 times lower when heated carbon dioxide was used. This supports the hypothesis that peritoneal damage might occur immediately after starting

the laparoscopic procedure. This hypothesis was first proposed by Bergström et al., who found an early rise in peritoneal PAI-1 concentration during laparoscopy, suggesting an adverse effect of carbon dioxide insufflation.[20] Data from the current study suggest that it may not be the insufflation itself, but rather the cooling accompanying it causing this effect. Findings of Erikoglu further support the observation that the use of heated carbon dioxide may be less traumatic for the peritoneal layer.[21] They have described that heated and humidified carbon dioxide results in less peritoneal morphological alterations compared to cold and dry carbon dioxide in an experimental study. The latter caused drastic alterations of the surface layer, including extreme desquamation of the mesothelial layer. In rats operated with heated and humidified carbon dioxide, in contrast, mesothelial cells had only bulged up to the surface layer and retracted. It remains to be demonstrated whether it was the heating, the humidifying or the combination of those, which was responsible for this difference.

Results of the current study are seemingly in contrast to the observations of Binda, who described that a decreased body temperature, which may be caused by the environment temperature and the pneumoperitoneum, decreases peritoneal adhesion formation in transgenic mice.[22] They have suggested hypoxia as a driving mechanism, since hypothermia decreases the toxic effects of hypoxia and of the ischaemia-reperfusion process. Data from the current study, however, clearly indicate an influence of temperature of the insufflation gas on the peritoneal fibrinolytic system, which has proven to be crucial in peritoneal healing processes and adhesiogenesis.[10,11] In addition to hypoxia and hypofibrinolysis, other mechanisms may also be involved. Glew et al. have demonstrated that heating and humidifying carbon dioxide leads to faster dissipation of residual gas, which is associated with a reduced duration of inflammation in piglets.[23] Further experimental and clinical studies are indicated to elucidate the specific role of both temperature and humidification of insufflation gasses on peritoneal healing processes.

In the current study we have observed a slight, but non-statistically significant, decrease in tPA antigen levels, regardless whether heated or unheated carbon dioxide was used. In open surgery a rapid decline of tPA levels has been described.[13,18] Since peritoneal trauma might be lower during laparoscopic surgery, the operation time in the current study might have been too short to detect any significant differences in peritoneal tPA expression. Further studies on prolonged laparoscopic surgery are indicated to elucidate this subject.

In conclusion, we have demonstrated that heating of carbon dioxide, used for insufflation during laparoscopic surgery, affects peritoneal biology by decreasing the expression of PAI-1, which is an important protein in peritoneal repair processes.

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Peritoneal transforming growth factor beta 1 expression during laparoscopic surgery

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Surgical Endoscopy 2007 Sep;21(9):1537-41

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.[1] 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 peritoneum healing.

TGF-beta1 appears to be a major stimulator of peritoneal adhesion formation[2], 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.[3] Moreover, TGF-beta1 is a major stimulator of extracellular matrix deposition by inducing the production of collagen, fibronectin and integrins.[4,5] Increased TGF-beta1 concentrations have been found in peritoneal fluid of patients with adhesions and in adhesion tissue itself.[6] Moreover, postoperative peritoneal administration of TGF-beta1 increased adhesion formation in mice while its inactivation is reported to reduce the incidence of adhesions.[7]

TGF-beta1 regulates chemotaxis, mitogenisis and angiogenesis and thereby involved in dissemination processes.[8] 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, lung, endometrium, prostate, breast, brain, and bone.[9,10] 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.[11] 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 videolaparocopic 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

Group	Pressure	Light intensity	Dissection device
A	10	50%	Electrocautery
B	13	50%	Electrocautery
C	16	50%	Electrocautery
D	13	80%	Electrocautery
E	13	50%	Ultrasonic scalpel

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

taken with forceps and scissors immediately after CO_2 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 Thurrax IKA 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 immuno sorbent 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.

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 intraperitoneal 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.

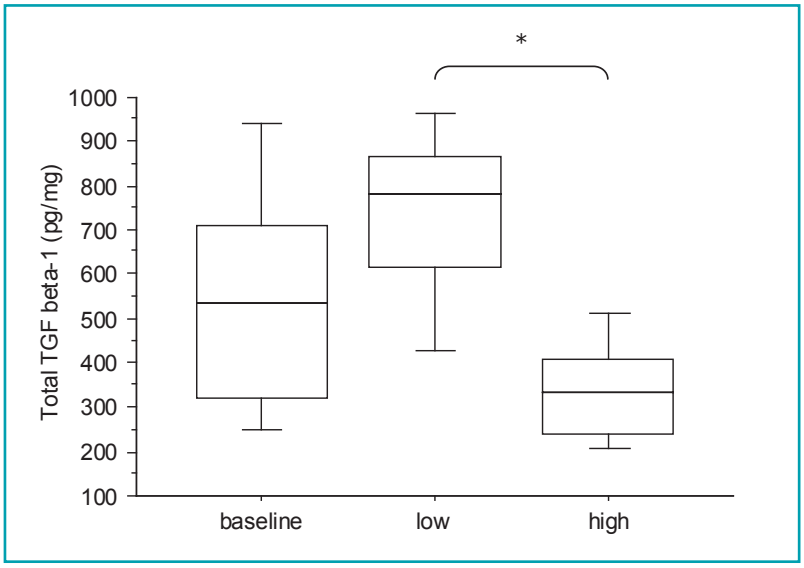

Figure 1a *Total transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with low and high light intensity (B and D). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). * = p<0.005.*

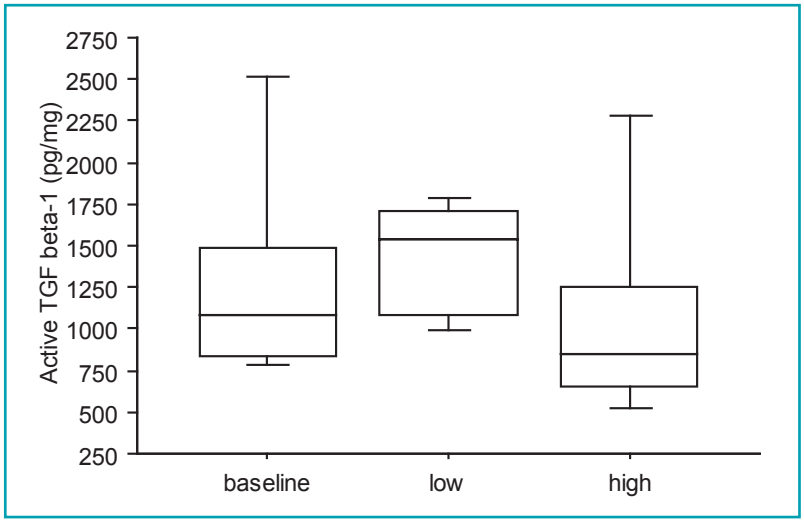

Figure 1b *Active transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with low and high light intensity (B and D). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

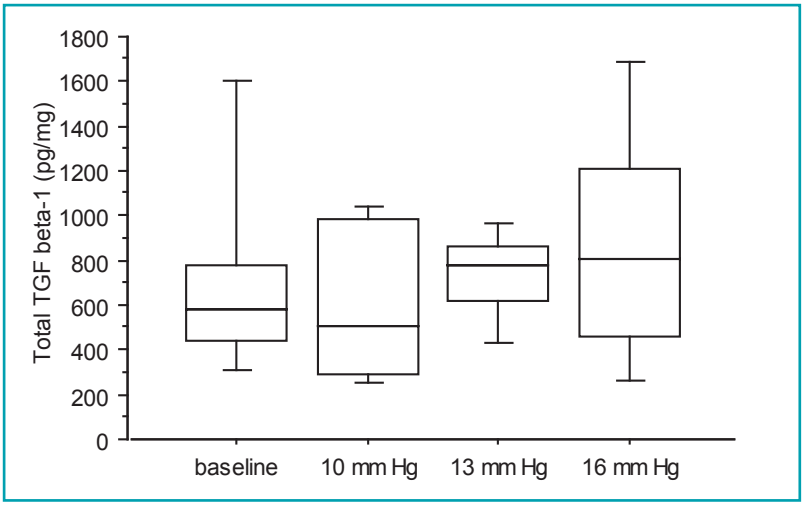

Figure 2a *Total transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with an intra-abdominal pressure of 10, 13 and 16 mmHg (A, B and C). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

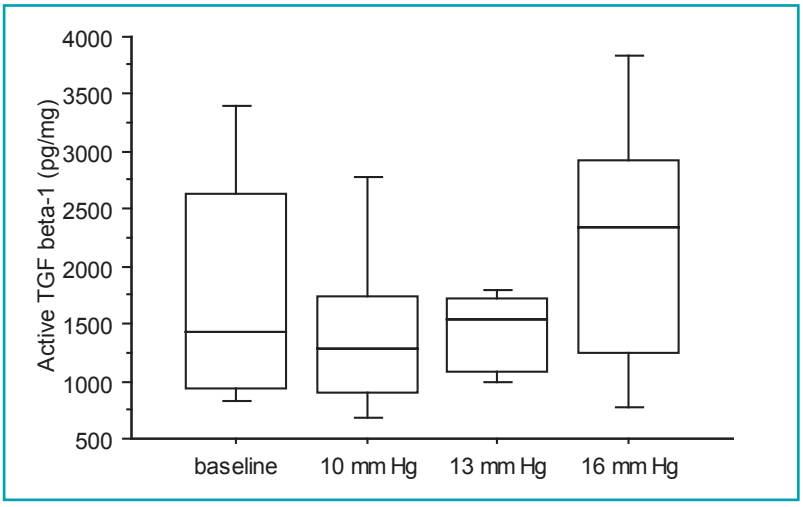

Figure 2b *Active transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups with an intra-abdominal pressure of 10, 13 and 16 mmHg (A, B and C). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

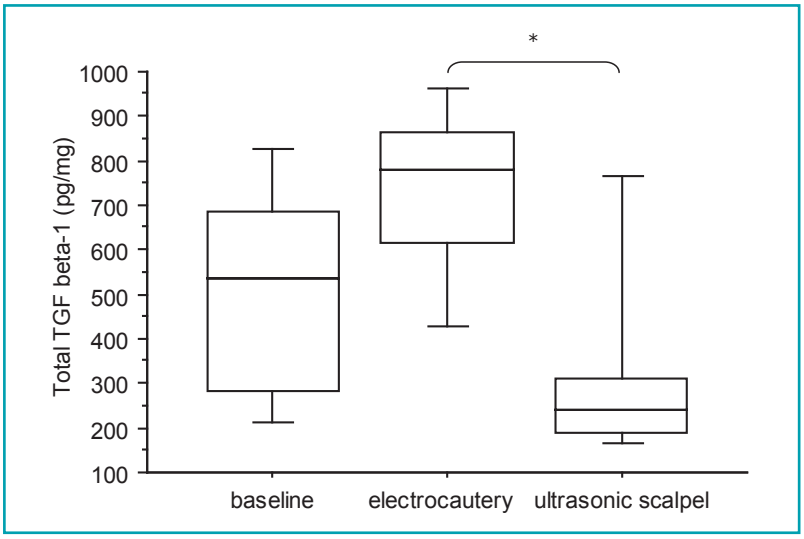

Figure 3a *Total transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups operated with either electrocautery or ultrasonic scalpel (B and E). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). * = p<0.005.*

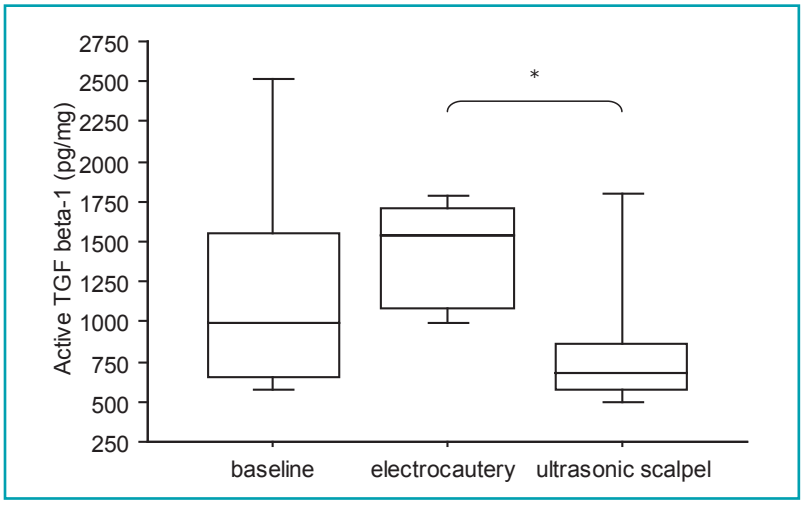

Figure 3b *Active transforming growth factor beta 1 (TGF-beta1) concentrations in peritoneal samples in the groups operated with either electrocautery or ultrasonic scalpel (B and E). Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). * = p<0.01.*

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.[12-15] 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 significant shorter.[14] 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 a laparoscopic cholecystecomy.[16] Schemmel et al. found no differences in peritoneal adhesion formation between traditional incision, electrosurgery and the ultrasonic scalpel in a rabbit uterine horn model.[17] Similar observations were described by Tulandi at al. in a rat uterine horn model.[18] 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.[19-21] An increasing part of laparoscopic procedures is performed for oncologic pathology such as laparoscopic colectomie, nefrectomy and hysterectomy. Experimental studies have demonstrated that laparoscopy is associated with less intra-peritoneal tumor growth compared to laparotomy, while insufflation of CO_2 may promote peritoneal tumor growth compared to gasless laparoscopy.[22,23] Lecuru et al, however, did not find any deleterious effect of CO_2 insufflation on ovarian tumor growth when compared to gasless laparoscopy or midline laparotomy in a rat model.[24] 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.[25] 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.[26] 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 CO_2, 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.[27] 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.[28] 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-beta1 in oncologic and peritoneal repair processes urges the need for further clinical trials.

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Decreased peritoneal tissue plasminogen activator during prolonged laparoscopic surgery

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Journal of Surgical Research 2009 Jan;151(1):89-93

Abstract

Background
Peritoneal fibrinolysis is crucial in peritoneal healing processes and subsequent adhesion formation. During conventional surgery, the peritoneal fibrinolytic system is rapidly disturbed. Short-term laparoscopy does not seem to affect peritoneal fibrinolysis. The aim of the current study was to assess the effect of prolonged laparoscopic surgery on peritoneal fibrinolysis.

Methods
Twelve consecutive patients undergoing laparoscopic gastric bypass surgery for morbid obesity were included in the study. During the procedure, biopsies of the parietal peritoneum were taken at the start of the procedure and each 45 minutes afterwards. Tissue samples were homogenized and tPA antigen, tPA activity, uPA antigen and PAI-1 antigen were measured using commercial assay techniques.

Results
Both tPA antigen and its activity progressively decreased during the procedure, reaching significant levels after 90 minutes of surgery. The levels of uPA antigen and PAI-1 antigen did not significantly change throughout the procedure.

Conclusion
As for conventional surgery, prolonged laparoscopic surgery causes a decreased fibrinolytic activity in the peritoneum due to decreased tPA levels.

INTRODUCTION

Peritoneal healing processes after open abdominal surgery have been studied extensively during the last decades. The peritoneal plasmin system appears to play a key role in peritoneal healing processes.[1-3] During conventional surgery, the peritoneal fibrinolytic system is rapidly depressed, which eventually might lead to peritoneal adhesion formation.[4] The peritoneal organ is capable of producing both activators and inhibitors of the plasmin system. Tissue-type plasminogen activator (tPA) is the main plasminogen activator, exerting about 95% of plasminogen activator activity in the peritoneum.[2] A second, but less potent plasminogen activator is urokinase-type plasminogen activator (uPA), which also may play a role in tissue remodeling processes. Their activity is restricted by plasminogen activating inhibitors, predominantly type 1 (PAI-1).

Endoscopic surgery minimizes the surgical trauma, thereby reducing recovery time and possibly the incidence of postoperative complications.[5] A small number of trials have suggested that laparoscopic surgery induces fewer and less severe adhesions.[6,7] Laparoscopic surgery induces the activation of both coagulation and fibrinolytic pathways in plasma.[8] The effect of laparoscopy on peritoneal fibrinolytic response has not been studied extensively. We have recently demonstrated that short-term laparoscopic surgery does not affect the peritoneal fibrinolytic activity.[9] However, in that study the operation time did not exceed 38 minutes and might have been too short to detect any peri-operative changes.

The current study was performed to assess the effect of prolonged laparoscopic surgery on the peritoneal biology, focusing on peritoneal fibrinolytic activity.

MATERIAL AND METHODS

Twelve consecutive patients undergoing laparoscopic gastric bypass surgery for morbid obesity were included in the study. Institutional Review Board approval was obtained and written informed consent was given before enrolment.

Operative procedure

The patient was positioned in the lithotomic position. After establishment of a pneumoperitoneum, using a Veress needle in the left upper quadrant of the abdomen, five trochars were placed. The stomach was sized into a small pouch using a stapler device. The omentum was divided longitudinal to the level of the colon transversum. The jejunum then was anastomosed side to side approximately 20 cm after the ligament of Treitz to the pouch of the stomach. A 100 to 150 cm of length of the jejunum was measured distal to this anastomosis and stapled side

to side to the proximal jejunum. Afterward, the bowel was divided between the gastrojejunostomy and the entero-enterostomy. The gastrojejunostomy was tested for leakage by applying air through a nasogastric tube.

Sampling and processing

Immediately after creating the pneumoperitoneum, a biopsy was taken from the parietal peritoneum in the right upper quadrant. During the procedure, each 45 minutes an additional biopsy was taken from the parietal peritoneum.
The parietal peritoneum was carefully and sharply dissected, without using electrocautery, 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, each biopsy was cut into smaller pieces and placed into ice-cold phosphate buffered saline (PBS) with 0.5M sodium chloride and 0.01% Triton X-100 (Sigma, St.Louis, MO, USA) to a final concentration of 40 mg tissue/mL buffer. The tissue was homogenized for 60 s on ice using a Polytron homogenizer (Ultra Thurrax IKA T-25, Janke & Kunkel, Staufen, Germany); following centrifugation at 10.000 g for 4 min at 4°C, the supernatant was stored in aliquots at -70°C until further analysis. Tissue processing and assays were done in batches.

Biochemical assays

Determinations of plasminogen activators and inhibitors were done using commercially available assay-kits. Levels of tPA and PAI-1 antigen were assessed using TintElize tPA and PAI-1 (ELISA) kits and tPA activity was measured using a chromogenic assay (Chromolize tPA) from Biopool (Umeå, Sweden). uPA antigen was measured using an ELISA kit from Technoclone (Vienna, Austria). All results were normalized to total protein content using a commercial protein assay (Bio-RAD, Hercules, California, USA).

Statistical analysis

Values are given as mean and standard error normalized to protein content. Analysis of differences between the different time points was performed using the ANOVA test followed by Fisher's post-hoc test.

RESULTS

Clinical Results

The patients included in the study had a mean age of 38 ± 9 years and an average BMI of 49 ± 8 kg/m^2. There was an equal distribution between men and women. Four patients had previously undergone laparoscopic gastric banding and one of

them also underwent laparoscopic cholecystectomy. Two others had a history of laparoscopic cholecystectomy and one of them also underwent a laparoscopic appendectomy. The average operation time was 183 ± 35 minutes. In one patient the procedure was converted to a laparotomy, after 135 minutes of laparoscopic surgery, due to extensive adhesion formation after a previous gastric banding.

Biochemical results

In the study population 7 out of 12 patients had prior abdominal surgery. In 3 of these patients intra-abdominal adhesions were observed during the procedure. We did not find any differences in the measured plasmin activators or inhibitors between patients with or without previous abdominal surgery. No significant differences were found between patients with or without intra-abdominal adhesions present in the abdominal cavity.

tPA antigen

The tPA antigen concentration immediately after initiating the pneumoperitoneum was 29.8 ± 5.8 ng/mg protein. During the first ninety minutes of surgery, the peritoneal concentrations of tPA antigen decreased with approximately 30% ($p<0.05$). (figure 1) Afterwards the peritoneal levels of tPA antigen remained at the same level.

tPA activity

The active fraction of tPA, the tPA activity, was at the start of the procedure 18.3 ± 3.4 IU/mg protein. As for its antigen levels, tPA activity also decreased during laparoscopic surgery. After 90 minutes of surgery the tPA activity was 50% lower, when compared to the values measured in biopsies taken immediately after insufflation ($p<0.05$). (figure 2)

uPA antigen

Peritoneal uPA levels immediately after insufflation were 4.1 ± 1.0 ng/mg protein. During the surgical procedure, there was no significant change in peritoneal uPA expression. (Figure 3)

PAI-1 antigen

Peritoneal PAI-1 antigen concentration was at the beginning of the operation 0.05 ± 0.05 ng/mg protein. There was a slight, but not significant, increase to 0.21 ± 0.12 ng/mg, observed after 90 minutes of surgery. (figure 4)

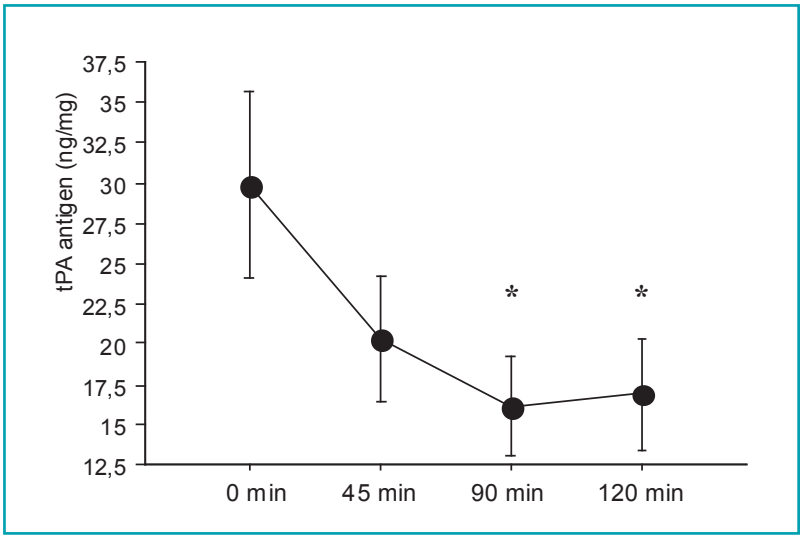

Figure 1 *Peritoneal tPA antigen concentrations (ng/mg protein) measured immediately after initiation of the procedure and every 45 minutes during a laparoscopic gastric bypass. * = p<.05 when compared to t=0 min.*

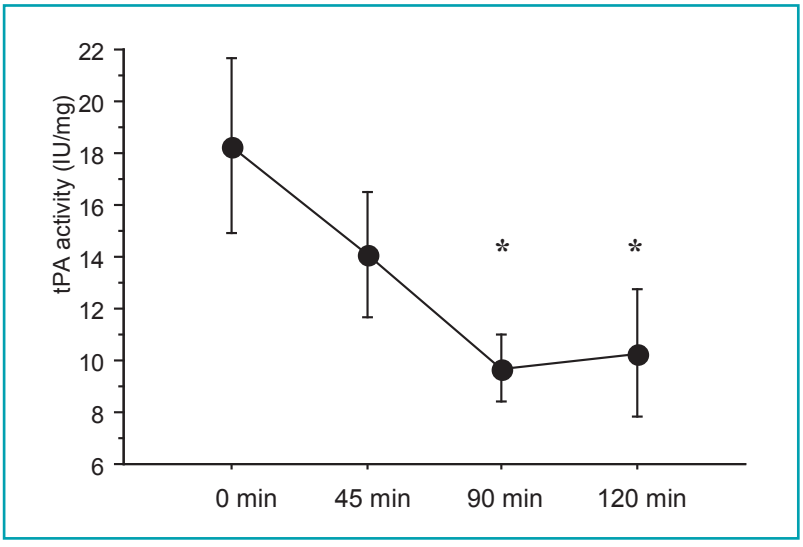

Figure 2 *Peritoneal tPA activity concentrations (IU/mg protein) measured immediately after initiation of the procedure and every 45 minutes during a laparoscopic gastric bypass. * = p<.05 when compared to t=0 min.*

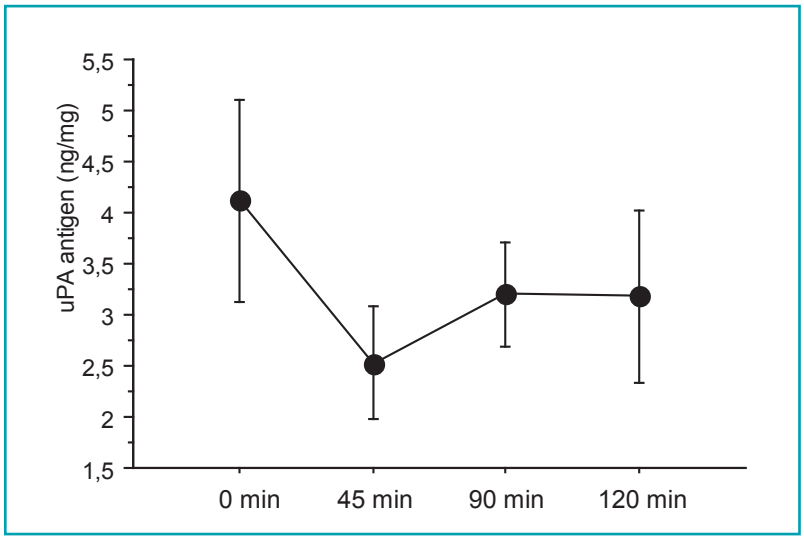

Figure 3 *Peritoneal uPA antigen concentrations (ng/mg protein) measured immediately after initiation of the procedure and every 45 minutes during a laparoscopic gastric bypass.*

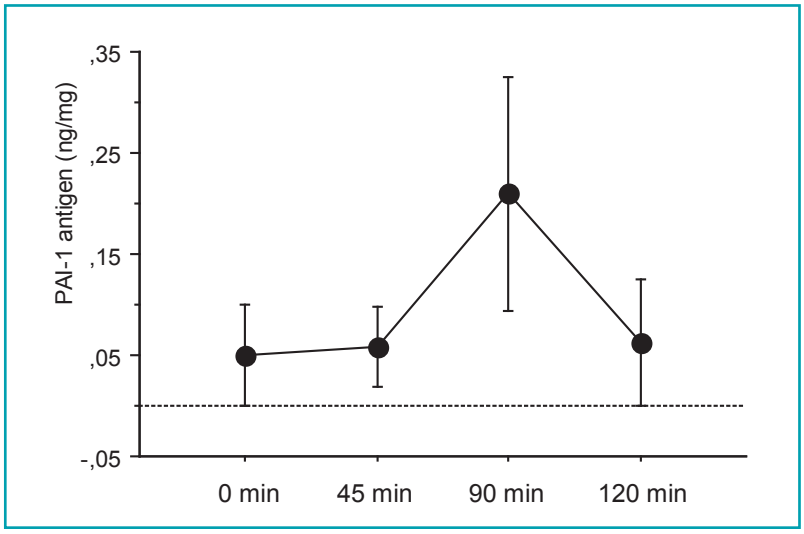

Figure 4 *Peritoneal PAI-1 antigen concentrations (ng/mg protein) measured immediately after initiation of the procedure and every 45 minutes during a laparoscopic gastric bypass.*

DISCUSSION

In the current study we demonstrate that prolonged laparoscopic surgery affects peritoneal biology by decreasing the presence of tPA and its specific activity. Various studies have shown that tPA is an important protein in peritoneal tissue repair processes needed to reduce adhesion formation.[1,2] The enzymatic activity of tPA is a resultant of the amount of tPA antigen and the concentration of its inhibitors, mainly PAI-1. In a free form, tPA is rapidly inactivated by PAI-1, by forming inactive one-on-one complexes.[10] Both the significant decrease in tPA antigen, and the slight, but not significant, increase in PAI-1 antigen expression might have contributed to the decreased peritoneal tPA activity, found in the current study.

A number of studies have demonstrated that the balance between plasminogen activators and inhibitors in the peritoneal cavity is decisive in peritoneal healing processes and subsequent adhesion formation.[2,11-15] Clinical studies on the peritoneal fibrinolytic response to conventional surgery, using sequential biopsies taken during the procedure, showed a progressive reduction in peritoneal tPA antigen and its activity, implying that tPA expression is reduced during conventional surgery.[4,14] Sampling of the peritoneal fluid 24 hours after elective open surgery did also reveal reduced tPA antigen levels.[16] In the current study we have observed that also during prolonged laparoscopic surgery, tPA antigen and its activity levels decrease. This is in accordance with the findings of Neudecker et al. associates who reported a decreased peritoneal tPA activity during laparoscopic colorectal resection.[17] Our results give further support to the hypothesis that the peritoneal fibrinolytic response to conventional and laparoscopic surgery is similar.

In a previous study we noted that short-term laparoscopic surgery did not significantly change the peritoneal expression of proteins from the plasmin system.[9] During laparoscopic cholecystectomy, peritoneal biopsies were taken immediately after insufflation and at completion of the procedure, which meant sampling at a mean of 38 minutes of laparoscopic surgery. These results are in accordance with the current study, where no significant differences in peritoneal expression of fibrinolytic proteins were found in biopsies taken immediately after initiation, compared with those taken after 45 minutes. These observations indicate that a prolonged period of laparoscopic surgery is needed before tPA levels decrease, a finding in contrast with the situation during open surgery, where a rapid decline of tPA levels has been shown in several studies.[2,4,13,14,18,19] This might be due to a less intensive or different peritoneal trauma during la-

paroscopic surgery, compared with open surgery, and supports the idea that laparoscopic surgery is less traumatic for the peritoneal organ.

The reasons for peritoneal hypofibrinolysis during laparoscopic surgery are unidentified. Peritoneal hypofibrinolysis has been suggested to be a marker of peritoneal trauma.[20] Besides the surgical trauma, several specific aspects of laparoscopy may cause damage to the peritoneal layer, including an increased intra-abdominal pressure with peritoneal distension, peritoneal exposure to CO_2, and an intense illumination and cooling of the peritoneal cavity. In a previous study we could not demonstrate any effect of light intensity or intra-abdominal pressure on peritoneal fibrinolysis during laparoscopic cholecystectomy.[9] However, the temperature of the insufflation gas used, increased peritoneal PAI-1 concentrations, indicating that cooling of the peritoneum might be involved.[21] Additionally, the choice of dissection device affected peritoneal transforming growth factor-beta1 expression (TGF-beta1).[22] TGF-beta1 is naturally occurring growth factor and is involved in various biological processes including peritoneal wound healing. It decreases the fibrinolytic activity of human peritoneal mesothelial cells by down-regulation of tPA production and increasing the expression of PAI-1.[23] Further experimental studies are required to elucidate the mechanisms involved in the reduction of peritoneal fibrinolysis during prolonged laparoscopic surgery.

It might be debatable whether the results of the current study in a group of obese patients reflect general surgical practice. However, the same would be true for studies in patients suffering from an inflammatory bowel disease or malignancy, making generalizations difficult. However, to our knowledge, a variation in peritoneal wound healing between an obese and a healthy population has never been described. The same is true for adhesion formation; obesity has never been proposed as a risk factor for peritoneal adhesion formation.

In conclusion, we have demonstrated that prolonged laparoscopic surgery affects the peritoneal fibrinolytic activity by reducing tPA antigen levels and its activity. Further studies are required to enhance the understanding of peritoneal damage and repair during laparoscopic surgery.

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The peritoneal fibrinolytic response to conventional and laparoscopic colonic surgery

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Journal of Laparoendoscopic and Advanced Surgical Techniques 2009 Aug;19(4):489-93

Abstract

Background

Laparoscopic surgery is considered to induce less peritoneal trauma than conventional surgery. The peritoneal plasmin system is important in the processes of peritoneal healing and adhesion formation. The current study assessed the peritoneal fibrinolytic response to laparoscopic and conventional colonic surgery.

Methods

Twenty four patients scheduled for a right colonic resection were enrolled in the trial. Twelve underwent conventional surgery and 12 were operated laparoscopically. Biopsies of the parietal peritoneum were taken at standardized moments during the procedure. Tissue concentrations of tissue-type plasminogen activator (tPA) and its specific activity (tPA activity), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1) were measured using commercial assays.

Results

After mobilisation of the colon, peritoneal levels of tPA antigen and activity were significantly higher in the laparoscopic group ($p<0.005$), due to a decrease in the conventional group ($p<0.05$). At the end of the procedure, the concentrations of tPA antigen and activity significantly ($p<0.05$) decreased in the laparoscopic group to levels comparable with the conventional group. Neither uPA antigen or PAI-1 antigen did change throughout the procedures.

Conclusion

Both conventional and laparoscopic surgery inflict a decrease in tPA antigen and its specific activity. Peritoneal hypofibrinolysis initiates more rapidly during conventional surgery compared to laparoscopic surgery, but at the end of the surgery the effect was the same.

INTRODUCTION

Several lines of evidence indicate that the plasmin system plays a crucial role in peritoneal healing processes following conventional surgery.[1-3] The peritoneum is lined by mesothelial cells which are capable of producing both activators and inhibitors of plasmin, which initiates the degradation of fibrin clots.[4] Tissue-type plasminogen activator (tPA) is the main plasmin activator in the peritoneum, followed by the less potent urokinase-type plasminogen activator (uPA).[1] Both activators are inhibited by plasminogen activating inhibitors, predominantly type 1 (PAI-1). During conventional surgery the peritoneal fibrinolytic activity is rapidly diminished.[1,4-6] This may lead to persistence of fibrin clots that, eventually, may develop into permanent adhesions. We have recently described that, in contrast to short-term laparoscopic surgery,[7] prolonged laparoscopic surgery also causes a decreased fibrinolytic activity in the peritoneum due to decreased tPA levels.[8] Few studies have actually investigated the differences between laparoscopic and conventional procedures. Bergstrom et al. found that during cholecystectomy the concentrations of tPA in peritoneal tissue decline during both conventional and laparoscopic surgery.[9] There was an increase in PAI-1 concentration during laparotomy, but not during laparoscopy. At the end of the operation, there was no difference between the groups. Neudecker et al. have described that peritoneal concentrations and activities of tPA and PAI-1 are similar during laparoscopic and conventional colorectal resections.[10] In that study, however, all patients underwent an initial laparoscopy.
The current study was conducted to clarify the peritoneal fibrinolytic response to both conventional and laparoscopic colonic surgery.

MATERIAL AND METHODS

Twenty-four patients scheduled for a right colonic resection were enrolled in the trial. The choice of operation was based upon the experience of the treating surgeon and the preference of the patient. Institutional Review Board approval was obtained and written informed consent was given before enrolment.

Operative procedure

The conventional procedure was performed through a midline laparotomy. First, the lateral parietal peritoneum was incised and the right hemicolon was mobilised. Then, the terminal ileum and colon where divided using a liniair stapler (GIA™, Autosuture, Gosport, UK). Afterwards the mesocolon was devided and the specimen was removed. The ileum was anastomosed to the colon in a side-to-side manner

using a resorbable monofilament suture (PDS, Johnson & Johnson Medical BV, St-Stevens-Woluwe, Belgium) and the mesocolon was closed. Then, the fascia and skin were closed.
The laparoscopic procedure was started with an open introduction of the first trocar at the umbilicus. The pneumoperitoneum was esthablished and three trocars where placed, two caudal from the umbilicus, one cranial. The colon was mobilised and mesocolon was devided. Subsequently, the colon was brought out through a 5 to 10 cm incision. Extra-abdominal, the terminal ileum and colon where divided using a lineair stapler. Then a side to side anasomosis was performed in a similar way as the conventional procedure. Afterwards the bowel was brought inside the abdomen and the fascia and skin were closed.
Biopsies of the parietal peritoneum were taken at three standardized moments during surgery. The first one was taken at the earliest moment after starting the procedure. The second one was taken after mobilisation of the colon and division of the mesocolon. In the laparoscopic group this implicates just before the minilaparotomy. The last one was taken at the end of surgery.

Tissue sampling and processing

The parietal peritoneum was carefully and sharply dissected, without using electrocautery, 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, each biopsy was cut into smaller pieces and placed into ice-cold phosphate buffered saline (PBS) with 0.5M sodium chloride and 0.01% Triton X-100 (Sigma, St.Louis, MO, USA) to a final concentration of 40 mg tissue/mL buffer. The tissue was homogenized for 60 s on ice using a Polytron homogenizer (Ultra Thurrax IKA T-25, Janke & Kunkel, Staufen, Germany), following centrifugation at 10.000 g for 4 min at 4°C, the supernatant was stored in aliquots at -80°C until further analysis. Tissue processing and assays were done in batches.

Biochemical assays

Determinations of plasminogen activators and inhibitors were done using commercially available assay-kits. Levels of tPA and PAI-1 antigen were assessed using TintElize tPA and PAI-1 (ELISA) kits and tPA activity was measured using a chromogenic assay (Chromolize tPA) from Biopool (Umeå, Sweden). uPA antigen was measured using an ELISA kit from Technoclone (Vienna, Austria). The inter- and intra-assay variation (CV) were for tPA antigen 3.5-5.4% and 4.9-5.5%, for PAI-1 2.4-3.3% and 1.9-2.9%, for tPA activity 5.2-5.3% and 3.9-7.0% and for uPA antigen <10% and <5%, respectively. The lower detection limits were 1.5 ng/mL for tPA antigen, for PAI-1 antigen 0.5 ng/mL, for tPA activity 0.1 IU/mL and for uPA antigen

0.5 ng/mL, according to the instructions from the manufacturer. All results were normalised to total protein content using a commercial protein assay (Bio-RAD, Hercules, California, USA).

Statistical analysis

Data are presented as mean and standard deviation. Two-tailed statistical analysis of differences between groups was performed using the non-parametric Mann-Whitney U test and the Fisher's Exact test. A p-value <0.05 was considered statistically significant.

RESULTS

Clinical results

There was no difference in gender between groups (M : F = 1 : 4). The age of patients was 55 ± 20 years in the laparoscopic group and 72 ± 10 years in the conventional group ($p<0.05$). The indication for surgery was malignancy or dysplastic leasion in 17 patients and inflammatory bowel disease in 7 patients, without significant differences between groups. Furthermore, there were no differences in the incidence of previous laparotomies or the occurrence of intra-peritoneal adhesions during surgery.

Timing of biopsies

In the conventional group the first biopsy was taken immediately after opening the abdomen. Mobilisation of the colon and division of the mesocolon was completed after 26 ± 16 minutes of surgery. The last specimen was taken after 54 ± 16 minutes. In the laparoscopic group the first biopsy was taken immediately after initiating the pneumoperitoneum, the second biopsy after 41 ± 13 minutes of surgery and the last one after 78 ± 12 minutes.

Biochemical results

tPA antigen

During conventional surgery the mean tPA antigen concentration at start of the procedure was 16.9 ng/mg. (figure 1) After mobilisation of the colon the concentration decraesed 45% to 9.0 ng/mg ($p<0.05$) and remained at the same level at the end of surgery.

During laparoscopic surgery the initial concentration was 19.3 ng/mg. The levels did not change during the mobilisation of the colon, but were significantly lower at the end of the procedure (13.4 ng/mg, $p<0.05$).

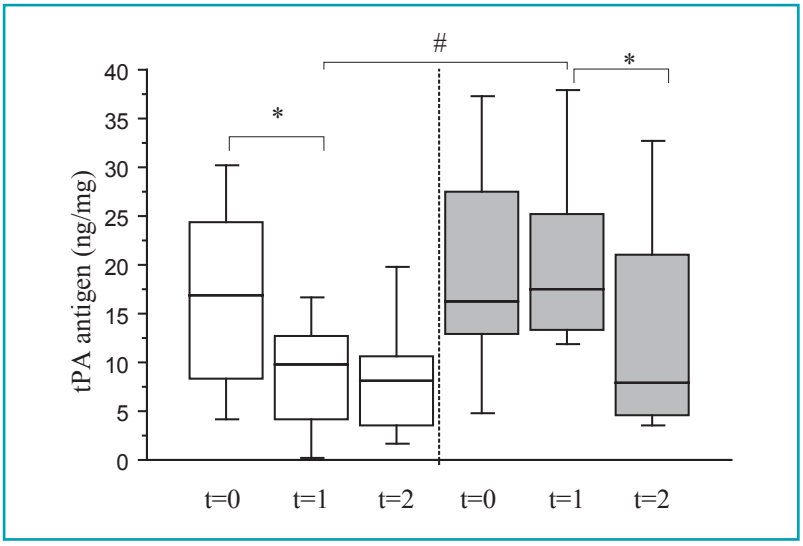

Figure 1 *Tissue-type tissue-type plasminogen activator (tPA) concentration in peritoneal samples taken at the start of surgery (t=0), after mobilisation of the colon (t=1) and at the end of surgery (t=2). Open boxes represent conventional group and closed boxes the laparoscopic group. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). *=p<0.05 and #=p<0.005, respectively.*

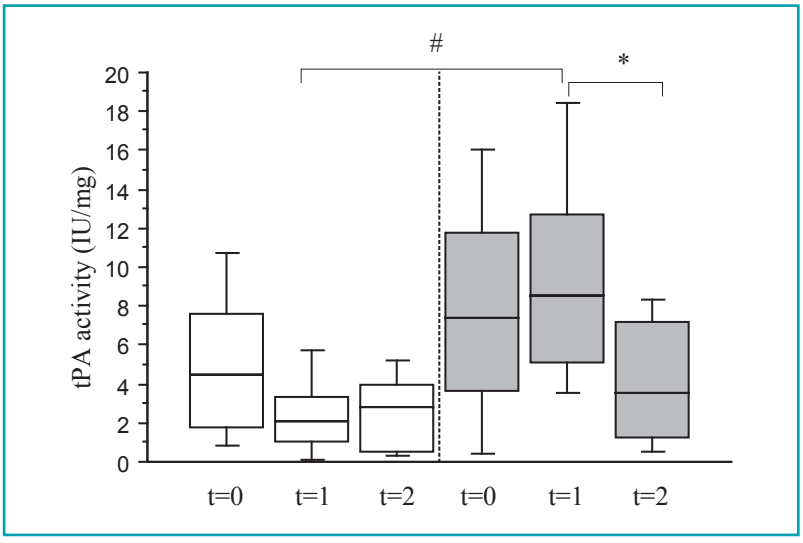

Figure 2 *Functional plasminogen activator (tPA) concentration in peritoneal samples taken at the start of surgery (t=0), after mobilisation of the colon (t=1) and at the end of surgery (t=2). Open boxes represent conventional group and closed boxes the laparoscopic group. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars). *=p<0.05 and #=p<0.001, respectively.*

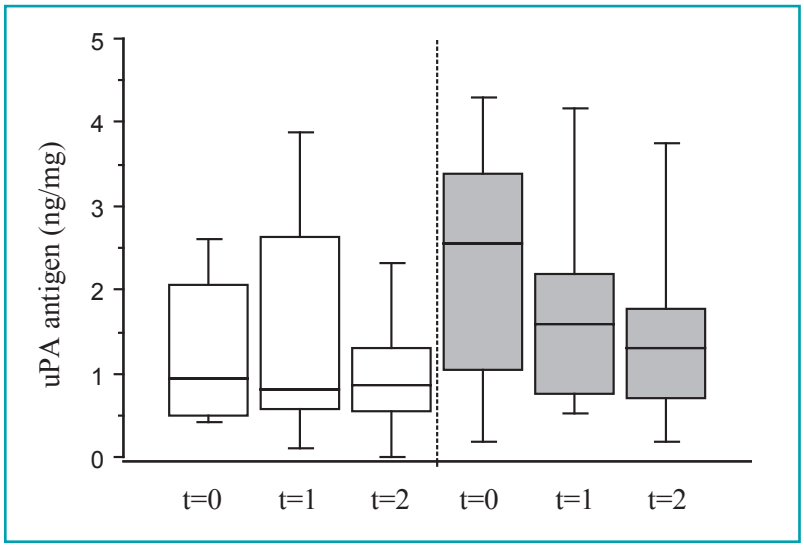

Figure 3 *Urokinase plasminogen activator (uPA) concentration in peritoneal samples taken at the start of surgery (t=0), after mobilisation of the colon (t=1) and at the end of surgery (t=2). Open boxes represent conventional group and closed boxes the laparoscopic group. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

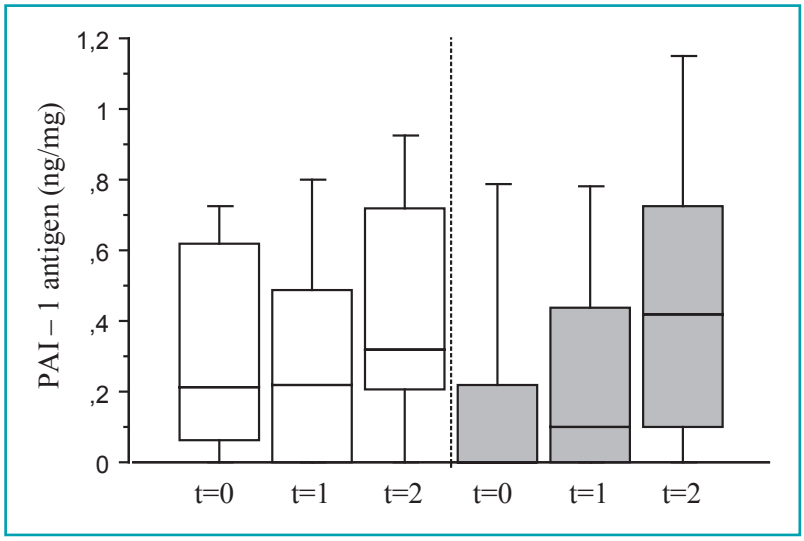

Figure 4 *Plasminogen activator inhibitor type 1 (PAI-1) concentration in peritoneal samples taken at the start of surgery (t=0), after mobilisation of the colon (t=1) and at the end of surgery (t=2). Open boxes represent conventional group and closed boxes the laparoscopic group. Values are median (horizontal line), interquartile range (boxes) and 10th and 90th percentiles (error bars).*

At the start of the procedure there was no significant difference between the two surgical procedures. After mobilisation of the colon the tPA antigen concentration was significantly lower in the conventional group ($p<0.005$). At the end of surgery this difference was abolished.

tPA activity

During conventional surgery the mean tPA activity concentration at start of the procedure was 5.1 IU/mg. (figure 2) During mobilisation of the hemicolon levels decreased over 50% to 2.4 IU/mg ($p=0.09$) and remained at the same level afterwards.

During laparoscopic surgery the peritoneal concentrations of tPA activity were similar at start of the procedure and after mobilisation of the hemicolon (8.10 IU/mg and 9.42 IU/mg, respectively). At the end of the procedure levels had significantly decreased to 4.27 IU/mg ($p<0.05$).

At the start of the procedure there were no differences in concentration of tPA activity between the groups. After mobilisation of the right hemicolon, peritoneal tPA activity was significantly lower in the conventional group compared to the laparoscopic group ($p<0.001$). As for tPA antigen, tPA activity levels at the end of the procedure were similar in both groups.

uPA antigen

During both conventional and laparoscopic surgery peritoneal uPA antigen concentrations did not change throughout the procedure. (figure 3) At all measured time points, there were no differences between the both groups.

PAI-1 antigen

Peritoneal PAI-1 levels did not change during the right hemicolectomy, regardless whether it was performed conventionally or laparoscopically. (figure 4) At none of the measured time points there was any difference in peritoneal PAI-1 levels between both surgical modalities.

DISCUSSION

In the current study we have demonstrated that the peritoneal fibrinolytic response to laparoscopic colonic surgery differs from conventional surgery. Both tPA antigen and its activity decrease more rapidly during conventional surgery. At the end of the procedure, however, peritoneal tPA concentrations were the same.

Various studies have demonstrated that the incidence of postoperative adhesions is lower after laparoscopic resection when compared to conventional surgery.[11-15] A reduced peritoneal fibrinolysis is considered to be an important factor in adhesiogenesis.[2,3,16] Several aspects of laparoscopic surgery might contribute to peritoneal hypofibrinolysis, including intense illumination, cooling of the peritoneum, disse-

cation and peritoneal acidification. All of these are in relation to peritoneal damage and biological dysfunction.

In the current study we did not find a difference in the final concentrations of plasmin activators and inhibitors between conventional and laparoscopic resection. During the procedure, however (i.e. after mobilization of the right hemicolon), concentrations of plasmin activators were significantly higher in the laparoscopic group. The decrease afterwards is this group might have been induced by several factors. First, the prolonged laparoscopic procedure may have induced the reduced tPA concentrations. This hypothesis is supported by a previous study of our group wherein we observed that tPA antigen and its activity progressively decrease during laparoscopic surgery, reaching significant levels after 90 minutes of surgery.[8] Second, tissue handling during the minilaparotomie, performed to complete the resection and anastomosis, may have contributed to the decreased tPA levels. A laparotomy is associated with a rapid decline of tPA antigen and activity.[1,6] Further studies on complete laparoscopic resection, including the construction of the anastomosis, or even during natural orifice transluminal endoscopic surgery, are indicated to clarify this subject.

Results of the current study are in accord with the findings of Neudecker et al[10] Decreased tPA activity levels were observed in their study at the end of both conventional and laparoscopic colonic resection. In contrast to our study, they did not find a difference in peritoneal tPA antigen levels. Since they took specimens only at start and end of the procedure it is unclear if there were any differences between groups at some stage in the procedure. Moreover, their study might have been biased by its design, since all patients underwent an initial laparoscopy in order to assess whether a laparoscopic resection was feasible. Tarhan et al. have recently decribed the fibrinolytic changes in peritoneal fluid after a cholecystecomy and observed similarities in conventional and laparoscopic cholecystectomies with regard to tPA activity and PAI-1 levels.[17]

The timing of the biopsies is an issue that should be addressed. In the current study we choose to take biopsies at specific moments during the operation. Generally, laparoscopic surgery is taking more time than conventional surgery. This was also true in our study. The second biopsy and third biopsy were taken approximately ten and twenty minutes later in the laparoscopic group. Increased operation time is associated with a progressive decrease or both tPA antigen and activity.[1,2,8] Thus, one might have expected lower tissue levels of tPA in the group operated laparoscopically. However, the opposite was true; at completion of the strictly laparoscopic part of the procedure, levels of both tPA antigen and its activity were still significantly higher in the laparoscopic group, when compared to the group operated conventionally. This supports the concept that laparoscopic surgery is less trauma-

tic for the peritoneum compared to conventional surgery. This timing issue might have been overcome by another study design wherin biopsies are taken at specific time points during the procedures. That design, however, would have biased the results due to the diverse extent of tissue trauma at each time point.
In the current study, groups were clinically identical except for the age of patients. The age was significantly higher in the conventional group. This difference, however, does not seem to have affected the biochemical results of the current study. An age-related change in peritoneal fibrinolysis has never been described or hypothesised. Moreover the occurrence of peritoneal adhesions does not seem to change due to age.
In conclusion, we have demonstrated that both conventional and laparoscopic surgery inflict a decrease in tPA antigen and its specific activity. The peritoneal hypofibrinolysis initiates more rapidly during conventional surgery compared to laparoscopic surgery, but at the end of the surgery, the effect was the same.

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Summary and Future Directions

SUMMARY

Laparoscopic surgery has been incorporated in common surgical practice.In similarity with open surgery, laparoscopic surgery may affect both the integrity and biology of the peritoneum. The peritoneum is an organ with various biological functions that may be influenced by laparoscopic and open techniques in different ways. In *chapter 2* an overview is given on the effects of laparoscopic surgery on peritoneal biology. A literature search identified articles focusing on the key issues laparoscopy, peritoneum, inflammation, morphology, immunology and fibrinolysis using the Pubmed and Cochrane databases. Laparoscopic surgery was shown to induce alterations in the peritoneal integrity and to cause local acidosis, probably due to peritoneal hypoxia. The local immune system and inflammation are modulated by pneumoperitoneum and the peritoneal plasmin system is inhibited leading to peritoneal hypofibrinolysis.It was concluded that, in similarity with open surgery, laparoscopic surgery affects both the integrity and biology of the peritoneum. These observations may have implications for various clinical conditions.

Peritoneal fibrinolysis is important in peritoneal wound healing processes and adhesion formation. The peritoneal fibrinolytic response to laparoscopy is merely unknown. In *chapter 3* we have studied the effect of short-term laparoscopy on the peritoneal fibrinolytic response and the influence of intra-abdominal pressure, light intensity and choice of dissection device on this response. Fifty patients that were scheduled for laparoscopic cholecystectomy were randomised in five groups operated with various pressures, light intensities and dissection devices. Peritoneal biopsies were taken at the beginning and the end of the procedure. Tissue concentrations of tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA),plasminogen activator inhibitor type 1 (PAI-1) and the tPA activity were measured using ELISA techniques. There were no differences in tPA antigen, tPA activity, uPA antigen or PAI-1 antigen concentrations in biopsies taken at the beginning compared to samples taken at the end of the operation. Different intra-abdominal pressures, light intensities and the choice of dissection device did not affect any of the measured parameters. It was concluded that short-term laparoscopy does not affect the peritoneal fibrinolytic activity. The used intra-abdominal pressure, light intensity and choice of dissection device did not affect peritoneal activity during short-term laparoscopy.

Cooling of the peritoneum, due to insufflation during pneumoperitoneum, is another aspect of laparoscopic surgery that might traumatize the peritoneum and disturb peritoneal fibrinolysis. In *chapter 4* we have studied the effect of the tem-

perature of insufflation gas on the peritoneal fibrinolytic response to laparoscopic surgery. Thirty patients that were scheduled for laparoscopic cholecystectomy were randomised in two groups. In one group the pneumoperitoneum was created with carbon dioxide at room temperature, and in the other carbon dioxide at body temperature was used. Again, peritoneal biopsies were taken at the start and end of surgery and tissue concentrations of tPA antigen, tPA activity, uPA antigen and PAI-1 antigen were measured using ELISA techniques. Peritoneal PAI-1 antigen levels were significantly higher at the end of the procedure in patients operated with carbon dioxide at room temperature ($p<.05$). A slight, but not significant, decrease in tPA antigen and activity was observed in both groups during the procedure. Peritoneal concentrations of uPA antigen did not change during the procedure. Increased PAI levels have been associated with an increased post surgical adhesion formation. High PAI concentrations are found in adhesions and peritoneal tissue of patients with extensive adhesions. Accordingly, PAI-1 is considered to be an important factor in peritoneal tissue repair. The observation that peritoneal PAI-1 levels are lower in patients operated with heated carbon dioxide might indicate that cooling of the peritoneum traumatizes the peritoneal layer, leading to decreased fibrinolytic activity.

Transforming growth factor beta 1 (TGF-beta1) is a growth factor involved in various biological processes, including peritoneal wound healing and dissemination of malignancies. It is unknown if laparoscopic surgery affects the peritoneal TGF-beta1 expression. In *chapter 5* we have studied the peritoneal TGF-beta1 expression during laparoscopic surgery. As in chapter two, 50 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 and tissue concentrations of total and active TGF-beta1 were measured using ELISA techniques. 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 patients operated with low light intensity. It was concluded that 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. Since TGF-beta1 is involved

in various biological processes in the peritoneal cavity, this observation may have important clinical consequences.

In the studies described in chapters 3, 4 and 5, the overall concentrations of fibrinolytic enzymes and TGF-beta1 did not change during the laparoscopic cholecystectomy. The operating time of a laparoscopic cholecystectomy, however, might have been too short to detect significant differences at the protein level. Additional studies on prolonged endoscopic procedures were required to further elucidate the possible effects of endoscopic surgery. In *chapter 6*, we describe the results of a study performed to assess the effects of prolonged laparoscopic surgery on peritoneal fibrinolysis. Twelve consecutive patients undergoing laparoscopic gastric bypass surgery for morbid obesity were included in the study. During the procedure biopsies of the parietal peritoneum were taken at the start of the procedure and each 45 minutes afterwards. Tissue samples were homogenized and tPA antigen, tPA activity, uPA antigen and PAI-1 antigen were measured using commercial assay techniques. Both tPA antigen and its activity progressively decreased during the procedure, reaching significant levels after 90 minutes of surgery. The levels of uPA antigen and PAI antigen did not significantly change throughout the procedure. It was concluded that, as for conventional surgery, prolonged laparoscopic surgery causes a decreased fibrinolytic activity in the peritoneum due to decreased tPA levels.

To evaluate differences in the peritoneal fibrinolytic response to laparoscopic and conventional colonic surgery we performed a study wherein twenty four patients scheduled for a right colonic resection were enrolled (*chapter 7*). Twelve patients underwent conventional surgery and 12 were operated laparoscopically. Biopsies of the parietal peritoneum were taken at standardized moments during the procedure. Tissue concentrations of tPA and its specific activity (tPA activity), uPA and PAI-1 were measured using commercial assays. After mobilization of the colon, peritoneal levels of tPA antigen and activity were significantly higher in the laparoscopic group ($p<0.005$), due to a decrease in the conventional group ($p<0.05$). At the end of the procedure, the concentrations of tPA antigen and activity significantly ($p<0.05$) decreased in the laparoscopic group to levels comparable with the conventional group. Neither uPA antigen or PAI-1 antigen did change throughout the procedures. In conclusion, both conventional and laparoscopic surgery inflict a decrease in tPA antigen and its specific activity. Peritoneal hypofibrinolysis initiates more rapidly during conventional surgery compared to laparoscopic surgery, but at the end of the surgery the effect was the same.

FUTURE DIRECTIONS

In the studies, presented in this thesis, we have shown that laparoscopic surgery has a clear effect on peritoneal biology. Whereas short-term laparoscopy did not affect the fibrinolytic response, long-term laparoscopy did. Moreover, the temperature of carbon dioxide, used to establish a pneumoperitoneum, affected peritoneal concentrations of PAI-1. During open abdominal surgery, the peritoneal hypofibrinolysis appeared to initiate more rapidly, when compared to laparoscopic surgery, emphasizing the minimal invasive character of laparoscopic surgery. Minimizing trauma to the peritoneum should be the goal for further research. With regard further minimalisation of surgical trauma, the NOTES technique seems a promising technique, but both comparative and pathophysiological studies are indicated.

The effects of laparoscopic surgery on the peritoneal fibrinolytic response may have been induced by several pathways. Elucidating the role of each of these pathways and the search for possible interventions are challenges for further studies. First, the abdominal temperature seems of importance, as was suggested in chapter 4. A decreased temperature has been shown to inhibit the plasmin system and affect local cytokine concentrations. Second, an intense illumination of the peritoneum may affect the peritoneum, either directly or indirectly by causing local dissecation. Local TGF-beta1 levels were affected by the intensity of light. Other possible components are the intra-abdominal pressure, duration of procedure, choice of dissection devices, dissecation and the insufflation gas. CO_2 is the most frequently used insufflation gas. CO_2 has been shown to affect the peritoneal morphology and to cause local hypoxia leading to acidosis. Moreover it affects the local inflammatory response and disturbs peritoneal fibrinolysis. Other gases, such as helium or air, do not always induce similar effects. Additional studies are indicated to elucidate the individual effects of all described components.

The effects of laparoscopy on peritoneal integrity and biology may have various clinical repercussions. Although the incidence of adhesion formation appears to be reduced following laparoscopic surgery, adhesion formation remains a major concern. Efforts to reduce their occurrence are indicated, since they may cause severe and life-threatening complications such as small bowel obstruction and require further studies. There is considerable evidence that the plasmin system plays a key role in the pathophysiology of intra-abdominal adhesions. Clinical studies on the peritoneal fibrinolytic response to conventional surgery, using sequential biopsies taken during the procedure, have shown a progressive peritoneal hypofibrinolysis. During laparoscopic procedures, a prolonged period of surgery was needed before

tPA levels decreased, a finding in contrast with the situation during open surgery, where a rapid decline of tPA levels was found. This might be due to a less intensive or different peritoneal trauma during laparoscopic surgery, compared with open surgery. These observations warrant further research focusing on various components of laparoscopic and open surgery and their consequences on peritoneal healing processes.

Abdominal surgery is frequently performed for malignant diseases. Despite the fact that dissemination to the peritoneum is not exceptional, mechanisms of action have not been fully elucidated. Only when the mesothelial cell layer has been breached, tumor cells may infiltrate and proliferate within the submesothelial connective tissue matrix. The early bulging of mesothelial cells during laparoscopic procedures may facilitate this phenomenon. Experimental studies have suggested a prominent role of neutrophils in the occurrence of peritoneal metastasis. Others have proposed that tumor-conditioned media or exogenous inflammatory cytokines may induce mesothelial cells retraction and disaggregation to gain access to the submesothelial connective tissue. Studies focusing on the effects of laparoscopic surgery on the expression of these adhesion molecules are therefore indicated.

The peritoneal effects of laparoscopic procedures may also be important in infectious diseases, such as a perforated appendicitis or diverticulitis. A laparoscopic peritoneal lavage is frequently performed under these circumstances. Animal studies have suggested that the microbial adherence to the peritoneum is high, which may negatively affect the results of the lavage. Whether the effects of laparoscopic surgery on peritoneal biology affect this microbial adherence either positively or negatively, when compared to open lavage, remains to be investigated. This may be clinically important since lavage-resistant bacteria may be a source of persistent infection and might cause, through translocation, septic complications.

In conclusion, laparoscopic surgery affects the integrity of the peritoneum and its biological activity. Information about separate components of laparoscopic surgery and their specific effects are the challenges for the future.

SAMENVATTING

Samenvatting

In de huidige chirurgische praktijk is laparoscopie, ofwel sleutelgatchirurgie, inmiddels niet meer weg te denken. Bij zowel conventionele, open chirurgie als laparoscopische chirurgie wordt het peritoneum beschadigd. Er ontstaan verschillende biologische reacties, die ertoe bijdragen dat de continuïteit van het peritoneum weer wordt hersteld. Deze reacties zouden bij laparoscopische ingrepen anders kunnen zijn dan bij open chirurgie.
In *hoofdstuk 2* wordt een overzicht gegeven van alle bekende reacties van het peritoneum op laparoscopische chirurgie. In de Pubmed en de Cochrane databases is gezocht met de sleutelwoorden: "laparoscopy", "peritoneum", "inflammation", "morphology", "immunology" en "fibrinolysis".
Laparoscopische chirurgie geeft een verandering van de cellulaire integriteit van het peritoneum en veroorzaakt lokale acidose, waarschijnlijk door hypoxie van het peritoneale weefsel. Het immuunsysteem en de ontstekingsreactie op peritoneaal niveau worden beïnvloed door het aanleggen van het pneumoperitoneum. Daarnaast wordt de peritoneale fibrinolyse verstoord. In het tweede hoofdstuk wordt geconcludeerd dat laparoscopische chirurgie, net als open chirurgie, de integriteit en de biologie van het peritoneum beïnvloedt.

De peritoneale fibrinolyse is van belang voor de genezing van het peritoneum en is gerelateerd aan de vorming van postoperatieve verklevingen. Wat de invloed is van laparoscopische chirurgie op de fibrinolytische respons is onbekend. In *hoofdstuk 3* wordt een studie beschreven, waarbij de invloed van de intra-abdominale druk, lichtintensiteit en het gebruik van ultracision dan wel elektrochirurgie op de peritoneale fibrinolyse onderzocht is. Daartoe is een groep van vijftig patiënten, die een laparoscopische cholecystectomie moesten ondergaan, verdeeld in vijf groepen. Er is gevariëerd met druk, lichtintensiteit en het gebruik van ultracision of elektrochirurgie. Aan het begin en einde van de procedure zijn biopten genomen van het peritoneum. Na homogenisatie zijn hierin de concentraties van tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1) en de tPA activiteit bepaald. In deze studie zijn er geen significante verschillen in de concentraties tPA antigen, tPA activiteit, uPA antigen en PAI-1 antigen waargenomen tussen de biopten, genomen aan het begin en aan het einde van de ingreep. Ook de verandering van druk, lichtintensiteit, danwel keuze van het dissectie instrument lieten geen veranderingen zien. Er is geconcludeerd dat bij kortdurende laparoscopische ingrepen het verschil van druk, licht en gebruikt chirurgisch instrument geen invloed heeft op de peritoneale fibrinolytische respons.

Door het aanleggen van het pneumoperitoneum vindt een afkoeling plaats van het peritoneum door het gebruikte koolstofdioxidegas. Deze afkoeling zou van invloed kunnen zijn op de locale fybrinolytische activiteit. In *hoofdstuk 4* is deze relatie bestudeerd. Dertig patiënten die een laparoscopische cholecystectomie moesten ondergaan, zijn gerandomiseerd in twee groepen. In een groep werd koolstofdioxidegas gebruikt op kamertemperatuur en bij de andere groep werd het pneumoperitoneum aangelegd met koolstofdioxidegas op lichaamstemperatuur. De eerder genoemde parameters werden bepaald in de biopten van het peritoneum. Er is een significant hogere peritoneale PAI-1 antigen concentratie gevonden in de biopten genomen aan het einde van de procedure bij het gebruik van koolstofdioxidegas op kamertemperatuur, vergeleken met de groep geopereerd met gas op lichaamstemperatuur ($p<0,5$). Verhoogde PAI-1 antigen concentraties worden geassocieerd met een toegenomen adhaesievorming. Hoge PAI-1 concentraties zijn gevonden in het peritoneum bij patiënten met verklevingen. De gevonden lage peritoneale PAI-1 concentraties bij de groep van patiënten die geopereerd zijn met verwarmd koolstofdioxidegas zouden kunnen betekenen dat koeling van het peritoneum de peritoneale cellen beschadigt, waarbij de fibrinolytische activiteit daalt.

Transforming growth factor beta1 (TGF-beta1) is een groeifactor betrokken bij verschillende biologische processen, waaronder de genezing van het peritoneum en verspreiding van eventuele tumorcellen. Het is onbekend of laparoscopische chirurgie de peritoneale TGF-beta1 expressie beïnvloedt. In *hoofdstuk 5* wordt deze relatie onderzocht. Vijftig patiënten die een laparoscopische cholecystectomie moesten ondergaan zijn gerandomiseerd in vijf groepen, waarbij is gevariëerd met druk, lichtintensiteit en het gebruikte chirurgische instrument. Aan het begin en aan het einde van de procedure werden biopten genomen, waaruit de totale en actieve TGF-beta1 concentraties werden bepaald. Patiënten die geopereerd waren met ultracision hadden significant lagere peritoneale concentraties van totale ($p<0,005$) en actieve TGF-beta1 ($p<0,01$) in vergelijking tot de patiënten die geopereerd waren met elektrochirurgie. Tevens was in de groep patiënten geopereerd met een hogere lichtintensiteit de totale TGF-beta1 concentratie lager ($p<0,005$), terwijl de actieve TGF-beta1 concentratie gelijk bleef ten opzichte van de groep met de lagere lichtintensiteit. Geconcludeerd is dat de keuze van het chirurgisch instrument en de gebruikte lichtintensiteit van invloed is op de peritoneale TGF-beta1 concentraties, hetgeen impliceert dat de peritoneale biologie wordt beïnvloed door laparoscopische chirurgie. Gezien het feit dat TGF-beta1 betrokken is bij verschillende biologische processen zou deze observatie klinische consequenties kunnen hebben.

In de studies beschreven in hoofdstukken 3, 4 en 5 waren de concentraties van de fibrinolytische enzymen en TGF-beta1 aan het begin van de ingreep niet anders dan die aan het einde van de laparoscopische cholecystectomie. De operatieduur zou in het geval van een laparoscopische cholecystectomie echter te kort kunnen zijn om significante verschillen van de enzymen te kunnen detecteren. Om die reden werd in de studie, beschreven in *hoofdstuk 6*, de peritoneale fibrinolytische reactie onderzocht bij langdurige laparoscopische ingrepen. Twaalf patiënten die een laparoscopische gastric bypass moesten ondergaan vanwege morbide obesitas zijn hiervoor geïncludeerd. Er zijn aan het begin van de procedure en daarna om de 45 minuten biopten van het peritoneum genomen. tPA antigen, tPA activiteit, uPA antigen and PAI-1 antigen zijn wederom bepaald in deze biopten. tPA antigen en tPA activiteit daalden gedurende de procedure, waarbij een significant verschil werd aangetoond na 90 minuten. Geconcludeerd is, dat langdurige laparoscopische chirurgie de fibrinolytische activiteit van het peritoneum vermindert, door een dalende tPA expressie. Dit is in overeenstemming met de conventionele, open chirurgie, hoewel de reactie daar snel optreedt.

Om eventuele verschillen tussen laparoscopische en conventionele chirurgie aan te tonen, wordt in *hoofdstuk 7* een studie beschreven waar 24 patiënten die een hemicolectomie rechts ondergingen zijn geïncludeerd. De helft is open geopereerd en de andere helft laparoscopisch. Biopten van het peritoneum werden op gestandaardiseerde momenten genomen, waaruit tPA antigen, tPA activiteit, uPA antigen en PAI-1 antigen zijn bepaald. Na het mobiliseren van het colon was het tPA antigen en de tPA activiteit significant hoger in de laparoscopische groep ($p<0,005$). Dit is met name veroorzaakt door een daling van tPA antigen en tPA activiteit in de open groep. Aan het einde van de procedure waren de concentraties van tPA antigen en tPA activiteit in de laparoscopische groep ook significant gedaald ($p<0,05$) tot het niveau vergelijkbaar met de open groep. uPA antigen en PAI-1 antigen concentraties veranderden niet gedurende de procedure. Er is geconcludeerd dat zowel conventionele chirurgie als laparoscopische chirurgie een daling geven van de peritoneale concentratie van tPA antigen en tPA activiteit. De peritoneale hypofibrinolyse begint echter eerder tijdens open chirurgie in vergelijking tot de laparoscopische chirurgie.

DANKWOORD

DANKWOORD

Het is volbracht. Mijn proefschrift is klaar. Dit was nooit gelukt zonder de steun van anderen. Het is onmogelijk iedereen te noemen die een bijdrage heeft geleverd aan dit proefschrift, maar ik zou een aantal mensen in het bijzonder willen bedanken.

Dr. M.M.P.J. Reijnen, beste Michel,
Wie had het 20 jaar geleden gedacht? We begonnen tegelijk aan de studie Geneeskunde in Nijmegen. Ik elke dag fluitend naar college en jij je onnoemelijk ergerend aan dat frivole gedoe in de ochtend. Nu ben je mijn co-promotor en hebben we het mooi klaargespeeld.
Hoe je het vol hebt gehouden, weet ik niet. Maar dit proefschrift is er door jouw onvoorwaardelijke steun en enthousiasme gekomen. Een artikel, dat ik naar je stuurde, kreeg ik de volgende dag met nieuwe voorstellen terug. Door jouw overzicht van de materie, verzandde ik niet in oeverloze discussies. Van jouw manier van zaken verwoorden, heb ik veel geleerd en ik hoop dit te kunnen doorgeven. Dank hiervoor. Vanaf nu gaan we gewoon een biertje drinken, zonder steeds "het onderzoek" als onderwerp.

Dr. J.H.G. Klinkenbijl, beste Jean,
In 2000 begon ik bij jou de opleiding tot chirurg. Tijdens ons kennismakingsgesprek heb ik me cynisch uitgelaten over onderzoek. Ik wilde met name het snijden goed leren en dacht dat wetenschap maar bijzaak was.
In de 6-jarige opleiding heb jij me laten zien dat het vak meer inhoud heeft dan alleen maar "snijden". Inmiddels ben ik chirurg en heb ik een goede basis meegekregen om mijn kennis steeds verder uit te bouwen. Dit proefschrift is niet de afronding hiervan, maar een nieuwe basis. Dank daarvoor.

Prof. Dr. I.H.M. Borel Rinkes, beste Inne,
Vanaf het moment dat we elkaar ontmoetten, had ik het idee dat het proefschrift er inderdaad wel zou komen. Je liet me vrij in de tijdsplanning, maar toch zat je achter m'n broek aan. Op een congres was de zin: "ik zie de stukken wel komen, ik twijfel er niet aan" een vaste uitspraak. Het is gelukt en veel dank dat je mijn promotor hebt willen zijn.

Naast mijn promotor en co-promotoren ben ik uiteraard dank verschuldigd aan de beoordelingscommissie van dit proefschrift, voor de snelle en vakkundige beoorde-

ling: Prof. dr. F.L. Moll, Prof. dr. Ph.G. de Groot, Prof. dr. L.M.A. Akkermans, Prof. dr. I.A.M.J. Broeders, Prof. dr. H.J. Bonjer en Prof. dr. J.L.H.R. Bosch, ik dank u.

Lena Holmdahl, Peter Falk, Maria Bergström and Marie-Lois Ivarsson,
Thank you for all the work you have done besides your daily job in the lab. I am proud to show you this thesis.

Alle stafleden, (oud-)assistenten en OK-medewerkers van het Ziekenhuis Rijnstate. Jullie hebben ervoor gezorgd dat de biopten bij de geplande laparoscopische procedures (op tijd) werden genomen en direct konden worden ingevroren. Ik besef dat het storend werkte op de normale procedure, maar uiteindelijk heeft het toch tot iets moois geleid!

Beste maten,
Dank dat jullie mij de tijd hebben gegeven om dit proefschrift uiteindelijk te kunnen afronden. We gaan binnenkort een mooie, bloeiende chirurgische praktijk neerzetten in een nieuwe jas.

Fleur Bominaar,
Dank voor de hulp bij het vormgeven van dit proefschrift. Ik waardeer je geduld. Door jouw sturende bijdrage zijn het toch mijn beslissingen geworden en daardoor mijn eigen boekje.

Drs. R.B.G. Brokelman, beste Roy, broer en paranymf,
Dank voor de vele telefoongesprekken 's ochtends rond de klok van 7 uur, tijdens onze reis naar het werk. Het is mooi dat we allebei ons plekje in de maatschap hebben gevonden. Het is je niet gelukt om "me in te halen". Fysiek niet, omdat je elke dag naar Harderwijk rijdt, maar ook op professioneel vlak niet. Immers, jij koos voor de orthopedie en je promotiedatum moet je nog aanvragen. Dank dat je vandaag aan mijn zijde wilt staan.

Mr. J.H.M. ter Haar, beste Hans, vriend en paranymf,
Enkele jaren geleden zaten we op de Grote Markt in Groningen, op een druilerige zondagmiddag. Ook jij had een promotietraject voor jezelf uitgestippeld. Door dit meerjarenplan destijds aan mij duidelijk te maken, heb je mij laten zien hoe het eigenlijk moet. Om het doel te bereiken, moet je af en toe dingen doen die minder leuk zijn op dat moment, die minder goed bij je passen. In de jaren daarna maakten de gesprekken die ik met je voerde, dat ik op mijn ingeslagen weg door kon gaan.

Het heeft onze vriendschap verder verdiept. Dank hiervoor, en uiteraard ook dank dat je vandaag aan mijn zijde wilt staan.

Dank ook aan mijn ouders,
Zij hebben de basis gelegd voor hoe ik nu ben als persoon. “Vergett oe hoesnummer nie”, is de gevleugelde uitspraak van mijn vader.

Tenslotte, lieve, lieve Joyce,
Jij bent diegene met wie ik de weg samen bewandel. Het in opleiding komen, de onzekerheden tijdens de opleiding, het vinden van een goede werkplek en uiteindelijk de promotie. Altijd kan ik bij je terecht om mijn twijfels te bespreken. Jij maakt dat ik me steeds verder kan ontwikkelen.
Samen met Koen gaan we een toekomst tegemoet, waar ik ontzettend veel zin in heb. Ik hou van je.

Curriculum Vitae

Curriculum Vitae

Walter Johannes Antonius Brokelman werd op 20 augustus 1971 geboren in Enter, gemeente Wierden. Hij groeide op in het dorp Maria Parochie en behaalde zijn VWO-diploma aan het Pius X College te Almelo. In 1989 begon hij aan de studie Geneeskunde aan de Radboud Universiteit te Nijmegen. Zijn eerste baan als assistent chirurgie was in het St. Jansdal te Harderwijk in 1996.

Vanaf 1997 heeft hij twee jaar gewerkt als transplantatiecoördinator in het Academisch Ziekenhuis te Groningen. Vervolgens ging hij werken in het Elisabeth Ziekenhuis in Tilburg.

In 2000 begon hij zijn opleiding tot chirurg in het Ziekenhuis Rijnstate te Arnhem, onder leiding van dr. J.H.G. Klinkenbijl. Van 2003 tot 2005 doorliep hij zijn academische deel van de opleiding in het Radboud Ziekenhuis, met als opleider prof. dr. R.P. Bleichrodt. In 2006 rondde hij zijn opleiding tot chirurg af.

Hij is daarna een jaar werkzaam geweest als fellow laparoscopische chirurgie in het Ziekenhuis Rijnstate, waarna hij in 2007 begon als chef de clinique in het Jeroen Bosch Ziekenhuis te 's-Hertogenbosch. In maart 2008 is hij toegetreden tot deze maatschap, alwaar hij nu werkt als chirurg met aandachtsgebied laparoscopische chirurgie.

Hij woont samen met Joyce van Pul en zij hebben een zoon, Koen.