Influence of Peritoneal Trauma on Postoperative Adhesion Formation and Intra-Abdominal Tumour Recurrence

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Influence of Peritoneal Trauma on Postoperative Adhesion Formation and Intra-Abdominal Tumour Recurrence

Invloed van Peritoneaal Trauma op
Postoperatieve Adhesie Vorming en Intra-
Abdominale Tumor Recidivering

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General Introduction and Aims of the Thesis

Chapter I

General Introduction

Adhesions

Definition, incidence and complications

Intra-abdominal adhesions are abnormal unions between different peritoneal surfaces. The most common cause of intra-abdominal adhesion formation is prior abdominal surgery which accounts for 70–90% of all adhesions.[1-3] Remaining adhesions are due to inflammation or endometriosis or are congenital (18% and 2.8-6% respectively).[1, 3] Postoperative intra-abdominal adhesion formation is a major, up till now unavoidable complication of any kind of abdominal surgery. Postoperative adhesions occur in 55-100% of patients undergoing abdominal surgery, with an average of approximately 85%.[2, 3, 5-7] These adhesions can eventuate in bowel, fertility and abdominal syndromes which frequently require further surgery. Adhesion formation is the cause of 30% of all bowel obstructions,[8-10] of 15-20% of infertility in women[11-15] and of 13-26% of chronic pelvic pain in women.[16, 17] The cumulative risk of adhesive small-bowel obstruction after (sub)total colectomy is 11% within 1 year, increasing to 30% at 10 years.[18] After open abdominal or pelvic surgery in 29,790 patients studied by Ellis et al, 34.6% were readmitted a mean of 2.1 times in the subsequent 10 years for a disorder directly or possibly related to adhesions. Of these readmissions 22.1% occurred in the first year after initial surgery, but readmissions continued steadily throughout the 10 year study period.[19] About 1% of all surgical admissions and 3% of all laparotomies take place for intestinal obstruction due to adhesions.[7] The mortality rate from intestinal obstruction as a result of adhesions is 6-13%.[1, 4, 20] Adhesions also increase the technical difficulty and the risk of intraoperative complications at subsequent surgery depending on the organs involved and severity of adhesions. One of five patients undergoing adhesiotomy during reoperation suffers an inadvertent enterotomy, possibly resulting in extensive postoperative morbidity and mortality. Morbidity involves bowel obstruction, anastomotic leak, wound dehiscence, sepsis and pneumonia.[21] Clearly in addition to the obvious morbidity and mortality due to postoperative adhesion formation the costs of adhesion related health care are significant.[19, 20, 22-24] Until now there is no clinically relevant cost effective method available to reduce or prevent postoperative intra-abdominal adhesion formation.

Clinical diagnosis

Intra-abdominal adhesions cannot be shown by non-invasive modalities. The combination of medical history and physical examination fail to adequately predict adhesion formation and invasive as well as non-invasive imaging studies have poor sensitivity for detecting adhesions.[25-27] In general, the presence of adhesions can only be confirmed by either laparoscopy or laparotomy.

Aetiology and pathophysiology

Adhesion formation is a physiological consequence of peritoneal tissue repair. Surgical trauma of the peritoneal surfaces induces a sequence of events which effectuates wound healing but which can also ultimately lead to fibrous adhesions between different peritoneal surfaces. Damage to the peritoneum causes desquamation of injured mesothelial cells leaving a denuded area, and a local inflammatory reaction which leads to the formation of a serosanguineous exudate.[28-31] The primary inflammatory response, the acute phase response, is characterised by an increased vascular permeability and the migration of neutrophils (PMNs) and subsequently macrophages and leucocytes to the site of the inflammation during the first 48 to 72 hours after trauma.[32-34] Chemoattractants (IL-8, MCP-1), cytokines (TNF-α, IL-1β and IL-6) and growth factors (TGF-β, IGF-I, PDGF), produced and released by damaged mesothelial cells and resident and invading inflammatory cells are the key mediators of this inflammatory reaction.[35-39] In the resulting serosanguineous exudate fibrin deposition will take place through the activated complement- and coagulation systems and when two surfaces contact each other during this process a fibrinous adhesion will appear between day 2 and 4.[30, 31, 40] Whether these adhesions are permanent of character or will eventually be lysed depends upon the fibrinolytic capacity of the peritoneum.[30, 41-44] Intra-abdominally deposited fibrin has to be lysed to free attached structures, restore the patency of the peritoneal cavity and open up the way to adhesion free healing. When the peritoneum is slightly damaged and mesothelial cells are mostly intact, there will be a dynamic balance between fibrinogenesis and fibrinolysis, between tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitors (PAIs).[41, 42, 45, 46] Adhesion-free healing may then take place by conversion of plasminogen into plasmin effectuating the sufficient break-down of fibrin by day 4 to 5 followed by proliferation and centripetal migration of residual mesothelial cells and proliferation and differentiation of interspersing mesothelial stem cells.[28, 32, 47-49] Reepithelialisation occurs through continuous simultaneous dividing of adjacent mesothelial cells and "islands" of multipotential sub-mesothelial cells until the surface of the entire site of injury is covered by new mesothelium 5 to 8 days after the initial peritoneal trauma.[28, 32, 48] The remesothelialisation process appears to be mostly the result of mediators released by the mesothelial cells themselves.[50]

Loss of mesothelial integrity due to the initial peritoneal trauma and invading leucocytes, which have been shown to enhance peritoneal injury by adhering to and damaging mesothelial cells through the release of active oxygen species,[51-54] will expose the underlying connective tissue (ECM) and normal mesothelial fibrinolytic activity will be lost for at least 48 hours post trauma.[43, 55, 56] Exposed endothelial cells, damaged mesothelial cells, as well as resident and invading inflammatory cells, produce plasminogen activator inhibitor 1 and 2 (PAI 1 and 2), factors mediating a reduced functional fibrinolytic activity by down-regulating t-PA activity and thus plasminogen-plasmin conversion and fibrin demolishment.[42, 57] Cytokines and growth factors, also produced by damaged mesothelial cells and inflammatory cells, decrease t-PA and increase PAI concentrations intra-peritoneally diminishing fibrinolytic activity of the mesothelium likewise.[58, 59] The autonomous plasminogen activator activity (PAA) of the peritoneal exudates itself (result of local t-PA release and clearance) is also reduced during several hours postoperatively which contributes even more to the disbalance between t-PA and PAIs and thus to decreased fibrinolytic activity.[60, 61] Reduced plasminogen-plasmin conversion i.e. low fibrinolytic activity declines fibrin degradation and allows the fibrinous adhesion to organise to a fibrous permanent adhesion through invasion, proliferation and differentiation of fibroblasts and endothelial cells. This is followed by capillary formation and incorporation of collagen, all stimulated by cytokines, and growth factors (day 4 to 10).[29, 62-67] Cytokines increase expression of integrins, cell surface receptors mediating fibroblast and mesothelial cell adhesion to ECM.[68] The mesothelial cells recover the exterior aspects of the adhesion, isolating the ECM from the peritoneal cavity and reconstituting an intact peritoneal mesothelial surface.[69]

The results of the inflammatory and fibrinogenetic response to peritoneal trauma eventuate in ultimate resolution of the process, by triggering events that lead to cell regeneration and wound healing. Normal intra-abdominal inflammatory and fibrinogenetic defences become detrimental to the host if the balance between mediators of the inflammatory response and the balance between fibrinogenesis and fibrinolysis is lost and two peritoneal surfaces are apposed (Figure 1.1).

Treatment

Relaparotomy or laparoscopy for adhesiolysis can be a technical challenge with elevated peroperative complication risks and significant postoperative morbidity and even mortality. Clinical studies involving second look operations show that operative adhesiolysis is also complicated by a high recurrence rate of adhesions at the operated site (adhesion reformation) or at other sites (de novo adhesions).[70-73] Laparoscopy does appear to be associated with equal adhesion reformation as laparotomy, between 38 and 97%, whereas de novo adhesions seem to be significantly less common after laparoscopy (12%) than

laparotomy (51%).[70-74] Recurrence rate of adhesions possibly depends on technique of adhesiolysis and time between initial surgery and evaluation of reformation.[72, 73] Furthermore the degree of reformed adhesions is often more severe than that of the primary adhesions.[75] Adhesive bowel obstructions can be treated conservatively, by intravenous drip and nasogastric suction, or by operation. The danger of conservative measures is of course that a loop of obstructed bowel is strangulated and is going to progress to gangrene with eventual perforation and subsequent increased mortality rate. Even for experienced surgeons the distinction between simple and strangulated obstruction is extremely difficult since there are no clinical signs, diagnostic laboratory tests or radiological features that will accurately confirm or refute the diagnosis of bowel ischemia. There are no fixed rules regarding indications for or timing of operation and the decision is ultimately at the discretion of the individual surgeon. Conservative management is attempted in 27 to 83% of the patients while surgery rates also vary widely, from 22 to 54%.[4, 76-78] Non-operative treatment results in a shorter hospital stay and similar recurrence and reoperation quantities, but a reduced interval to reobstruction when compared with operative treatment.[78] During adhesiolysis the bowel resection rate ranges from 5 to 20% and mortality following resection varies from 15 to 20%.[77] After an operation for an episode of adhesive bowel obstruction the risk of further episodes of obstruction ranges from 11-21%.[79, 80] The likelihood of reobstruction increases and the time to reobstruction decreases with an increasing number of previous episodes of obstruction.[78, 81] Patients with multiple dispersed adhesions have a greater recurrence rate than those with band adhesions but strangulation occurs more frequently in patients with a single obstructive band.[78, 82]

Prevention of adhesion formation

Many chemical agents and procedures to prevent or reduce the formation of postoperative adhesions have been studied. The purpose of all studies has been to interfere with one or several of the pathogenetic steps in the dynamic process of peritoneal healing and adhesion formation (Figure 1.2).

Reduction of the inflammatory reaction

The use of anti-inflammatory drugs like corticosteroids, nonsteroidal anti-inflammatory drugs (NSAID's) and antihistamines can lead to a decreased permeability of vessels and to reduced posttraumatic intra-peritoneal influx of inflammatory cells. Experimental nor clinical studies could show significant adhesion reducing effects of these agents and various side effects like impaired wound healing, immunosuppression, gastrointestinal bleeding and even the occurrence of psychoses eliminated this treatment modality.[83-86] The efficacy of antibiotic peritoneal lavage (also quite an a-specific anti-inflammatory method) in the prevention of

adhesion formation has long been controversial and recent experimental studies even revealed adhesion promoting qualities of lavage fluids and antibiotics.[87, 88]

Attempts at decreasing adhesion formation by specifically altering macrophage function have also been considered. Results of experiments using calcium channel blockers have not yielded benefit of sufficient magnitude in animal models to implement these in large scale human trials.[89, 90] "Simply" reducing the number of macrophages infiltrating the traumatised peritoneal surface by using neutralising antibodies to monocyte chemotactic protein-1 (MCP-1) diminished adhesion formation in an animal study.[91] It is too early to know whether these experiments will lead to a treatment option in humans, but to focus on the cellular constituents of the inflammatory reaction during wound healing appears to be a worthwhile avenue of investigation.

Efforts to attenuate the inflammatory reaction by mastering the mediators of this process have also been suggested as an adhesion abating alternative. An experimental study showed a decrease of adhesion formation after the administration of antibodies against the cytokines TNF-α and IL-1.[92] Again, it is not certain that these results can be repeated in clinical studies, but selective immunosuppression, at a molecular level, might be part of future solutions to the problem of postoperative adhesion formation.

Prevention of fibrin deposition

In the absence of a coagulated serosanguineous exudate or a fibrinous adhesion, organisation of adhesions may not occur. To prevent coagulation of the inflammatory exudates, dilution and neutralisation of the coagulation factors (comprising prothombin, calcium, trombokinase, fibrinogen and trombin) has been effectuated by sodium citrate, heparin, dicoumarol and dextran. Experimental as well as clinical trials demonstrated that these agents also had inconclusive effects on postoperative adhesion formation while side effects like intra-abdominal haemorrhage were imposing.[93, 94] This treatment modality was abandoned because it simply had too many side effects without a clear demonstration of a sufficient efficacy.

Removal of fibrinous exudates

Fibrin is a prerequisite for normal tissue repair; on the other hand the removal of fibrin is needed to restore preoperative conditions and guarantee adhesion free peritoneal healing. The earliest methods to forestall adhesion formation were methods removing fibrin depositions. Removal of fibrinous exudates by peritoneal lavages theoretically erases the source of fibrous adhesions but experimental results showed no adhesion reducing effect, on the contrary, peritoneal lavage even seemed to induce adhesion formation in some studies.[95, 96]

Stimulation of fibrinolysis

The disturbed dynamic balance between fibrinogenesis and fibrinolysis to the detriment of fibrinolysis prohibits adhesion free healing. The actual cause of adhesions is the excess of fibrin that is not broken down fast enough as result of an insufficient fibrinolytic capacity. Experimental and clinical reports on the intra-peritoneal use of digestive ferments with supposed natural fibrinolytic activity like pepsin, trypsin and papain did not offer any acceptable evidence for its effectiveness in adhesion prevention. The instability of the preparation, the neutralisation of the solutions by the peritoneal fluid and the occurrence of peritonitis did not support the usage of these materials.[97-100] Animal studies in which the fibrinolytic activity was enhanced by streptokinase and urokinase did also show conflicting results while carrying the risk of bleeding.[101, 102] The rate-limiting factor of the fibrinolysis is t-PA which converts plasminogen into plasmin that in turn will stimulate demolishment of fibrin. Eliminating the intra-abdominal posttraumatic t-PA deficiency by adding recombinant tissue-plasminogen activator (rt-PA) might increase the fibrinolytic activity and reduce adhesion formation. Experimental studies demonstrated that rt-PA decreased postoperative adhesions[103-105] but that the levels of rt-PA required to prevent adhesion formation also produced a significant impairment of the early phase of wound healing, as measured by the wound content of hydroxyproline and bursting strength of the wound.[106]

Inhibition of fibroblast proliferation and organisation of fibrinous adhesion

Through blocking of integrins, and thus binding of fibroblasts to the fibrinous adhesion/ECM components, fibroblast invasion and reorganisation of fibrinous into fibrous adhesions may be prohibited and adhesion formation decreased. Administration of viscous agents containing arginine-glycine-aspartic acid (RGD) peptides (blockers of integrin-ligand interactions) at the end of surgery significantly reduced adhesion formation in animals.[107] Whether this treatment modality will proof to be effective in humans remains to be investigated, but given the complexity of the cell adhesion apparatus, blocking of one single receptor is not likely to prohibit adhesion through another and therefore this pathway may prove difficult for the clinical situation.

Inhibition of fibroblast proliferation by the use of steroids and 5-fluorouracil was not effective in adhesion prevention and these chemotherapeutics had side-effects that made them inappropriate as anti-adhesive treatment modality.[108, 109]

Averting the production of procollagen by invading fibroblasts might forestall the formation of collagenous fibers and thereby prohibit transformation of fibrinous into fibrous adhesions. An *in vitro* inhibitor of procollagen (cianidanol) substantially inhibited adhesion formation but was toxic in low concentrations to animals and consequently not suitable for further therapeutic research in humans.[110]

Mesenchymal stem cell seeding

Mesothelial damage is the primary event in adhesion formation. Mesothelial stem cells differentiate into mesothelial cells and hypothetically, adhesiogenesis could be reduced by seeding mesothelial stem cells onto peritoneal defects. One experimental study demonstrated this effect in a dose-dependent manner in rats but clinical trials have yet to be carried out.[111]

Adhesion prevention barriers

Theoretically with the barrier technique two, damaged peritoneal surfaces are mechanically separated during mesothelial regeneration, thereby preventing adhesion formation. Different animal and clinical studies have indicated that placement of an absorbable barrier like expanded polytetraflouroethylene (Preclude® Surgical Membrane), polaxamer 407, oxidised regenerated cellulose (INTERCEED®), or hyaluronic acid/carboxymethylcellulose (Seprafilm™) between injured peritoneal sites or the peroperative intra-abdominal application of a viscous solution such as ionically cross-linked 0.5% hyaluronic acid (Intergel™), 32% dextran 70 (Hyskon®) or low viscosity 0.04% hyaluronic acid (Sepracoat™) can reduce postoperative adhesion formation.[112-129] In our prospective clinical randomised multicenter trial performed by Vrijland et al, the effectiveness of bioresorbable Seprafilm™ membrane in the prevention of postoperative adhesion formation was assessed in patients requiring a Hartmann's procedure for either sigmoid diverticulitis or obstructing sigmoid carcinoma. The intra-abdominal placement of Seprafilm™ at the midline abdominal incision and in the pelvis was effective in significantly abating the severity of the formed adhesions, but did not diminish the incidence of adhesion formation. No results are available yet about the effect of this on the postoperative incidence of small bowel obstruction, infertility and chronic abdominal pain but it seemed to facilitate abdominal re-exploration.[130] Nevertheless major disadvantages of the site-specific adjuvants are that the surgeon must predict adhesion formation sites to determine barrier placement and that the materials in itself may be injurious to the peritoneum.[131] Non site-specific adjuvants show doubtful adhesion diminishing qualities and are associated with undesirable local and systemic side effects like oedema, an increase in body weight and central venous pressure, transient liver function disturbances and anaphylactic shock.[132-135] Furthermore, most barrier materials are in practice difficult to handle and apply.[130] Despite the great variety of agents employed, there is no standard treatment for adhesions yet. The ideal anti-adhesive barrier would be composed of a material that (1) does not injure the peritoneum, (2) does not elicit or activate peritoneal neutrophils, macrophages or mesothelial cells, (3) does not impede the natural fibrinolytic activity of the peritoneal surface (4) is removed from the peritoneum by simple absorption

and not by degradation, (5) is not trombogenic, (6) covers all peritoneal surfaces, (7) remains intact and in situ for 5 to 7 days and (8) can be used at both laparotomy and laparoscopy.

Other drugs

Nitric oxide has been shown to modulate a number of inflammatory and vascular disease processes with effect on macrophages and endothelial cells. Its natural precursor, L-arginine, is used to increase levels of nitric oxide in tissues and turned out to diminish adhesions in rats after intra-peritoneal administration.[136]

Because oxygen free radicals are believed to play a role in adhesion induction through damaging of the mesothelial cells, intra-abdominal adhesion formation may be reduced by limiting the intra-peritoneal levels of reactive oxygen. Drugs that can function as free radical scavengers indeed have been shown to decrease adhesion formation in animals. Further research has to be executed to estimate the clinical value of these kind of pharmaceutics.[137, 138]

Minimalisation of peroperative peritoneal trauma

The only consensus concerning the prevention of postoperative adhesions, that has remained valid over time, comprehends the avoidance of peroperative damage to the peritoneum through evading procedures with known adhesion-provoking consequences.[139-141] Forestalling of unnecessary drying, handling, clamping or suturing (ischaemia) of tissue and leaving behind as little foreign material as possible is the sole clinically available option to reduce postoperative adhesion formation.[142, 143] Universally accepted agreement also exists about the fact that closure of the peritoneum is superfluous, since the peritoneum regenerates rapidly without reapproximation.[28, 32, 48, 56] Non-closure even reduced non adhesion related postoperative complications,[144-147] and is desired because of its adhesion preventing effect.[148-141]

Laparoscopic surgical procedures with their minimal access to the abdominal cavity, and thus minimal parietal peritoneal trauma, are likely to be associated with fewer postoperative adhesions compared to open surgery.[152, 153]

Intra-Abdominal Tumour Recurrence

Incidence and aetiology

Peritoneal dissemination is a common cause for post-surgical tumour recurrence after potentially curative resection of gastro-intestinal adenocarcinomas and represents terminal stage of the disease.[154-157] Loco-regional recurrence incidence may amount up to 45% for colorectal carcinoma[158-161] and lies around 50% for gastric carcinoma.[162] Distribution patterns of first peritoneal recurrence show that the resection site is preferential, and combined recurrence on peritoneal surfaces and resection site is common.[154, 159, 163, 164]

Early preoperative tumour cell seeding and peroperative intra-abdominal shedding of tumour cells, due to handling the tumour and leakage from dissected lymphatic channels, are the most likely causes of peritoneal carcinosis.[154, 155, 165-167] With current techniques, disseminated cancer cells are detectable in the peritoneal cavity in patients with colorectal, gastric and pancreatic cancer.[147, 168, 169] Previous experimental data have demonstrated that the proliferative and metastatic potentials of these spilled tumour cells are very well preserved.[165, 170] Consequently exfoliated carcinoma cells may undergo further division and give rise to implantation of metastases. Furthermore, the peroperative occurrence of tumour cells in the peritoneal cavity has been shown to correlate with the postoperative survival rate. Positive peritoneal (tumour) cytology in abdominal washings of patients with colorectal, gastric and pancreatic cancer is associated with poor prognosis.[157, 171-173] The process of haematogenic or lymphogenic cancer metastasis consists of a series of sequential, interrelated steps including invasion, embolism and transport, arrest in organs, adherence and growth.[174] In case of peritoneal carcinosis due to pre- or peroperatively seeded tumour cells, the metastatic process only depends on adherence and subsequent growth of these cells on the peritoneal surfaces. Prevention of peritoneal tumour recurrence by impeding these steps offers a promising mode of improving disease free survival.

Mechanism of action in peritoneal tumour recurrence

Several theories speculate on the mechanisms of intra-peritoneal tumour recurrence as a consequence of peroperatively spilled intra-abdominal tumour cells. Presumed implantation strategies of tumour cells include the theory of metastatic efficiency and the tumour cell entrapment hypothesis.[154] According to the theory of metastatic efficiency the implantation of spilled tumour cells on raw tissue surfaces is very efficient as opposed to the inefficient implantation on intact surfaces. The tumour cell entrapment hypothesis proposes that the fibrinous exudate, formed as an initial response to surgical trauma of the peritoneum, facilitates implantation of cancer cells onto raw tissue due to entrapment of the spilled tumour cells in the fibrin. This would secure tumour emboli at surgically injured peritoneal sites

particularly. These trapped tumour cells may also be protected from natural host defences and systemic chemotherapy by their coating of fibrin. During peritoneal wound healing after peritoneal trauma, exposed endothelial cells, damaged mesothelial cells, as well as resident and invading inflammatory cells produce an abundance of chemoattractants (Il-8, MCP-1), cytokines (TNF-α, IL-1β and IL-6) and growth factors (TGF-β, IGF-I, PDGF).[35-39] The micro environment of the peritoneal wound hereby contains a plethora of factors creating a prerequisite area for cell proliferation and differentiation. Accordingly surgical peritoneal damage induces the release of substances that not only participate in the local healing process but that also, unfortunately, could be beneficial for proliferation and growth of spilled and trapped tumour cells. This should make the surgical wound in particular, a fertile soil for tumour growth and could result in augmented growth of an intra-abdominal tumour cell deposit at traumatised peritoneal sites. Indeed previously described clinical and experimental studies showed that surgical trauma may promote intra-abdominal tumour recurrence and in clinical situations, it appears that peritoneal tumour implants may recur within a fibrous adhesion resulting from surgical trauma.[175-179] Apart from stimulated growth, tumour cells may profit from the enriched wound micro-environment by enhancing the process of cell adhesion.[170-172] This would explain tumour recurrence at not specifically traumatised peritoneal surfaces. Most likely, metastatic cells respond to physiologic signals produced when homeostasis is disturbed. Tumour cells that either originate from or have an affinity for growth in a particular organ can also respond to these signals.[174]

Recapitulating, the dynamic cascade of peritoneal healing, induced by peritoneal damage, sometimes leading to adhesion formation also seems to be important in the process of intra-peritoneal adhesion and growth of free intra-abdominal tumour cells. Consequently the trauma ensuing tissue regeneration may affect tumour recurrence in various ways (Figure 1.1).

ADHESION FORMATION AND TUMOUR CELL ADHESION AND GROWTH

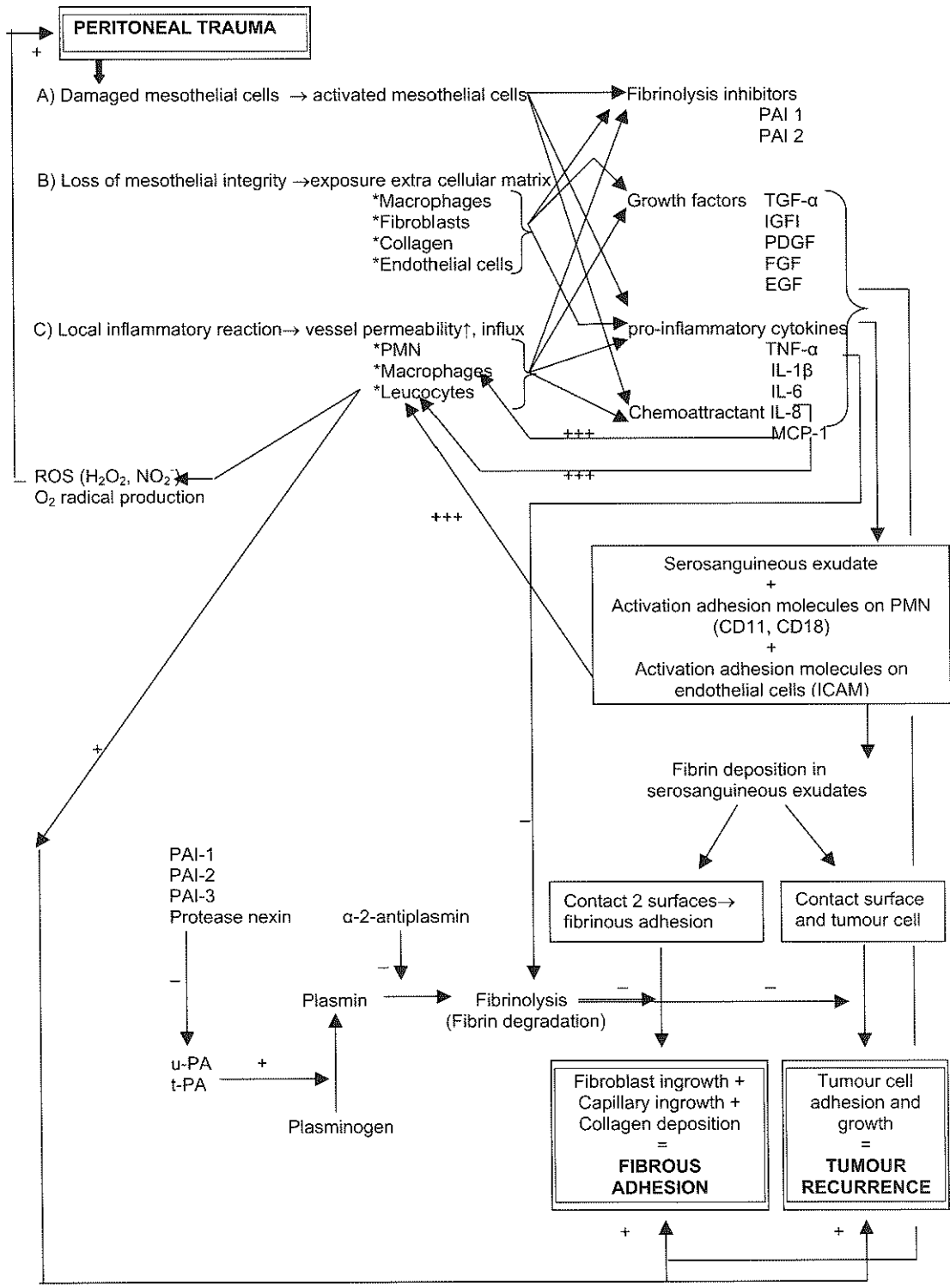

Figure 1.1

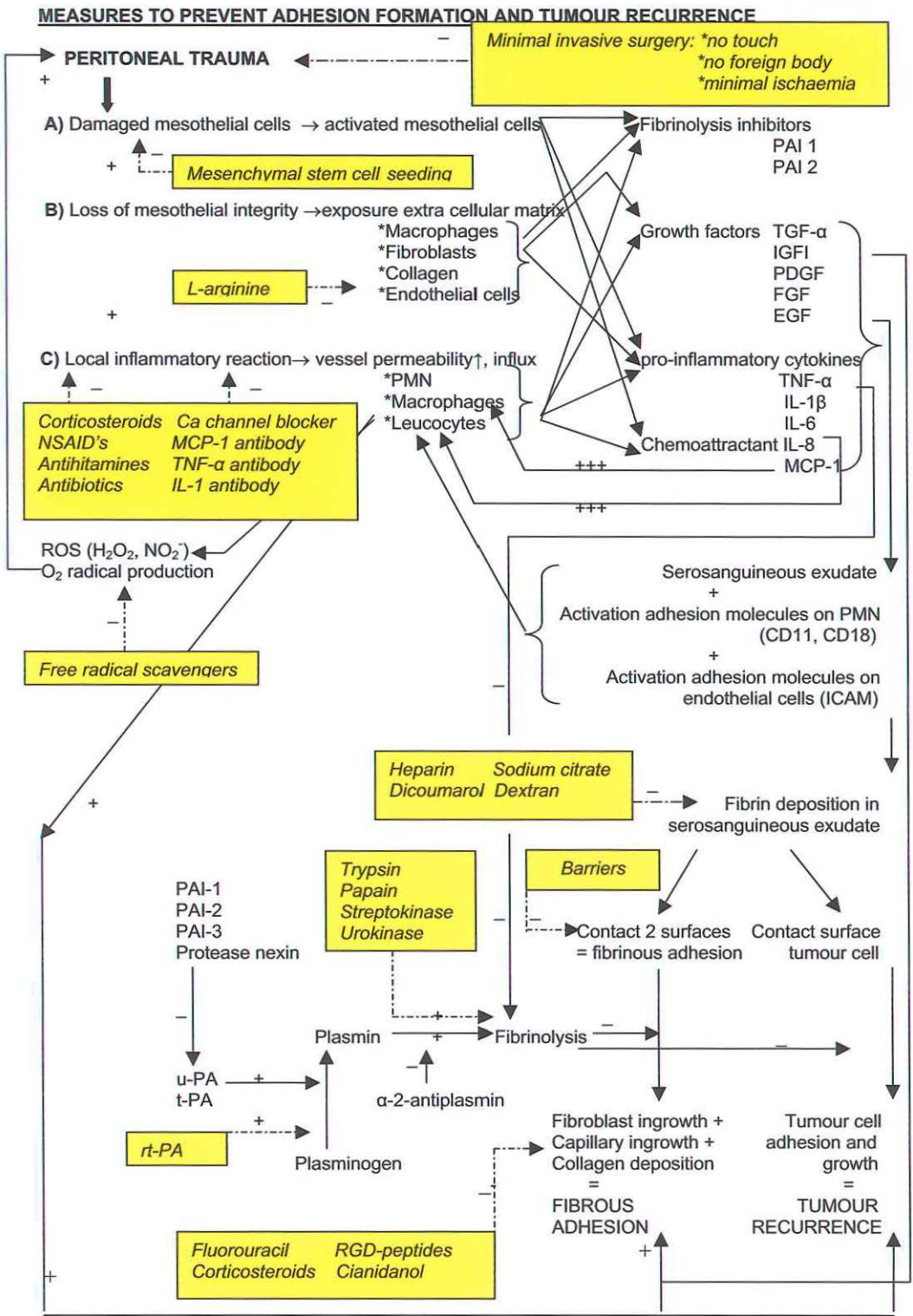
MEASURES TO PREVENT ADHESION FORMATION AND TUMOUR RECURRENCE
PERITONEAL TRAUMA
Minimal invasive surgery: *no touch
*no foreign body
*minimal ischaemia
A) Damaged mesothelial cells → activated mesothelial cells
Mesenchymal stem cell seeding
Fibrinolysis inhibitors
PAI 1
PAI 2
B) Loss of mesothelial integrity →exposure extra cellular matrix
*Macrophages
*Fibroblasts
*Collagen
*Endothelial cells
L-arginine
Growth factors
TGF-α
IGFI
PDGF
FGF
EGF
C) Local inflammatory reaction→ vessel permeability↑, influx
*PMN
*Macrophages
*Leucocytes
pro-inflammatory cytokines
TNF-α
IL-1β
IL-6
Chemoattractant IL-8
MCP-1
+++
+++
Corticosteroids
NSAID's
Antihitamines
Antibiotics
Ca channel blocker
MCP-1 antibody
TNF-α antibody
IL-1 antibody
ROS (H_2O_2, NO_2^-)
O_2 radical production
Free radical scavengers
Serosanguineous exudate
+
Activation adhesion molecules on PMN (CD11, CD18)
+
Activation adhesion molecules on endothelial cells (ICAM)
Heparin
Dicoumarol
Sodium citrate
Dextran
Fibrin deposition in serosanguineous exudate
Trypsin
Papain
Streptokinase
Urokinase
Barriers
PAI-1
PAI-2
PAI-3
Protease nexin
Contact 2 surfaces = fibrinous adhesion
Contact surface tumour cell
Plasmin
Fibrinolysis
u-PA
t-PA
α-2-antiplasmin
rt-PA
Plasminogen
Fibroblast ingrowth + Capillary ingrowth + Collagen deposition = FIBROUS ADHESION
Tumour cell adhesion and growth = TUMOUR RECURRENCE
Fluorouracil
Corticosteroids
RGD-peptides
Cianidanol

Figure 1.2

Chapter II

Aims of the Thesis

Surgical peritoneal trauma and adhesion formation

Adhesions may be regarded as the most frequent complication of surgery, with a high impact on health-care.[19, 20, 22-24] Adhesion formation produces burden to the patient and a clinical challenge for general surgeons due to complications of adhesions, prolonged incision time and substantial time necessary for division of adhesions at abdominal re-operations and inadvertent enterotomy during difficult re-interventions.[21, 183] Adhesions were considered to be a natural occurring phenomenon by most surgeons. It is only recently that surgeons, realising that there might be precautions that may be useful at the time of surgery, are becoming interested in identifying the magnitude of adhesion-related problems. A wide variety of therapeutic modalities to reduce the incidence of postoperative adhesion formation have been evaluated in clinical and experimental work.[83-139] The results achieved with the different treatment modalities are inconsistent and associated with many side effects. At the present time no agent seems to be overwhelmingly suitable for general use in humans. Accordingly, it is the surgeon herself who seems to play the most important role by operating in a non-traumatising fashion to prevent peritoneal injury.[139-151] Studies, in which peritoneal trauma was proven to be a cause of adhesion formation did not mention whether there was a connection between the degree of trauma, and the extent of formed adhesions.[28, 29, 55] Therefore one of the aims of this thesis was to analyse whether the extent and type of post-surgical adhesion formation correlates with the degree of peritoneal damage. Furthermore we tried to identify whether agents like surgical gauze, lavage solutions and glove powder, not yet generally considered as traumatic for the peritoneum, and commonly used in general surgical practice, indeed were traumatic and could consequently cause adhesion formation. and whether there were less traumatic and feasible alternatives for these agents. In order to achieve these aims we first developed a reproducible rat adhesion model allowing semiquantitative and qualitative scoring of adhesions.

Surgical peritoneal trauma and intra-abdominal tumour recurrence

Pre- and peroperatively seeded tumour cells influence the prognosis of patients with gastro-intestinal malignancies dramatically.[147, 161-163] Tumour cells disseminated during surgical dissection will not be treated using the conventional therapeutic modalities. Peroperative lavage will remove some of the spilled cells, but sufficient irrigation of the peritoneal cavity is not possible. In addition several experimental approaches to control the local recurrence have been initiated. Clinical trials are investigating the possibility of intra-peritoneal chemotherapy.[164, 165] Lavaging the abdominal cavity with chemotherapeutic agents will deteriorate the micro milieu for residual tumour cells but may have adverse side-effects on wound healing and peritoneal adhesion formation.[186, 187] Photodynamic therapy (PDT) may be another promising approach to attack manifest peritoneal tumour recurrence. PDT is a surface oriented, locally cytotoxic intervention. Defined tumour foci are specifically eradicated, hereby only traumatising restricted areas of the peritoneum.[188-190] The above mentioned therapies are mainly aimed at annulling manifest peritoneal tumour foci but do not focus on prohibiting the postoperative arising of peritoneal tumour recurrence. A profound understanding of the pathophysiology of tumour recurrence may lead to more specific tools to confront the initial process of tumour cell implantation. One hypothesis, trying to clarify the pattern of surgical treatment failure after potentially curative resection of gastro-intestinal adenocarcinoma, suggests the implantation of free tumour cells is most efficient on damaged tissue while the coating of these trapped cells with reactive fibrin protects them from natural host defences.[154] The fact that the resection site and surgical wounds are predestined sites for recurrence,[191] indeed indicates there may be a mechanistic relation between surgical tissue trauma and tumour recurrence. The dynamic cascade of peritoneal healing, induced by peritoneal damage, sometimes leading to adhesion formation also seems to be important in the process of intra-peritoneal adhesion and growth of free intra-abdominal tumour cells. This thesis tries to demonstrate the similarities between the process of adhesion formation and tumour recurrence by evaluating whether there was a relationship between degree of peritoneal trauma and the extent of tumour cell adhesion and growth and if this presumed relationship was merely a local phenomenon or whether systemic effects might also be involved. Furthermore we tried to identify whether exposure of the peritoneum to surgical gauze and glove powder known to cause adhesion formation also could induce more tumour recurrence. Next we investigated whether the less traumatising alternatives were valid in reducing tumour recurrence as well. To fulfil these tasks we developed a reproducible rat tumour adhesion and growth model, analogous to the rat adhesion model, allowing semiquantitative scoring of tumour load.

Pathways of adhesion formation and intra-abdominal tumour recurrence

Adjuncts to good surgical techniques are needed for adhesion and tumour recurrence prevention. Better understanding of the underlying pathogenesis of both processes is a prerequisite to rational prophylaxis and therapy of both surgical complications. Further experiments described in this thesis aim at unravelling pathways of enhanced tumour cell adhesion and growth after surgical peritoneal trauma. Soluble and cellular constituents of the peritoneal cavity are believed to both reflect the events occurring during peritoneal healing and the potential to modulate the outcome of tissue response to injury. We analysed the effect of cellular and non-cellular soluble inflammatory components using our *in vivo* and *in vitro* reproducible tumour adhesion and growth models.

Results of the analysis of the inflammatory cells in these experiments demonstrated a trauma-related influx of neutrophils (PMN) in the abdominal cavity. We evaluated the role of these PMN in postoperative adhesion formation and peritoneal tumour recurrence by treating rats intra-peritoneally with anti-neutrophil serum (ANS).

Adjuvant measures to prevent adhesion formation and tumour recurrence

Since peroperative peritoneal trauma cannot entirely be prevented in practice, the need for supplementary measures to reduce adhesions and tumour recurrence remains urgent. Accordingly, we performed experiments evaluating the capacity of a new glucose polymer solution, icodextrin®, to reduce adhesion formation and whether icodextrin® might reduce or possibly promote the adhesion and growth of intra-peritoneally injected tumour cells.

The results obtained from these experiments will lead to feasible measures that may ultimately support the surgeon to prevent peritoneal injury and thereby reduce postoperative adhesion formation and intra-abdominal tumour recurrence.

A more thorough understanding of the impact of neutrophils (PMN), macrophages, cytokines and integrins on the wound healing process and its derangement will undoubtedly help in the development of more specific therapeutic strategies to reduce postoperative adhesion formation and local tumour recurrence without causing serious complications.

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PART II

Surgical Peritoneal Trauma and Adhesion Formation

Chapter III

Reduction of Intra-Peritoneal Adhesion Formation by Use of Non-Abrasive Gauze

Adapted from the original publication in the British Journal of Surgery 1997; 84: 1410-1415

Adhesion formation is potentially harmful. Surgical swabs may contribute to adhesions by traumatising the peritoneum. The purpose of this study was to evaluate whether standard surgical gauze (Medipres) has an adhesion promoting effect, and to determine whether a soft textile (Fastsorb), used in the electronics industry, might be less traumatic and therefore lead to less adhesion formation.

A reproducible rat model allowing semiquantitative scoring of adhesion formation was used. Three different adhesion models representing increasing degrees of peritoneal trauma (minimal, moderate and severe) were employed. The model inflicting minimal peritoneal trauma was combined with standardised rubbing of the peritoneum with surgical gauze or non-surgical textile.

Minimal peritoneal trauma resulted in a significantly lower adhesion percentage (21%) than moderate (44%) or severe (60%) peritoneal trauma ($p \leq 0.005$). Rubbing of the peritoneum with surgical gauze after infliction of minimal peritoneal trauma did induce significantly extra adhesion formation (58% versus 23%, $p < 0.0001$). After infliction of minimal peritoneal trauma, rubbing with surgical gauze did produce significantly more adhesions than rubbing with non-surgical textile (63% versus 19%, $p < 0.0001$). Moreover, rubbing the peritoneum with non-surgical textile after infliction of minimal peritoneal trauma did not induce additional adhesion formation at all (35% versus 24%, $p = 0.23$).

The extent of adhesion formation correlates significantly with the inflicted degree of peritoneal damage. Standard surgical gauze is traumatising to the peritoneum and promotes adhesion formation whereas a less abrasive non-surgical textile does not.

INTRODUCTION

Postoperative adhesions are abnormal unions between tissue surfaces which occur after almost every intra-abdominal surgical intervention and can lead to a number of complications. Adhesion formation after intra-abdominal surgery accounts for 70–90% of all adhesions,[1-3] the rest being caused by inflammation of the peritoneum, endometriosis (18%) and congenital adhesions (2.8-6%).[4] Abdominal adhesions can cause intestinal obstructions which often requires immediate surgical intervention. Thirty percent of all bowel obstructions are caused by postoperative adhesions.[5-8] About 1% of all surgical admissions and 3% of all laparotomies are done for intestinal obstruction due to adhesions.[7] The mortality rate from intestinal obstruction due to adhesions is 6-13%.[1, 2, 4] Some 15-20% of infertility in women is a consequence of adhesion formation[9-13] and adhesions were identified as the primary cause of chronic pelvic pain in 13-26% of female patients.[14, 15]

A wide variety of therapeutic modalities to reduce the incidence of postoperative adhesion formation has been studied in clinical and experimental work. The results achieved with the different treatment modalities are inconsistent and associated with many side effects such as intra-abdominal haemorrhage and impaired wound healing. Until now only one strategy exists to reduce adhesion formation, namely the reduction, at operation, of all forms of peritoneal trauma and ischaemia. This necessitates avoiding desiccation and vigorous handling of intra-abdominal tissue and introducing as little foreign material as possible.[16-18] Manipulation with abdominal gauze might be traumatic to the peritoneum either because it is rough and abrades the peritoneum and serosa or because remnants of gauze are left behind in the abdomen.[19, 20] The present study was undertaken to evaluate whether a soft textile used in the electronics industry might have a less traumatic effect on the peritoneum than standard surgical gauze, and consequently lead to less adhesion formation. A reproducible animal model that allowed semiquantitative scoring of adhesion formation was used.

MATERIALS AND METHODS

Animals

Female Wistar rats of reproductive age weighing 200-250 g were obtained from Harlan, Zeist, The Netherlands. They were bred under specific pathogen-free conditions and kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light and 12 hours dark). The rats were given standard rat food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam, The Netherlands.

Gauzes

The evaluated "gauzes" were surgical Medipres gauze (van Heek Medical, Losser, The Netherlands), consisting of 100% cotton and commonly used in abdominal surgery, and non-surgical Fastsorb cleanroom wiper (Berkshire Corporation, Great Barrington, Massachusetts, USA), used in the electronics industry (Figure 3.1). Fastsorb is a rayon-polyester blend which possesses strength and softness combined with a high absorbing capacity. It has low particle generation, minimal extractable matter and a low ion content. Fastsorb is used on abrasion-sensitive surfaces.

Absorbing capacity

The absorbing capacity of both materials was evaluated using the following method. Ten 5 x 5 cm-pieces of surgical Medipres gauze (double layer) and non-surgical Fastsorb textile (monolayer) were saturated by immersion in water. After saturation, the materials were drained for 30 seconds. The pieces were weighed before and after saturation; results were expressed in millilitres of water absorbed per gram gauze.

Surgical techniques

The three adhesion models employed in this study were derived from a model described by van Bakkum et al.[21]

Model 1. Under ether anaesthesia and aseptic but not sterile conditions, using Biogel sterile gloves (Regent Hospital Products, London, UK), the abdomen was shaved and cleaned with alcohol. Laparotomy was performed using a lower midline incision of 5 cm. In the lateral abdominal wall, 1.5 cm downwards from the abdominal incision, a standard area of 1.5 x 1.2 cm, 3 mm deep, which contained the mesothelial as well as both underlying muscular layers, was excised with an oval shaped punch. This severe peritoneal defect was then closed with three 5-0 Surgilene sutures (Braun, Melsungen, Germany). Subsequently the uterus horn was sutured to the lateral peritoneum, both proximally and distally from the closed peritoneal defect, with 6-0 Surgilene. All knots were double and fastened tightly to ensure ischaemia. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures.

Model 2. The same type of operation was performed with only one difference: the damage inflicted to the lateral peritoneum was of moderate severity and consisted only of an incision of 1.5 cm long, 3 mm deep, which was also closed with three 5-0 Surgilene sutures.

Model 3. In this model minimal trauma was applied to the lateral peritoneum. No wound was inflicted. The trauma was ischaemic only and was brought about by three 5-0 Surgilene sutures at 0.7 cm intervals.

Figure 3.1

On the left surgical Medipres gauze and on the right non-surgical Fastsorb textile.

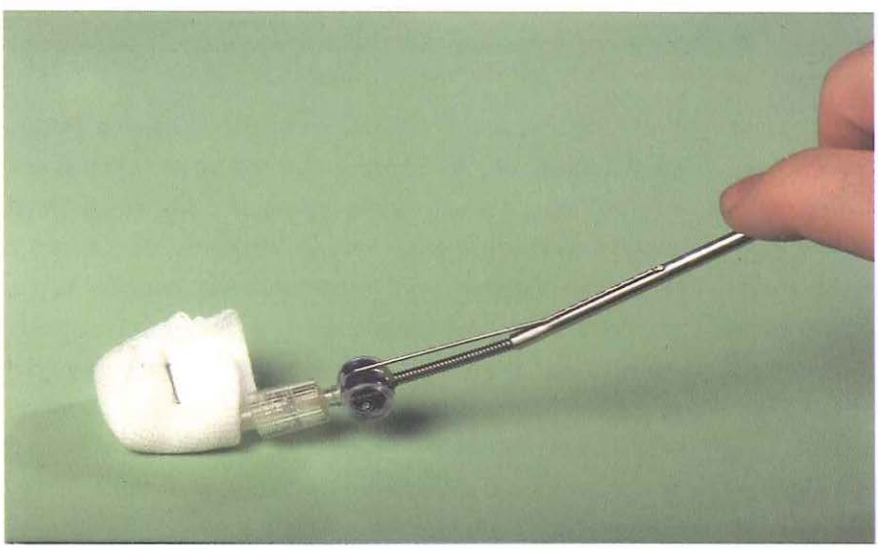

Figure 3.2

Rubbing device used to apply standardised trauma to the uterine horn. A spring attached to the handle enables rubbing under a certain pressure. The device is constructed in such a manner that a pressure of 120 g/cm^2 is applied when the indicator needle (above the spring) touches the blue roll. The gauze is wrapped and fixed around a hooked injection needle (1.1 x 40 mm Luer-Lock; Braun, Melsungn, Germany).

Rubbing with surgical gauze and non-surgical textile was performed using a device enabling the application of a constant pressure of 120 g/cm^2 (Figure 3.2). The uterus horn was rubbed ten times over its total length.

Experimental design

Adhesion formation after different amounts of peritoneal trauma.

In 15 rats one lateral abdominal wall side was operated on according to adhesion model 1 (severe peritoneal trauma). In nine of these animals the other side was treated according to adhesion model 2 (moderate peritoneal trauma) and six were treated according to adhesion model 3 (minimal peritoneal trauma).

Adhesion formation in model 3 after rubbing with surgical gauze or non-surgical textile.

In 30 rats minimal peritoneal trauma was inflicted to both lateral peritoneal sides (model 3). Both uterine horns were exposed and sutured to the lateral peritoneum. In ten rats one uterine horn was rubbed with surgical Medipres gauze whereas the other was not touched (group I); in ten animals one uterine horn was rubbed surgical Medipres gauze and the other one with non-surgical Fastsorb textile (group II); and in the last ten rats one uterine horn was rubbed with Fastsorb textile while the other was not touched (group III). In all cases randomisation was used to determine which rubbing model was to be carried out on which side.

Evaluation of adhesion formation.

Some 14 days after operation the rats were sacrificed by a humane method for assessment of post-surgical adhesion formation. Macroscopically the adhesions were scored according to their extent (quantity) and type (quality) by two independent observers. The extent of adhesion formation was quantified by dividing the area to be scored into eight by means of the three 5-0 sutures by which the defect was closed (Figure 3.3). The presence or absence of adhesions in the eight demarcated areas was scored. If there were adhesions in an area this accounted for 12.5% adhesions; a maximum of 100% adhesions could be scored. In each rat two lateral peritoneal sides were assessed. The type of adhesions formed was classified macroscopically using the Zühlke classification (Table 3.1). [22]

Evaluation of peritoneal damage

Microscopic analysis of peritoneal reaction after rubbing ten times with surgical Medipres gauze and non-surgical Fastsorb textile was performed. Peritoneal reaction was evaluated directly after, and 2 and 4 hours after rubbing the uterus horn. For each time point one-third of the four rubbed uterus horns (two rubbed with Medipres and two with Fastsorb) were excised and fixed in formalin. The samples were stained with haematoxylin and eosin. The appearance of the mesothelial and underlying muscular layers and the infiltration of leucocytes were observed and compared.

Statistical analysis

Statistical analysis was performed using the *t*-test for paired samples. In two instances the unpaired Student's *t* test for independent samples was used, as indicated in the text. Statistical significance was defined as $p < 0.05$. Data were expressed as mean adhesion percentage ± SD.

RESULTS

Gauzes: absorbing capacity

The mean absorbing capacity of non-surgical Fastsorb textile ($n = 10$) was 8.4 ml per g gauze (SD: 0.2) and that of surgical Medipres gauze ($n = 10$) was 6.6 ml per g gauze (SD: 0.6). This difference is statistically significant ($p < 0.0001$, Student's *t* test for independent samples).

Adhesion formation after inflicting different amounts of peritoneal trauma.

On almost all peritoneal defects adhesion formation was found after 2 weeks. No pathological conditions as bowel obstruction, peritonitis or abscesses were found.

Table 3.2 shows that a significantly more adhesions were found at sites where severe (model 1) trauma had been applied, compared with moderate (model 2) and minimal (model 3) peritoneal trauma ($p \leq 0.05$). Adhesions formed after severe and moderate trauma of the peritoneum were dense and thick and could be classified as Zühlke type 2-3 and Zühlke type 2. The pelvic fat and the uterine horn took part in the adhesion formation. The adhesions formed after minimal trauma of the peritoneum were filmy and could be classified as Zühlke type 1-2. Only pelvic fat took part in the adhesion formation.

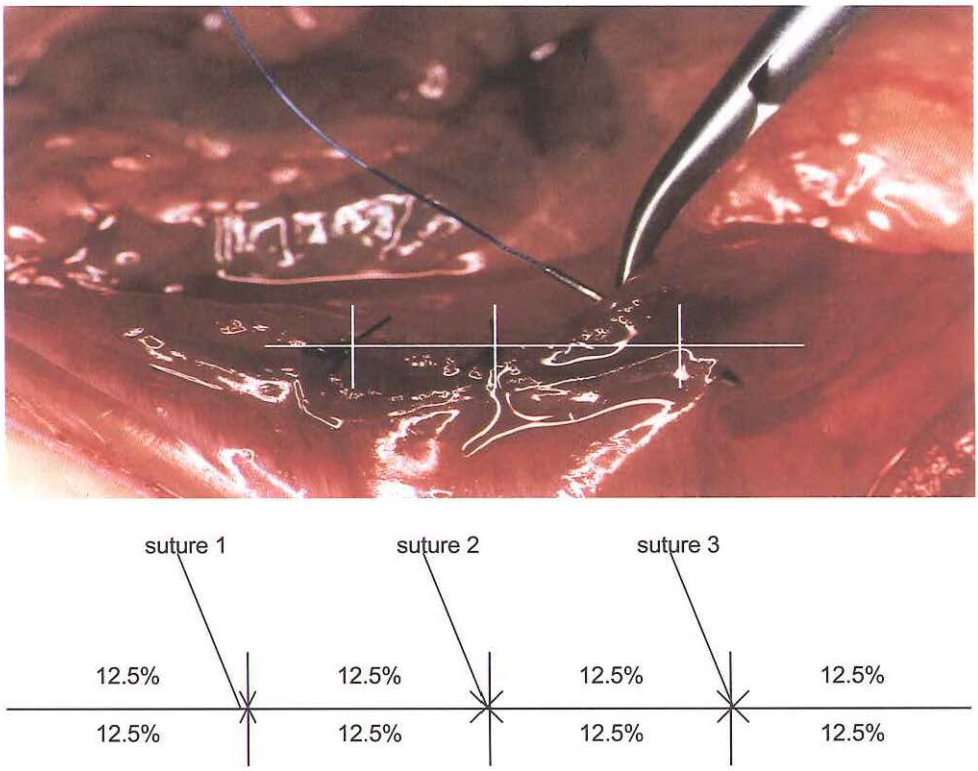

Figure 3.3

The extent of adhesion formation was quantified by dividing the "defect" area to be scored into eight areas of 12.5% by means of the three sutures used to close the defect.

Score	Characteristics
1	Filmy adhesion, easy to separate by blunt dissection
2	Stronger adhesion: blunt dissection possible, partly sharp dissection necessary; beginning of vascularisation
3	Strong adhesion: lysis possible by sharp dissection only; clear vascularisation
4	Very strong adhesion: lysis possible by sharp dissection only; organs strongly attached with severe adhesions: damage of organs hardly preventable

Table 3.1

Macroscopic (morphological) classification of abdominal adhesions according to Zühlke et al.[22]

Adhesion formation in model 3 after rubbing with surgical gauze or non-surgical textile.

Table 3.3 shows that rubbing the uterine horn with surgical Medipres gauze after infliction of minimal peritoneal trauma gave rise to a significantly higher mean adhesion percentage than infliction of minimal peritoneal trauma alone ($p \leq 0.0001$). The adhesions in the latter group were often filmy (Zühlke type 1-2) involving only pelvic fat. Adhesions at sites also rubbed by Medipres gauze were denser (Zühlke type 2-3); pelvic fat, uterine horn and the bowel were involved in these adhesions.

Table 3.3 shows that, after infliction of minimal peritoneal trauma, rubbing the uterus horn with surgical Medipres gauze induced a significantly higher mean adhesion percentage than rubbing the (opposite) uterus horn with non-surgical Fastsorb textile ($p \leq 0.0001$). The adhesions formed after rubbing with Medipres gauze were more dense (Zühlke type 2-3) than those formed after rubbing with Fastsorb textile (Zühlke type 1-2). In the first group the uterine horn, the bowel and pelvic fat were involved in adhesion formation, whereas in the Fastsorb group the bowel was never involved and the uterine horn rarely.

The mean adhesion percentage found after infliction of minimal peritoneal trauma did not significantly differ from that observed after combining this minimal trauma with rubbing the uterus with non-surgical Fastsorb textile ($p = 0.23$). The adhesions found were all filmy, Zühlke type 1 in the first group and type 1-2 in the second group; only pelvic was involved in adhesion formation.

Light microscopy

In all cases the surface of the uterine peritoneum was more damaged after rubbing with surgical Medipres gauze than after rubbing with non-surgical Fastsorb textile. After rubbing with Medipres gauze, the mesothelial layer had disappeared and the underlying muscular layer was partly lost and looked frayed. After rubbing with Fastsorb textile the mesothelial layer had disappeared for the most part but the underlying muscular layer looked smooth and was intact. At 2 and 4 hours after causing peritoneal damage with Medipres there were more leucocytes infiltrating the severely damaged area than after rubbing with Fastsorb (Figure 3.4).

Abdominal trauma	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
Model 1	15	60 (12.7)	2-3	0.011		
Model 2	9	44 (15.5)	2		0.004	
Model 3	6	21 (6.5)	1-2			0.005

Table 3.2

Mean adhesion percentages (SD), and Zühlke classification of found adhesions, after inflicting severe (model 1), moderate (model 2) and minimal (model 3) peritoneal trauma. Fifteen rats underwent an operation according to adhesion model 1 at one lateral abdominal wall side. In 9 of these rats the other, opposite, side was operated according to adhesion model 2 and in 6 of these rats according to adhesion model 3. Therefore, n is the number of peritoneal sites (uterine horns) assessed. Statistics p1 and p3: *t* test for paired samples (model 1 *versus* model 2 and model 1 *versus* model 3). Statistics p2: Student's *t* test for independent samples (model 2 *versus* model 3).

Uterine horns	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
I^a. No touch	10	23 (9.9)	1-2			
I^b. Medipres rubbing	10	58 (10.5)	2-3	< 0.0001		
II^a. Medipres rubbing	10	63 (11.8)	2-3			
II^b. Fastsorb rubbing	10	19 (12.2)	1-2		< 0.0001	
III^a. No touch	10	24 (11.6)	1			
III^b. Fastsorb rubbing	10	35 (24.3)	1-2			ns

Table 3.3

Mean adhesion percentages (SD), and Zühlke classification of found adhesions, after inflicting minimal peritoneal trauma alone (the uterine horn was not touched) and after rubbing the uterine horn with Medipres gauze or Fastsorb textile following the infliction of minimal peritoneal trauma. N is the number of defected peritoneal sites (uterus horns) assessed. Statistics p1 (I^a *versus* I^b), p2 (II^a *versus* II^b) and p3 (III^a *versus* III^b): *t*-test for paired samples (95% CI = 95% confidence interval).

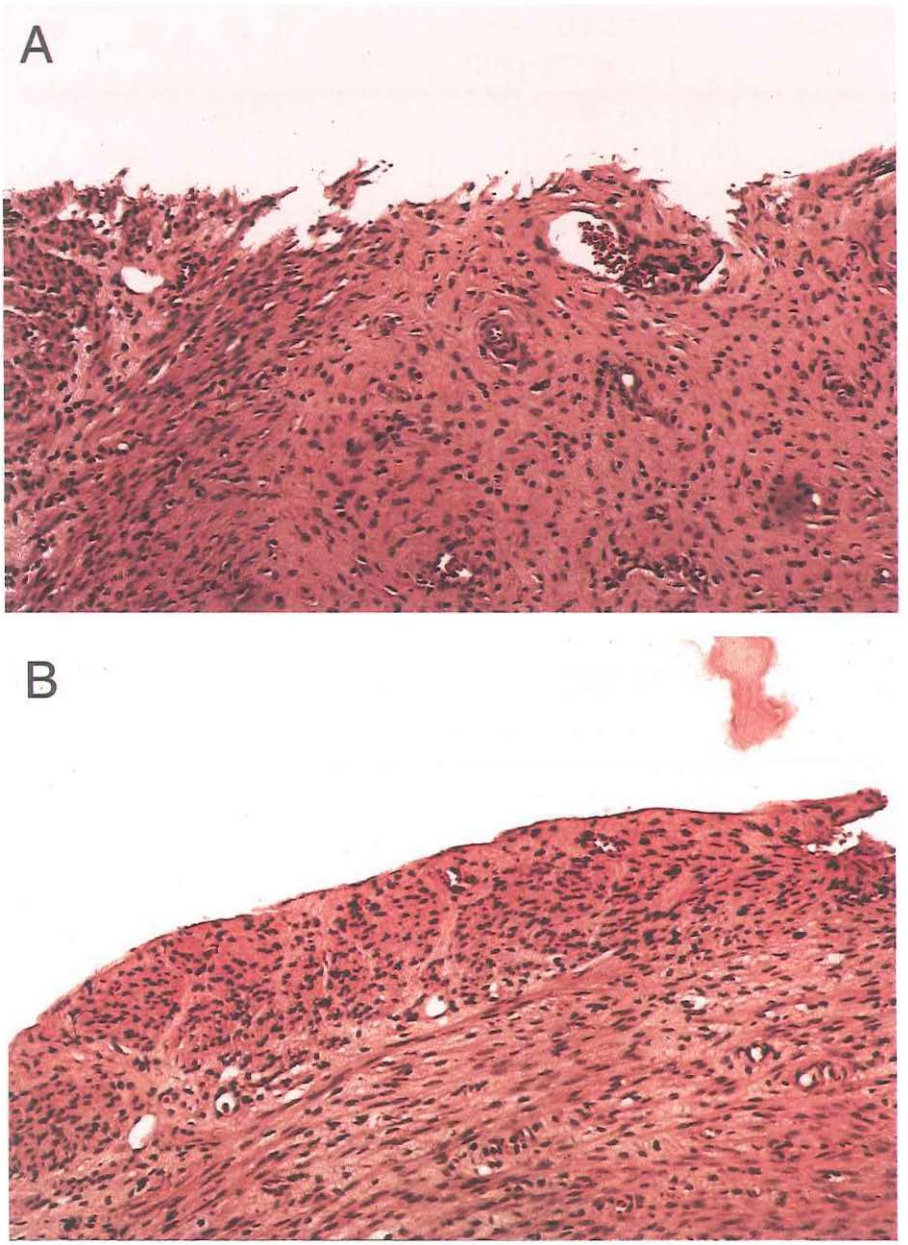

Figure 3.4

Light micrograph of uterus horn surfaces after rubbing with Medipres gauze (A) and Fastsorb textile (B). A: the mesothelial layer has disappeared, the underlying muscular layer is partly lost and frayed. Reactive leucocyte cells infiltrate the severely damaged surface area. B: The mesothelial layer has disappeared for the most part but the underlying muscular layer is intact. Magnification 10x.

DISCUSSION

Numerous attempts in clinical and animal studies to prevent or reduce the incidence of intra-peritoneal adhesions have yielded limited success.

Adhesion formation is a dynamic process consisting of several consecutive stages following damage to the parietal or visceral peritoneum. Different studies, in which peritoneal trauma was proven to be a cause of adhesion formation,[23, 24, 32] did not mention whether there was an association between the degree of trauma inflicted, and the extent of formed adhesions. The present study shows that the extent and type of post-surgical adhesion formation correlates significantly with the degree of peritoneal damage. At sites where severe, moderate or minimal peritoneal trauma was inflicted, mean adhesion percentages of 60%, 44% and 21% respectively were found.

Damage to the peritoneum causes a local inflammatory reaction which leads to the formation of a serosanguineous exudate.[23-26] In the exudate fibrin deposition will take place,[25-27] and when two surfaces contact each other during this process a fibrinous adhesion will appear. Whether these adhesions are permanent of character or will eventually be lysed is dependent on the fibrinolytic capacity of the peritoneum.[25, 28-31] When the peritoneum is slightly damaged and mesothelial cells are mostly intact, there will be a dynamic balance between fibrinogenesis and fibrinolysis, and adhesion-free healing may take place. When the peritoneum is more severely damaged the underlying connective tissue will be exposed and normal serosal fibrinolytic activity will be lost.[32] Exposed endothelial and inflammatory cells, as well as damaged mesothelial cells, may produce plasminogen activator inhibitor 1 and 2, factors mediating a reduced functional fibrinolytic activity.[29] Low fibrinolytic activity allows the fibrinous adhesion to organise to a fibrous permanent adhesion.[24] Therapeutic modalities, focusing on the different stages in adhesion formation include the use of anticoagulants and anti-inflammatory agents,[33-35] removal of fibrinous exudates by peritoneal lavages,[36] the augmentation of fibrinolysis by use of polysorb, saccharose, vitamin E or recombinant tissue plasminogen activator (t-PA),[37] and the inhibition of fibroblast proliferation by use of steroids and 5-fluorouracil.[38] Proving clinical effectiveness remains difficult and there may be severe side-effects.

Recent animal and clinical studies evaluating mechanical separation during reperitonealisation of two adjacent anatomical structures are more promising.[39-42] Non-resorbable and resorbable barriers have up to now been used mainly in experimental settings, leaving minimalisation of peritoneal trauma as the main therapeutic modality in prevention of postoperative adhesion formation.

Limitation of peritoneal injury should include attempts to reduce trauma inflicted by surgical gauze. Down et al[19, 20] showed in two experimental studies that the abrasive effect of gauzes

causes adhesion formation. In various experimental models, used to evaluate the prevention of adhesions by different substances, peritoneal damage is created by gauze abrasion.[43-47] In 1968 Saxen and Myllarniemi reviewed 309 laparotomies with adhesions present and found foreign-body granulomas in 61% of all cases.[48] Remnants of gauze were the second most frequent observed foreign body materials (16%) found in these adhesions.[48]

The current study shows that peritoneal manipulation with surgical Medipres gauze after infliction of minimal peritoneal trauma produces significantly more adhesions than inflicting minimal trauma alone. The dense Zühlke type 2-3 adhesions were thereby formed not only between traumatised lateral peritoneum and pelvic fat but also between traumatised peritoneum and uterine horn and between uterine horn and bowel. This finding suggests that manipulation with surgical Medipres gauze causes severe trauma of the peritoneum. After minimal peritoneal trauma, manipulation of the peritoneum with non-surgical Fastsorb textile produced significantly fewer adhesions than manipulation with surgical Medipres gauze, suggesting a less traumatic influence on the peritoneum of Fastsorb textile. Indeed, there was no significant difference in the extent and type of adhesions formed after infliction of minimal peritoneal trauma alone and minimal trauma in combination with Fastsorb manipulation of the peritoneum. Thus peritoneal manipulation with non-surgical Fastsorb textile, as performed in the current study, does not seem to have a traumatising effect on the peritoneum. Microscopic analysis of rubbed uterine horns partly confirms this conclusion: after rubbing with Medipres, severe trauma of the surfaces was seen with many infiltrating cells, whereas rubbing with Fastsorb produced little tissue damage with only a small number of infiltrating cells.

Given the fact that non-surgical Fastsorb textile meets important criteria for a gauze used for surgical purposes and the fact that this non-abrasive textile produces fewer adhesions than the usual surgical gauzes, further study of the use of non-abrasive textile in abdominal surgery is indicated. Fastsorb textile might be a suitable substitute for Medipres gauze in surgical practice.

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Chapter IV

Peroperative Abdominal Lavage Promotes Intra-Abdominal Adhesion Formation in the Rat

Adapted from the original publication in the European Surgical Research 1999; 31: 196-201

Intra-abdominal adhesions continue to pose a serious postoperative clinical problem. Per-operative peritoneal trauma can lead to postoperative adhesion formation. In vitro studies show that exposure of the peritoneal surface to lavage solutions enhances peritoneal activity and provokes an inflammatory response. This study was designed to evaluate the in vivo effect of lavage solutions on the peritoneum and post-surgical adhesion formation.

A reproducible rat model allowing semiquantitative scoring of adhesions was used to study adhesion formation after lavage of the abdominal cavity with RPMI medium, NaCl 0.9%, polyvinylpyrrolidone iodine 1% (PVP-I), Viaspan and chlorhexidine 0.02% in dilution. All solutions were applied during a laparotomy in which standardised minimal peritoneal trauma was inflicted.

Peritoneal lavage of the abdominal cavity after the infliction of minimal peritoneal trauma induced significantly more adhesions than the infliction of minimal peritoneal trauma alone ($p = 0.0001$). Aggressive as well as non-aggressive lavage solutions caused extra adhesion formation.

The results found in the present study correlate with observations from in vitro experiments; exposure of peritoneal cavity to lavage solutions traumatises the peritoneum, enhances peritoneal activation and thus promotes intra-abdominal adhesion formation. During surgery in non-contaminated abdominal cavities peritoneal lavage should not be performed. Per-operative lavage of contaminated abdominal cavities should be viewed with caution.

INTRODUCTION

Peroperative lavage following contaminated or potentially contaminated abdominal surgery has become common practice. Although peritoneal lavage may diminish the bacterial count in the abdominal cavity,[1] it does not reduce the risk of postoperative peritonitis, sepsis or rates of other complications of peritonitis,[2] and it may enhance peritoneal reaction and provoke an inflammatory response on the surface. Human mesothelial cells in culture have been shown to have modulated inflammatory, fibrinolytic as well as procoagulant activity after exposure to lavage solutions in non-toxic dilutions.[3-5] When similar responses occur *in vivo*, peroperative irrigation of the abdominal cavity may disturb postoperative restoration of the mesothelium, physiological local defence systems and furthermore induce adhesion formation. It has been observed in both mice and rats that irrigation with solutions generally regarded as physiological acceptable, during intra-abdominal manipulations, caused more adhesions than no irrigation at all.[6, 7]

Lysis of fibrous adhesions depends on the presence of an adequate amount of plasminogen activator activity.[8, 9] Ischaemia or inflammation of the mesothelium results in a greater reduction of fibrinolytic activity, which leads to more fibrous adhesions, than does simple trauma alone.[10] Therefore, a standardised experimental rat model which induced peritoneal ischaemia was chosen to determine the effect of various solutions, commonly used in surgical practice, on peritoneal adhesion formation.

MATERIALS AND METHODS

Animals

Female Wistar rats of reproductive age weighing 150-230 g obtained from Harlan, Zeist, The Netherlands were used. They were bred under specific pathogen-free conditions and kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light and 12 hours dark periods). The rats were fed standard rat food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam, The Netherlands.

Lavage solutions

The evaluated lavage solutions were: RPMI 1640 medium (Gibco, Paisly, Scotland), NaCl 0.9% (Fresenius BV, 's Hertogenbosch, The Netherlands), polyvinylpyrrolidone iodine 1% (PVP-I, Asta Medica BV, Diemen, The Netherlands), Viaspan (an *ex vivo* storage solution, DuPont, Clakson, Canada) and chlorhexidine 0.02% (Vifor Medical SA, Huizen, The

Netherlands). Dilutions of the lavage solutions were prepared in medium (RPMI) for optimal pH (7.2-7.4) and osmolarity (280-300 mosmol/kg). All solutions were prepared under pathogen-free conditions. The analysed solutions were divided into non-aggressive and aggressive lavage solutions. Non-aggressive solutions caused only moderate activation of mesothelial functional properties in previous *in vitro* experiments.[2-5] Aggressive solutions provoked major mesothelial cytokine production and other functional activation of cultured human mesothelial cells.[3-5]

Operative procedures

To study the effect of the different solutions on adhesion formation in rats our previously described reproducible rat adhesion model was used.[11] Briefly, under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Three Surgilene 5-0 sutures (Braun, Melsungen, Germany) were applied to both lateral peritoneal sides 0.7 cm from each other and 1.5 cm downwards from the abdominal incision. All knots were double knots fastened tightly to ensure local ischaemia. Both uterine horns were exposed and sutured to the lateral peritoneum with Surgilene 6-0 (Braun) proximally and distally from the three 5-0 sutures. In this way a standardised amount of minimal peritoneal trauma was inflicted. Subsequently the abdominal cavity was exposed to 10 ml of lavage solutions (25°C) for 10 minutes. After 10 minutes the fluids were suctioned out carefully. At least 5 ml of the lavage solutions remained within the peritoneal cavity. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures.

Experimental design

Adhesion formation after peritoneal lavage with non-aggressive solutions.

In 15 rats, standardised minimal peritoneal trauma was inflicted to both lateral peritoneal sides by applying 3 sutures in, and fixating the uterine horns to the lateral peritoneum. In five rats (group I) no peritoneal lavage was performed. The other ten rats were treated with non-aggressive solutions. Five rats (group II) underwent lavage with 10 ml NaCl 0.9% and five (group III) with 10 ml povidone-iodine (PVP-I diluted 1:100 in RPMI).

Adhesion formation after peritoneal lavage with aggressive solutions.

Again minimal peritoneal trauma was inflicted to both lateral peritoneal sides in 18 rats. Six rats (group IV) underwent a lavage with the non-aggressive solution RPMI, six rats (group V) underwent a lavage with Viaspan and six (group VI) with the aggressive lavage solution chlorhexidine 0.02% (diluted in 1:10 in culture medium).

Evaluation of adhesion formation.

Fourteen days postoperatively the rats were sacrificed for assessment of post-surgical adhesion formation. Macroscopically the adhesions were scored according to their extent (quantity) and type (quality) by three independent observers. Quantification was assessed by dividing the area to be scored into eight by means of the three 5-0 sutures in the lateral peritoneum (Figure 4.1). The presence or absence of adhesions in the eight demarcated areas was scored. If there were adhesions in an area this accounted for 12.5% adhesions; a maximum of 100% adhesions could be scored. In each rat two lateral peritoneal sides were assessed. The type of adhesions formed was classified macroscopically using the Zühlke classification (Table 4.1).[12]

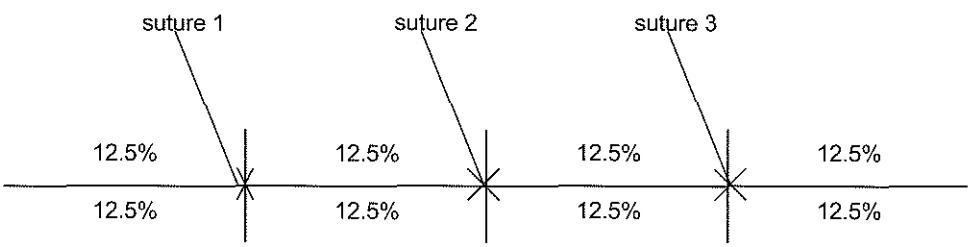

Figure 4.1

The extent of adhesion formation was quantified by dividing the "defect" area to be scored into eight areas of 12.5% by means of the three sutures.

Score	Characteristics
1	Filmy adhesion, easy to separate by blunt dissection
2	Stronger adhesion: blunt dissection possible, partly sharp dissection necessary; beginning of vascularization
3	Strong adhesion: lysis possible by sharp dissection only; clear vascularization
4	Very strong adhesion: lysis possible by sharp dissection only; organs strongly attached with severe adhesions: damage of organs hardly preventable

Table 4.1

Macroscopic (morphological) classification of abdominal adhesions according to Zühlke et al.[12]

Statistical analysis

Data were expressed as mean adhesion percentage ± SD. Statistical analysis was performed using the one-way ANOVA test to determine overall differences followed by a Bonferroni *post hoc* test to analyse differences between groups if the ANOVA test showed significance. Statistical significance was defined as $p < 0.05$

RESULTS

In almost all peritoneal cavities, adhesions were found after 2 weeks. No pathological conditions as bowel obstruction, peritonitis or abscesses had occurred.
Table 4.1 and 4.2 show that infliction of minimal (ischaemic) peritoneal trauma alone induced a mean adhesion percentage of 22.5% and that additional peritoneal lavage with all five lavage solutions gave rise to significantly higher mean adhesion percentages.

Adhesion formation after peritoneal lavage with non-aggressive solutions

Table 4.2 shows that peritoneal lavage with non-aggressive solutions as NaCl 0.9% or povidone-iodine (PVP-I) after infliction of minimal peritoneal trauma caused over 150% more adhesions than infliction of minimal peritoneal trauma alone ($p = 0.0001$). The mean adhesion percentage found after peritoneal lavage with NaCl 0.9% did not significantly differ from that observed after lavage with povidone-iodine ($p = 0.61$). Adhesions found after minimal ischaemic peritoneal trauma were all filmy (Zühlke type 1). The adhesions formed after peritoneal lavage with NaCl 0.9% or povidone-iodine were more dense and could be classified as Zühlke type 1-2. In all groups only pelvic fat took part in the adhesion formation.

Adhesion formation after peritoneal lavage with aggressive solutions.

Tables 4.2 and 4.3 show (again) that peritoneal lavage with non-aggressive solutions as RPMI or Viaspan after infliction of minimal peritoneal trauma induced about 100% more adhesion formation than infliction of minimal peritoneal trauma alone ($p \leq 0.01$). Table 4.3 also shows that peritoneal lavage of a minimally traumatised peritoneal cavity with chlorhexidine (0.02%; 1:10), an aggressive solution for mesothelial cells in culture, induced significantly more adhesion formation than lavage of minimally traumatised peritoneum with RPMI or Viaspan ($p \leq 0.012$). The adhesions found after peritoneal lavage with RPMI or Viaspan were dense (Zühlke type 2) and involved pelvic fat and seldom the uterine horn. Adhesions found after lavage with chlorhexidine were more dense (Zühlke type 3) and the uterine horn, the bowel and pelvic fat were involved in adhesion formation.

Lavage solution	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
I. No lavage	5	22.5 (8.4)	1	0.0001		
II. NaCl 0.9%	5	60.0 (11.4)	1-2		ns	
III. PVP-I 1%	5	63.8 (11.2)	1-2			0.0001

Table 4.2

Mean adhesion percentages (SD) and Zühlke classification of adhesions found after minimal peritoneal trauma alone (group I) and after peritoneal lavage with NaCl 0.9% (group II) or povidone-iodine 1% 1:100 (group III) following infliction of minimal peritoneal trauma. For each rat, the individual data concerning the 2 lateral abdominal wall sites (uterine horns) were averaged; n is the number of data (= rats) per group used for analysis. Statistics p1 (I *versus* II), p2 (II *versus* III) and p3 (I *versus* III): one-way ANOVA test, with Bonferroni *post hoc* test.

Lavage solution	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
IV. RPMI	6	47.7 (12.2)	1-2	ns		
V. Vispan	6	40.6 (13.0)	2		0.012	
VI. Chlorhexidine	6	70.8 (14.1)	2			0.003

Table 4.3

Mean adhesion percentages (SD) and Zühlke classification of adhesions found after peritoneal lavage with RPMI (group IV), Viaspan (group V) or chlorhexidine 0.02% 1:10 (group VI) following infliction of minimal peritoneal trauma. For each rat, the individual data concerning the 2 lateral abdominal wall sites (uterine horns) were averaged; n is the number of data (= rats) per group used for analysis. Statistics: p1 (IV *versus* V), p2 (V *versus* VI) and p3 (IV *versus* VI): one-way ANOVA test, with Bonferroni *post hoc* test.

DISCUSSION

Intra-peritoneal postoperative adhesion formation creates a major challenge to abdominal surgery, as it is the main cause of mechanical bowel obstruction, unexplained abdominal pain and female infertility. Furthermore the presence of dense adhesions makes reoperation technically difficult. By far the most common cause of intra-peritoneal adhesions is previous surgical intervention.[13] Intra-abdominal surgery will, by traumatising of the peritoneum, alter mesothelial activity and inflammatory responses of the peritoneum and disturb the fibrinolytic homeostasis.[14, 15] Previous *in vitro* studies show that exposure of the peritoneum to different lavage solutions enhances mesothelial functional features and provokes an inflammatory response.[2-5]

We have assessed the effect of peroperative peritoneal lavage, with non-aggressive and aggressive solutions, *in vivo* on postoperative adhesion formation. We used an established reproducible rat adhesion model in which standardised peritoneal trauma, which mimics peritoneal injury induced in patients during abdominal surgery, was inflicted before peritoneal irrigation took place. As in our previous studies the infliction of standardised minimal ischaemic peritoneal trauma caused a mean adhesion percentage of 22%. The present study shows that peritoneal lavage of areas with ischaemic injury is associated with increased adhesion formation. Aggressive as well as non-aggressive solutions caused significantly more adhesions. Peritoneal lavage with aggressive solutions increased adhesion formation significantly more than lavage with non-aggressive solutions. Irrigation with aggressive fluids gave rise to adhesions that were more dense and contained more organs than the adhesions formed after irrigation with non-aggressive solutions.

Possible explanations for the induction of adhesions by lavage solutions include cellular injury, increased peritoneal permeability and peritoneal activation.[16] We have studied cellular injury by measuring the release of an intracellular enzyme (LDH) *in vitro* and in peritoneal fluid of patients undergoing elective colonic surgery and noted increased levels of LDH after exposure to lavage solutions (unpublished data). Furthermore, permeability studies with a macromolecule (inulin) after treatment of mesothelial monolayers with lavage solutions showed disturbance of mesothelial integrity with increase permeability. This facilitated passage for macromolecules may cause increasing fibrous exudation to the abdominal cavity and thereby induce adhesion formation. *In vitro* studies also show an increased pro-inflammatory peritoneal cytokine response after exposure to various solutions. *In vivo* these increased cytokine concentrations may lead to an enhanced inflammatory response and increased adhesion formation.[2, 5] Moreover, procoagulant and fibrinolytic properties of human mesothelial monolayers were also affected by various solutions in earlier *in vitro* experiments.[5] Notwithstanding an overall increase of responses of human mesothelial cells in culture to

various kinds of clinically used lavage solutions, not all solutions induced an equal effect. In the current *in vivo* study no significant difference in the extent of postsurgical adhesion formation was observed between exposure of the peritoneal cavity to NaCl 0.9% or povidone-iodine 1%, 1:100. This discrepancy between *in vitro* and *in vivo* results may be explained as follows. Firstly, by observations indicating that the cytokine profile and peritoneal fibrinolytic activity differ in different species.[17-19] In humans, intra-peritoneal responses to stimuli may differ and be more complicated compared with responses in the rat. Secondly, povidone-iodine as a highly viscous solution reduced mesothelial cell activation *in vitro* probably by coating the cells. *In vivo* the coating effect over the entire peritoneal surface may not be as effective as *in vitro*.

Studies at our laboratories have emphasised the use of scavengers to minimise human mesothelial cell damage and activation during oxidative stress. The trauma induced in this rat model was mainly ischaemic injury. Therefore, we also tested the organ preservation fluid Viaspan, regarded as more biocompatible as it contains lactate and oxigen radical scavengers (glutathione and allopurinol). Nevertheless peroperative lavage with Viaspan also induced a significant increase of adhesion formation. However the effect of antioxidants on mechanisms underlying the initiation of fibrin appeared also to be species specific in another experimental study.[20]

In conclusion, this study is in accordance with previous *in vitro* experiments with human mesothelial cells. This study shows that exposure of the abdominal cavity to peritoneal lavage solutions may lead to more postoperative adhesion formation. Therefore, peroperative irrigation with any kind of lavage solution should be discouraged especially during surgery in non-contaminated abdominal cavities. Further studies to establish the composition of an ideal lavage solution which does not traumatise the peritoneum and which might even protect the peritoneum from the effects of trauma seems to be indicated.

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PART III

Surgical Peritoneal Trauma and Intra-Abdominal Tumour Recurrence

Chapter V

Reduction of Peritoneal Trauma by Using Non-Surgical Gauze Leads to Less Implantation Metastasis of Spilled Tumour Cells

Adapted from the original publication in the Annals of Surgery 1998; 227: 242-248

After potentially curative resection of colorectal carcinoma, the most common site for recurrence is loco-regional. We previously demonstrated that surgical trauma induces a cascade of events leading to adhesion formation. The same mechanisms may be responsible for improved tumour cell adherence and growth facilitation in early local recurrence. The objective of this study was to evaluate whether surgical peritoneal trauma affects tumour recurrence.

A reproducible rat model, in which peritoneal damage was inflicted by standardised rubbing of the peritoneum with surgical gauzes of different texture, was used to assess tumour cell adherence and growth at traumatised and non-traumatised peritoneal sites. In an analogue rat model the effect of peritoneal trauma on "ectopic" tumour growth was investigated. The final experiment evaluated how soon after peritoneal trauma tumour cell adhesion and growth promoting factors were active and whether they could be passively transferred to naive non-traumatised abdominal cavities.

A significant correlation between the amount of peritoneal trauma and degree of tumour load at damaged peritoneal surfaces was found ($p \leq 0.018$). Tumour load at not directly traumatised remote peritoneal sites was significantly higher after severe trauma than following moderate trauma of the peritoneum ($p \leq 0.005$). In addition, a significant correlation between the degree of peritoneal trauma and the growth of "ectopic" tumours, situated under the renal capsule, was observed ($p \leq 0.009$). Within a few hours after infliction of peritoneal trauma tumour cell adhesion and growth promoting effects could be passively transferred to naive recipients.

Surgical trauma is an important factor in the promotion of local tumour recurrence. The enhancing effect of trauma is not restricted to the inflicted site but rather has a generalised character. Avoidance of unnecessary surgical trauma by employing gentle techniques and materials is therefore indicated.

INTRODUCTION

Loco-regional recurrence of colorectal adenocarcinoma remains an important complication after potentially curative surgical resection. Its incidence varies between 0 and 45%.[1-4] Ways to prevent these loco-regional recurrences are the subject of various clinical and experimental studies.[5-9] The most common site for colorectal adenocarcinoma to recur is the site of the primary tumour; the second is the peritoneal surface.[2, 6, 10, 11] The tumour cell entrapment hypothesis might explain this pattern of surgical treatment failure resulting in loco-regional recurrence. When a tumour is removed, tumour cells can leak from transected lymphatics into the abdominal cavity.[10] Experimental studies have shown that the proliferative and metastatic potentials of these spilled cells are very well preserved. Consequently exfoliated carcinoma cells may undergo further division and give rise to implantation of metastases.[12, 13] However, implantation of spilled tumour cells on surfaces with intact basement membranes is an inefficient process, whereas implantation on damaged surfaces, resulting from intra-abdominal manipulation, is very efficient.[10, 14] The dynamic process of peritoneal healing following ischaemic damage of the peritoneal surfaces, sometimes leading to adhesion formation, also seems to be important in the process of adhesion and growth of spilled tumour cells to the peritoneum.[15] In clinical situations, it appears that peritoneal tumour implants may recur within a fibrous adhesion resulting from surgical trauma.[10]

In a rat model we recently showed that surgical trauma evoked by standard surgical gauze led to marked adhesion formation, which could significantly be reduced by using non-abrasive textile.[16] The present study was undertaken to evaluate whether the intra-abdominal use of this less traumatic non-surgical textile would also lead to less intra-peritoneal tumour cell adhesion and tumour growth of spilled carcinoma cells. In addition, experiments were performed to evaluate whether the relationship between degree of trauma and tumour growth was merely a local phenomenon, or whether systemic effects might also be involved.

MATERIALS AND METHODS

Animals

Female inbred WAG rats of reproductive age weighing 115-170 g (Harlan-CPB, Austerlitz, The Netherlands) were used. They were bred under specific pathogen-free conditions, kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/ 12 hours dark) and fed with standard rat food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands. Before

performing any new experiments we made sure that the model used in our adhesion studies,[16] executed on Wistar rats, were also valid in WAG rats.

Gauzes

The used "gauzes" were surgical Medipres gauze, consisting of 100% cotton, commonly used in abdominal surgery (van Heek Medical, Losser, The Netherlands), and non-surgical Fastsorb cleanroom wiper, used in the electronics industry on abrasion-sensitive surfaces (Berkshire Corporation, Great Barrington, Massachussets, USA). Fastsorb is a rayon-polyester blend which possesses strength and softness combined with a high absorbing capacity. In previous experiments we demonstrated that non-surgical Fastsorb textile was less traumatic for the peritoneum and caused less adhesion formation after intra-abdominal manipulation than surgical Medipres gauze.[16]

Tumour

Tumour CC531 is a moderately differentiated, weakly immunogenic colonic adenocarcinoma induced in WAG rats by 1,2-dimethylhydrazine.[17] It is transplantable in syngeneic WAG rats. The tumour is maintained as a cell culture in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), 1% penicillin (5000 U/ml), 1% streptomycin (5000 U/ml) and 1% L-glutamin (200 mM). Before use *in vivo* tumour cells were harvested from stationary cultures by gentle trypsinisation (5 minutes, 37°C), centrifugation (5 minutes, 700 g) and re-suspension in RPMI 1640, providing cell suspensions with a viability greater than 95%. CC531 is relatively insensitive to chemotherapy, but sensitive to the effects of biological response modifiers. To grow solid tumour, 1 x 10^8 tumour cells were injected into the right flank of a syngeneic WAG rat. After 6 weeks, a tumour mass with a volume of 2.5 cm^3 had grown and could be aseptically isolated from the outer membrane of the main lesion with a scalpel. The harvested tumour was cut into 1 mm^3 cubes (weighing 5.8 - 7.2 mg) and immersed in a culture solution stored at 4°C. Within 1 to 4 hours after collection of the solid CC531 tumour, the cubes were implanted sub-renally in syngeneic WAG rats.

Operative procedures

Under ether anaesthesia the abdomen was shaved and cleansed with alcohol 70%. Laparotomy was performed using a lower midline incision of 5 cm. Both horns of the uterus were exposed, and rubbed either with severely traumatising surgical Medipres gauze, or less traumatising non-surgical Fastsorb textile, or not manipulated at all by any gauze. Rubbing took place using a device enabling the application of a constant pressure of 120 gr/cm^2.[16] The uterus horn was rubbed 10 times over its total length. Thus, three different peritoneal traumas could be inflicted.

After performing one of these 3 procedures, the uterus horn was subsequently sutured to the lateral peritoneum, both proximally and distally, using single Surgilene 6-0 sutures (B Braun, Melsungen AG, Germany). The abdomen was closed in 2 layers with Dexon 5-0 and silk 2-0 sutures (B Braun).

Experimental design

Effect of uterus horn manipulation on intra-peritoneal tumour cell adhesion and growth

Ten rats (group I) underwent an operation in which one uterus horn was rubbed with surgical gauze and the other was not manipulated. In 10 rats (group II), one uterus horn was rubbed with non-surgical textile and the other was not touched. In 9 rats (group III), one uterus horn was rubbed with surgical gauze and the other with non-surgical textile. Directly after manipulation of the peritoneum and before closing the abdomen, 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally (0.5 ml along the left and 0.5 ml along the right abdominal wall). Three weeks after surgery, the rats were sacrificed and intra-peritoneal tumour load was scored semiquantitatively at the following sites: right uterus horn, left uterus horn, subcutaneously (at the site of the operative scar), parietal peritoneum (at the lateral abdominal wall sides where no uterus horns were fixed), kidney, liver, retroperitoneum and omentum. The scoring was performed by 2 independent observers and ranged from 0 to 5 per site according to the peritoneal cancer index derived from an index described by Steller.[18] A score of 0 meant there was no tumour growth, a score of 1 indicated an estimated tumour diameter less than 0.5 cm, a score of 2 a tumour diameter between 0.5 and 1 cm, a score of 3 a tumour diameter between 1 and 2 cm, a score of 4 a tumour diameter between 2 and 3 cm, and a score of 5 a tumour diameter of more than 3 cm (Figure 5.1). For each rat the score at all peritoneal sites, except for the uterus horns, were summarised, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is sometimes used to illustrate tumour load, which is the assumed net result of tumour cell adhesion and tumour growth, because we presume intra-peritoneal injecting of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Effect of uterus horn manipulation on established "ectopic" tumour growth

On day 1, 30 rats underwent a laparotomy using a midline incision of 2.5 cm. A solid cube of CC531 colon tumour weighing about 6 mg was placed under the capsule of both exposed kidneys under microscopic vision. Thereafter, the abdomen was closed in one layer. On day 3, all 30 rats were operated on again. Both uterus horns and 5 cm of the small bowel were rubbed, in 10 rats (group IV) with surgical gauze, and in 10 rats (group V) with non-surgical textile. Group

VI (n = 10) underwent a laparotomy only; neither the left nor the right uterus horn nor the small bowel were touched. Ten days after tumour implantation, the rats were sacrificed and growth of the sub-capsular tumours was measured by weighing the 60 e-nucleated lumps. (for each rat, the 2 individual data were averaged, 10 data per group were used for statistical analysis).

Passive transfer experiments

To evaluate whether the tumour-promoting effect of surgical trauma of the peritoneum could be passively transferred to naive non-traumatised rats, the following procedure was employed. Three rats were operated on. Two animals underwent a laparotomy, during which both uterus horns and a 5 cm long part of small intestine were rubbed with either surgical gauze (rat 1) or non-surgical textile (rat 2). The third rat only underwent a laparotomy. The abdomen was closed in one layer. After 5 hours, these rats underwent a second laparotomy during which the abdominal cavity was rinsed 5 times per rat with 5 ml RPMI 1640. Each time, 1 ml of the injected irrigant was collected.

Subsequently, 15 naive rats were treated. In 5 rats (group VII), 1 ml of irrigant collected from rat 1 (peritoneal manipulation with surgical gauze) and 0.5 x 10^6 CC531 tumour cells in 1 ml RPMI 1640 were injected intra-peritoneally along the inner left and right abdominal walls. In 5 rats (group VIII), the same procedure was performed with the irrigants collected from rat 2 (peritoneal manipulation with non-surgical textile). In the last group (group IX), irrigants collected from rat 3 (no peritoneal manipulation) were used. To ensure that all fluids were injected intra-peritoneally, the drop test was performed. In this test a drop of saline solution is placed within the open lumen of the injecting needle from which it should disappear as soon as the needle enters the peritoneal cavity because of its relative negative pressure.

After 3 weeks, the rats were sacrificed and tumour load was scored semiquantitatively at the sites depicted in Table 5.4.

Statistical analysis

The median and range of intra-peritoneal tumour load at each site, of the total tumour load and the means and standard deviations (SD) of the sub-renal tumour weights were calculated per group. Statistical analysis was performed using the Wilcoxon matched pairs test if two groups were compared and the non-parametric Kruskal-Wallis test if three groups were compared. If the latter overall test indicated significance, comparisons between groups were made using the Mann-Whitney *U* test. Statistical significance was defined as $p < 0.05$.

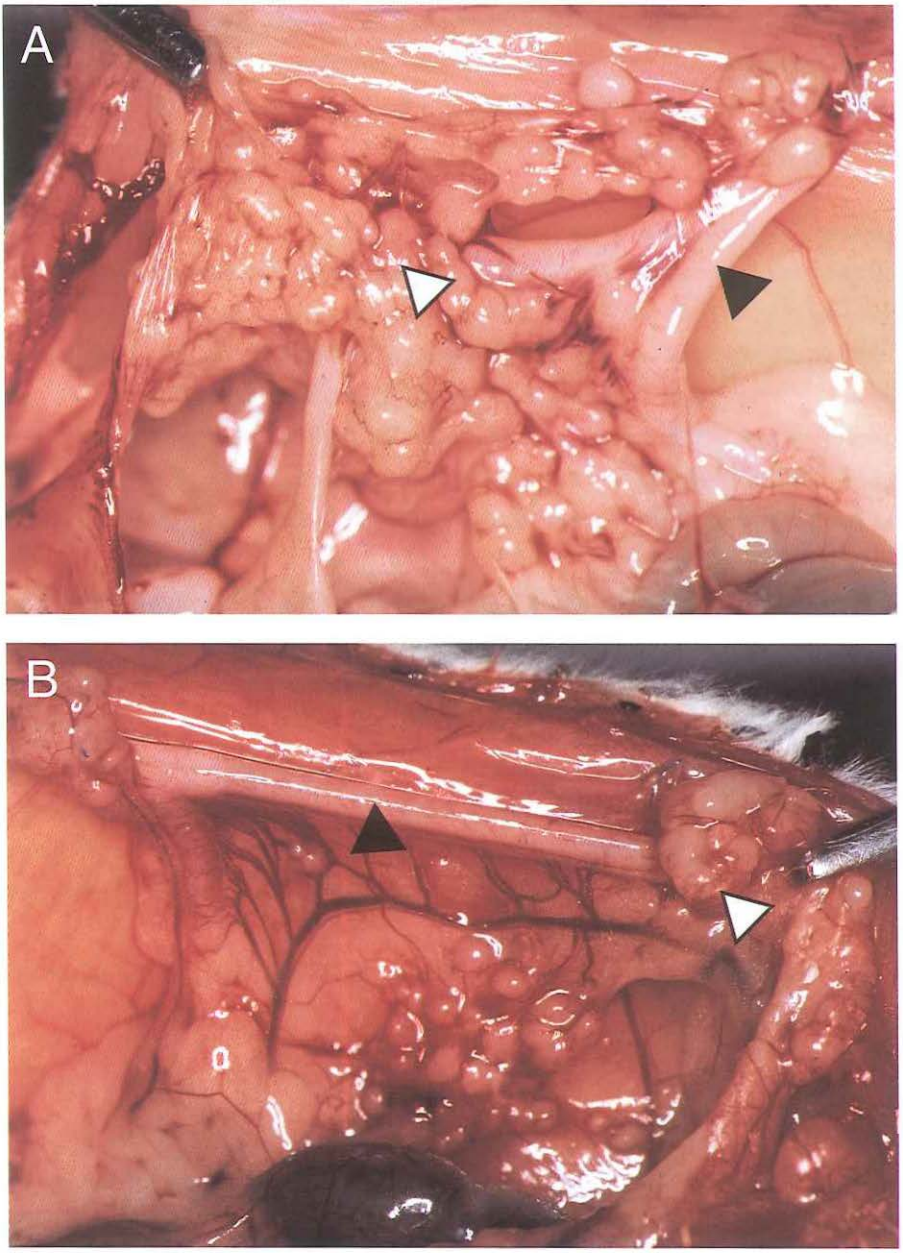

Figure 5.1

Examples of tumour load score at directly traumatised peritoneal sites. A: Tumour load at a uterus horn severely traumatised by rubbing with surgical Medipres gauze (score 5). B: Tumour load at a uterus horn mildly traumatised by rubbing with non-surgical Fastsorb textile (score 2). ▶ Uterus horn, ▷ tumour load.

RESULTS

Effect of uterus horn manipulation on intra-peritoneal tumour cell adhesion and growth

Table 5.1 summarises the results observed at the site of the uterus horns. In three different experiments, a significant correlation was found between tumour load and the degree of peritoneal trauma imposed by rubbing. Rubbing with severely traumatising surgical gauze produced the highest tumour load, whereas no rubbing resulted in the lowest (group I; $p = 0.005$). Rubbing with mildly traumatising non-surgical textile evoked a low degree of tumour load, but it was still significantly more than when no rubbing had taken place (group II; $p = 0.018$). At the site of the uterus horns, the tumours were often located in adhesions. Table 5.2 shows the tumour load at the non-manipulated remote peritoneal sites in rats from groups I, II and III. It shows significant differences in tumour load at 2 abdominal sites (the retroperitoneum ($p \leq 0.01$) and the omentum ($p \leq 0.01$)) between group II (non-surgical textile) and groups I and III (surgical gauze). A significant difference in total tumour load between the same groups (II *versus* I and II *versus* III) was found ($p \leq 0.005$). Differences in tumour load at the other 3 peritoneal sites were not significant.

Effect of uterus horn manipulation on established "ectopic" tumour growth

The mean weight of the sub-renal tumours was measured 10 days after tumour implantation, 7 days after manipulation with surgical gauze or non-surgical textile. Significant differences in mean tumour weight between the 3 groups were found (Table 5.3). Again, a significant correlation between degree of peritoneal trauma and tumour growth was observed: the mean weight of the "ectopic" tumour was the highest in rats that were rubbed with surgical gauze (group IV) and significantly lower in rats rubbed with non-surgical textile (group V) ($p = 0.009$). When the peritoneum was not touched (group VI), the lowest mean tumour weight was found, significantly lower than after rubbing with non-surgical textile ($p = 0.002$) or surgical gauze ($p < 0.0001$).

Passive transfer experiments

The median total peritoneal tumour load in rats injected with irrigants collected from abdominal cavities, manipulated with surgical gauze or non-surgical textile or not differed significantly from each other (Table 5.4, $p \leq 0.016$). These differences were mainly due to differences at the site of the omentum and the kidney. As in the previous experiments, a decreasing gradient of tumour load was found, from surgical (group VII) to non-surgical (group VIII) to non-traumatised (group IX) abdominal cavities.

Uterus horns	n	Median tumour load (range)	p1	p2	p3
I^a. No touch	10	0.0 (0-2)			
I^b. Medipres rubbing	10	4.5 (3-5)	0.005		
II^a. No touch	10	0.0 (0-2)			
II^b. Fastsorb rubbing	10	2.0 (0-3)		0.018	
III^a. Medipres rubbing	9	5.0 (4-5)			
III^b. Fastsorb rubbing	9	1.0 (0-3)			0.008

Table 5.1

Median tumour load (range) at uterus horns severely traumatised by rubbing with surgical gauze (group I^b and III^a), at uterus horns mildly traumatised by rubbing with non-surgical textile (group II^b and III^b) and at not directly traumatised uterus horns (group II^a and III^a). N is the number of uterus horns assessed. Statistics p1 (I^a *versus* I^b), p2 (II^a *versus* II^b) and p3 (III^a *versus* III^b): Wilcoxon Matched pairs test.

Abdominal sites	Tumour load I. Medipres n = 10	Tumour load II. Fastsorb n = 10	Tumour load III. M & F n = 9	p1	p2	p3
Subcutis	0 (1-3)	2 (0-3)	1 (0-4)	ns	ns	ns
Parietal peritoneum	0 (0-2)	0 (0-1)	0 (0-1)	ns	ns	ns
Kidney	1 (0-2)	1 (0-2)	1 (0-2)	ns	ns	ns
Liver	2 (0-3)	1 (0-2)	2 (1-3)	ns	ns	ns
Retroperitoneum	2 (1-3)	1 (0-2)	2 (1-3)	0.007	0.01	ns
Omentum	2 (0-3)	1 (0-1)	2 (2-4)	0.01	0.0001	ns
Total	1.5 (0-3)	1.0 (0-3)	1.5 (0-4)	0.005	0.001	ns

Table 5.2

Median tumour load (range) at different not directly traumatised peritoneal sites in rats having been intra-abdominally manipulated by severely traumatising surgical gauze (group I), mildly traumatising non-surgical textile (group II) or a combination of both materials (group III). N is the number of treated rats. Statistics p1 (I *versus* II), p2 (II *versus* III) and p3 (I *versus* III): Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

Uterus horns	n	Mean tumour weight (mg) (SD)	p1	p2	p3
IV. Medipres rubbing	10	34.14 (7.2)	0.009		
V. Fastsorb rubbing	10	28.01 (6.5)		<0.0001	
VI. No touch	10	17.80 (6.1)			0.002

Table 5.3

Mean tumour weight (SD) of the sub-renal tumours after intra-abdominal rubbing with severely traumatising surgical gauze (group IV), with mildly traumatising non-surgical textile (group V) and after no rubbing of the peritoneum at all (group VI). N is the number of operated rats, per rat the mean weight of two sub-renal tumours were assessed and used for analysis. Statistics p1 (IV *versus* V), p2 (V *versus* VI) and p3 (IV *versus* VI): Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

Abdominal sites	Tumour load VII. Medipres n = 10	Tumour load VIII. Fastsorb n = 10	Tumour load IX. No touch n = 10	p1	p2	p3
Left uterus horn	0 (0-0)	0 (0-0)	0 (0-0)	ns	ns	ns
Right uterus horn	0 (0-0)	0 (0-0)	0 (0-0)	ns	ns	ns
Subcutis	0 (0-0)	0 (0-0)	0 (0-0)	ns	ns	ns
Parietal peritoneum	0 (0-0)	0 (0-0)	0 (0-0)	ns	ns	ns
Kidney	2 (0-3)	1 (0-2)	0 (0-0)	ns	0.03	0.03
Liver	2 (1-3)	1 (0-2)	0 (0-2)	ns	ns	ns
Retroperitoneum	1 (0-3)	1 (0-2)	1 (0-2)	ns	ns	ns
Omentum	3 (2-5)	1 (0-3)	0 (0-2)	0.03	ns	0.008
Total	0 (0-5)	0 (0-3)	0 (0-2)	0.02	0.002	0.008

Table 5.4

Median tumour load (range) at different peritoneal sites in rats intra-abdominally injected with irrigant obtained from rats that underwent rubbing of the peritoneum with severely traumatising surgical gauze (group VII), mildly traumatising non-surgical textile (group VIII), or no rubbing at all (group IX). N is the number of treated rats. Statistics: p1 (VII *versus* VIII), p2 (VIII *versus* IX) and p3 (VII *versus* IX): Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

DISCUSSION

Experimental and clinical studies suggest that surgical trauma promotes tumour cell adherence and tumour growth.[15, 19-22] The mechanism by which surgical trauma promotes these processes is not completely understood but is probably multi-factorial, because tumour cell adherence as well as local and regional tumour growth can be enhanced. It seems that trauma leads to a process during which locally and regionally active tumour promoting agents are produced.[15, 19] We recently demonstrated that surgical Medipres gauze was more traumatising to the peritoneum than non-surgical Fastsorb textile, leading to significantly more adhesion formation.[16] Our current data suggest that the factors responsible for the formation of post-surgical adhesions also play a role in the adhesion and growth of tumour cells to the peritoneum. The most impressive tumour load was observed at sites where abrasion of the mesothelium was most severe. The degree of tumour recurrence at traumatised sites was highly correlated with the degree of trauma; abrasion with surgical gauze produced the highest tumour load whereas untouched peritoneum showed the lowest tumour burden and surfaces traumatised by non-surgical textile presented intermediate tumour encumbrance. The finding that traumatised surfaces are privileged sites for tumour cells has been demonstrated before.[15, 23, 24] It is conceivable that the process of enhanced tumour recurrence in traumatised tissue is biphasic. Firstly, trauma of the peritoneum and the ensuing inflammatory response will lead to upregulation of adhesion molecules, thus promoting the anchoring of tumour cells. Second, the subsequent healing of the peritoneum leads to growth promotion of the adhered tumour cells through the action of locally produced growth factors.

Using the same tumour model as in the present study, we recently demonstrated that the phenomenon of enhanced tumour recurrence as it relates to trauma and healing also occurs in other experimental settings. It was found that laparoscopic removal of a bowel segment led to less adherence and growth of intra-peritoneal tumour cells than when conventional surgery was performed, again indicating that the degree of surgical trauma was proportional to the extent of tumour recurrence.[22] In addition, we observed that the growth of a regenerating liver following partial hepatectomy led to a marked propagation of intra-hepatic tumour growth.[25, 26]

Interestingly, our present results indicate that the sequelae of peritoneal trauma with regard to tumour recurrence are not confined to the inflicted site itself, but appear to have a generalised character. We showed that trauma led to more tumour at the traumatised site and also at non-traumatised peritoneum. Again, the amount of tumour at these loco-regional sites correlated with the severity of the inflicted trauma. This clear correlation was also found in the experiment in which we studied the effect of peritoneal trauma on tumour growth under the renal capsule. Even in this "ectopic" tumour model, the consequences of the intra-abdominal trauma were

demonstrable. Because promotion of adherence was irrelevant in this model, this experiment also revealed that trauma could evoke enhancement of the growth of an established tumour. Gutman et al made comparable observations, finding that a regenerating liver induced enhanced tumour growth not only in the liver but also at distant sites.[27]

Our final experiment, in which we demonstrated that within a few hours after infliction of peritoneal trauma, the effects on tumour recurrence could be passively transferred to naive recipients, supports the notion that trauma *per se* has a marked effect, most likely on tumour cell adhesion.

Taken together, the current experiments suggest that both tumour cell adherence and tumour growth are modified by surgical trauma. It is clear that the present model provides unique possibilities to further unravel the similarities and differences between the processes of adhesion formation and tumour cell adhesion and tumour growth. Variables such as kinetics of adhesion molecule expression with regard to inflammatory cytokines and growth factors and the role of mesothelial hyaluronic acid and CD44 are currently being investigated. These studies may lead to sophisticated tools to prevent the unwanted side effects of surgery. On the other hand, the present study clearly indicates that these unwanted side effects already can partly be omitted by employing delicate surgery, using non-abrasive gauze material.

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Chapter VI

Glove Powder Promotes Adhesion Formation and Facilitates Intra-Peritoneal Tumour Cell Adhesion and Growth

Adapted from the original publication in the British Journal of Surgery 2001; 88: 1258-1263

The presence of foreign material in the abdominal cavity during surgery irritates the peritoneal surface leading to an inflammatory reaction of the peritoneum. This defensive mechanism can provoke adhesion formation. The same peritoneal defence cascade is thought to play a role in the process of intra-abdominal tumour recurrence. The aim of this study was to evaluate whether the adhesion provoking effect of glove powder could be reproduced in our rat adhesion model and to evaluate whether glove powder also promotes intra-abdominal tumour recurrence in our rat tumour cell adhesion and growth model.

Reproducible rat models allowing semiquantitative scoring of adhesion formation and tumour load were used to observe adhesion formation and tumour recurrence in three different groups of rats. One group was treated by intra-abdominal application of powder obtained from starch-powdered gloves, one by application of pure starch and in one group no application of powder took place.

Local application of glove powder or pure starch on minimally and severely traumatised peritoneum gave rise to a, comparable, significantly higher percentage of adhesion formation than infliction of peritoneal trauma alone ($p < 0.0001$). Peritoneal application of glove powder or pure starch induced significantly more intra-abdominal tumour load than when no powder was applied ($p \leq 0.002$)

Starch induced peritoneal trauma not only leads to more adhesion formation but also to increased adhesion and growth of free intra-abdominal tumour cells. This finding adds to the already existing evidence that intra-abdominal contamination with starch from starch powdered gloves should be avoided. Since good powder-free alternatives are available there is no longer any justification for the use of powdered gloves during intra-abdominal surgery.

INTRODUCTION

Postoperative adhesions are abnormal unions between peritoneal surfaces which occur after almost every intra-abdominal surgical intervention and can lead to a number of complications. A variety of therapeutic modalities to reduce postoperative adhesion formation has been studied but clinical useful therapy is currently not available. The sole available strategy to reduce postoperative adhesions is prevention by limiting peroperative trauma of the peritoneum. Over a period of 45 years, several studies have shown that exposure of peritoneum to starch powder present on surgical gloves leads to an inflammatory response of the peritoneum and contributes to adhesion formation.[1-6] Exposure of already injured peritoneal surfaces to starch enhances the intensity of the peritoneal reaction and gives rise to more and stronger adhesions than peritoneal trauma or starch alone.[7, 8] In previously described rat studies we showed that surgical trauma of the peritoneum promotes adhesion formation and that the intensity of inflicted trauma correlates with the extent of adhesion formation.[9] One aim of the present study was to evaluate whether the experimentally and clinically observed effect of glove powder on peritoneal adhesion formation could be reproduced in our rat adhesion model and to differentiate whether starch powder alone or also other glove contents are responsible for peritoneal damage and subsequent adhesions.
Following potentially curative resection of gastro-intestinal carcinoma, local recurrence and peritoneal dissemination are common in tumour recurrence.[10] Distribution patterns of first peritoneal recurrence show that the resection site is preferential, and combined recurrence on peritoneal surfaces and resection site is common.[11] Early preoperative tumour cell seeding and peroperative shedding of tumour cells, due to handling the tumour and leakage from dissected lymphatic channels, are the most likely causes of tumour recurrence.[11] The tumour cell entrapment theory proposes that the fibrinous exudate, formed as an initial response to surgical trauma of the peritoneum, facilitates implantation of cancer cells onto raw tissue.[11] The dynamic cascade of peritoneal healing, induced by peritoneal damage, leading to adhesion formation also seems to be important in the process of intra-peritoneal adhesion and growth of tumour cells. Indeed, previously described clinical and experimental studies showed that surgical trauma may promote intra-abdominal tumour recurrence.[12-14] The degree of inflicted trauma correlated with the extent of intra-abdominal tumour load.[14] The secondary aim of this study was, therefore, to analyse whether the traumatic effect of glove powder on peritoneum also promotes intra-abdominal tumour recurrence.

MATERIALS AND METHODS

Animals

Female inbred WAG rats of reproductive age weighing 120-160 g were obtained from Harlan-CPB, Austerlitz, The Netherlands. They were bred under specific pathogen-free conditions, kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light and 12 hours dark cycles) and fed with standard rat food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam, The Netherlands.

Surgical gloves; isolation of glove powder

Starch-powdered gloves (Baxter, Utrecht, The Netherlands) were cut into pieces of 1 x 1cm. The glove parts were washed in phosphate buffered saline (PBS) by gentle stirring during 24 hours. Subsequently the suspension was filtered over a stericup filter with a pore size of 0.22 µm (Millipore, Ettenleur, The Netherlands). The particles left behind on the filter were centrifuged (2500 rpm, 10 minutes). The supernatant was removed and the pellets were left to dry under sterile conditions for 24 hours. The obtained powder was used for further experiments.

Pure starch

It might be possible that enforcing the described process of isolating powder from starch powdered gloves accidentally extricated other glove contents. To investigate whether starch alone or in combination with other glove contents were responsible for any effects on adhesion formation and tumour cell adhesion and growth all experiments were also performed with pure starch obtained from Regent Hospital Products, London, UK.

Three mg of glove powder or pure starch was used in subsequent experiments. This dose is large compared to the amount of glove powder that might be left in the peritoneal cavity during laparotomy in humans. However laparotomy in humans lasts longer than in rats so glove powder will obviously irritate the peritoneum for a longer period.

Tumour

Tumour CC531 is a moderately differentiated, weakly immunogenic colonic adenocarcinoma induced in WAG rats by 1,2-dimethylhydrazine.[15] It is transplantable in syngeneic WAG rats. The tumour is maintained as a cell culture in RPMI 1640 medium supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), 1% penicillin (5000 U/mL), 1% streptomycin (5000 U/mL) and 1% L-glutamin (200 mM). Medium and all supplements were

obtained from Life Technologicals BV, Breda, The Netherlands. Cells were passaged once a week using trypsin (0.05%) and EDTA (0.02%). Before use *in vivo* tumour cells were harvested from stationary cultures by gentle trypsinisation (5 minutes, 37°C), centrifugation (5 minutes, 700 g) and re-suspension in RPMI 1640, providing cell suspensions with a viability greater than 95%. CC531 is relatively insensitive to chemotherapy but is sensitive to the effects of biologic response modifiers.

Operative procedures

To study the effect of starch-powdered glove particles on adhesion formation in rats our previously described reproducible rat adhesion model was used.[9] Briefly, under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Three Surgilene 5-0 sutures (Braun, Melsungen, Germany) were applied to both lateral peritoneal sides 0.7 cm apart and 1.5 cm away from the abdominal incision. All knots were double, fastened tightly to ensure local ischaemia. Both uterus horns were exposed, not handled and sutured to the lateral peritoneum with Surgilene 6-0 (Braun) proximally and distally from the three 5-0 sutures. In this way a standardised amount of minimal peritoneal trauma was inflicted. Standardised severe peritoneal trauma was inflicted by additively rubbing the exposed uterus horns with severely traumatising surgical Medipres gauze (van Heek Medical, Losser, The Netherlands) before suturing them to the lateral abdominal wall. Rubbing was performed with a device that enabled the application of a constant pressure of 120 g/cm^2. The uterus horns were rubbed 10 times over their total length.

Minimally and severely traumatised peritoneal sides were (further) treated locally by application of 3 mg of powder obtained from the starch-powdered gloves, by application of 3 mg of pure starch (Regent Hospital Products) or were not exposed to any kind of powder at all. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures (Braun).

To study the effect of glove powder and pure starch on local tumour recurrence our previously designed reproducible tumour adhesion and growth model was used.[14] Under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Both uterus horns were exposed and sutured to the lateral peritoneum both proximally and distally, using Surgilene 6-0 sutures. Peritoneum of the uterus horn and lateral abdominal wall side was treated locally by application of 3 mg of glove powder, by application of 3 mg of pure starch or was not exposed to any kind of powder at all. The abdomen was closed in two layers with Dexon 5-0 and 2-0 silk sutures.

Experimental design

Adhesion formation after infliction of minimal peritoneal trauma and additive application of glove powder or starch

In 20 rats standardised minimal peritoneal trauma was inflicted to both lateral peritoneal sides by applying the three sutures and fixating the uterus horns to the lateral peritoneum. In 10 rats (group I) glove powder was applied on one uterus horn while the other uterus horn was not exposed. In the other 10 rats (group II) pure starch was applied on one uterus horn while the other one was not exposed.

Adhesion formation after infliction of severe peritoneal trauma and additive application of glove powder or starch

In 19 rats standardised severe peritoneal trauma was inflicted to both lateral peritoneal sides by means of the three sutures in the lateral peritoneum and rubbing of the uterus horns. In 9 rats (group III) glove powder and in 10 rats (group IV) pure starch was applied on one uterus horn while in both groups the other uterus horn was not exposed to powder or starch.

Effect of glove powder and starch on intra-peritoneal tumour cell adhesion and growth

Nine rats (group V) underwent an operation during which both uterus horns were exposed and sutured to the lateral peritoneum. No powder was applied. Before closing the abdomen, 0.5×10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally (0.5 ml along the left and 0.5 ml along the right abdominal wall). Nine rats (group VI) underwent the same operative procedure with the additional application of 3 mg of glove powder on each uterus horn. Nine rats (group VII) underwent the same procedure during which 3 mg of pure starch was applied on both uterus horns.

Evaluation of adhesion formation

Fourteen days after laparotomy the rats were sacrificed for assessment of intra-abdominal adhesion formation. Macroscopically the adhesions were scored according to their extent (quantity) and type (quality) by two independent observers. The extent of adhesion formation was quantified by dividing the area to be scored into eight by means of the three 5-0 sutures in the lateral peritoneum (Figure 3.3, Chapter 3). The presence or absence of adhesions in the eight demarcated areas was scored. Adhesions in an area accounted for 12.5% adhesions; thus a maximum of 100% adhesions could be scored. In each rat two lateral peritoneal sides were assessed. The type of adhesions formed was classified macroscopically using the Zühlke classification (Table 3.1, Chapter 3).[16]

Evaluation of intra-peritoneal tumour cell adhesion and growth

Twenty-one days after surgery all rats were sacrificed and intra-peritoneal tumour load was scored semiquantitatively at the following sites: right uterus horn, left uterus horn, subcutaneously (at the site of the operative scar), parietal peritoneum (at the lateral abdominal wall sides where no uterus horns were fixed), kidney, liver, retroperitoneum and omentum. The scoring was performed by two independent observers and ranged from 0 to 5 per site (Table 6.1). For each rat the score at peritoneal sites, except for the uterus horns, was added and averaged, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is used to illustrate tumour load, which is the assumed net result of tumour cell adhesion and tumour growth, because we presume intra-peritoneal injecting of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Score	Characteristics
0	No tumour growth
1	Estimated tumour diameter of less than 0.5 cm
2	Estimated tumour diameter between 0.5 and 1 cm
3	Estimated tumour diameter between 1 and 2 cm
4	Estimated tumour diameter between 2 and 3 cm
5	Estimated tumour diameter more than 3 cm

Table 6.1

Tumour scoring system derived from the peritoneal cancer index described by Steller.[17]

Statistical analysis

The mean adhesion percentage and standard deviation was calculated per group. Statistical analysis was performed with a *t* test for paired samples where dependent samples were concerned and an unpaired Student's *t* test where independent samples were concerned.

The median and range of intra-peritoneal tumour load at each site and of the total tumour load were calculated per group. Statistical analysis was performed using the non-parametric Kruskall Wallis test to determine overall differences followed by the non-parametric Mann-Whitney *U* test to compare differences between groups. Statistical significance was defined as $p < 0.05$.

RESULTS

None of the rats were found to have adhesions at the initial operation. There were no postoperative complications e.g. bowel obstructions, peritonitis or tumour overgrowth.

Adhesion formation after infliction of minimal peritoneal trauma and additive application of glove powder or starch

Table 6.2 shows that application of glove powder, after minimal peritoneal trauma, led to significantly more adhesion formation than minimal peritoneal trauma alone ($p < 0.0001$). Local application of pure starch also induced a significantly higher mean adhesion percentage than minimal peritoneal trauma alone ($p < 0.0001$). The mean adhesion percentage found in the glove powder group did not differ significantly from that of the pure starch group ($p = 0.112$). Adhesions formed after minimal peritoneal trauma were filmy (Zühlke type 1-2) involving only pelvic fat. Adhesions found after local application of glove powder or pure starch were stronger (Zühlke type 2-3) and involved pelvic fat, uterus horn and small bowel.

Adhesion formation after infliction of severe peritoneal trauma and additive application of glove powder or starch

Table 6.3 shows that local application of powder obtained from starch-powdered gloves or pure starch induced additional adhesion formation, even in the presence of severe trauma to the peritoneal surface. Both gave rise to a significantly higher mean adhesion percentage than severe peritoneal trauma alone ($p < 0.0001$). As in the previous experiment there was no significant difference in the adhesion inducing capacity of glove powder and pure starch ($p = 0.47$). Adhesions found after Medipres rubbing of the uterus horn were dense and thick (Zühlke type 2-3) while application of glove powder or pure starch on severely traumatised peritoneum lead to even stronger adhesions (Zühlke type 3).

Effect of glove powder and starch on intra-peritoneal tumour cell adhesion and growth

Table 6.4 summarises results observed at peritoneal sites where glove powder or pure starch were applied (uterus horns) in comparison to controls. Glove powder as well as pure starch evoked a significant increase in tumour load at the uterus horns ($p < 0.0001$). Application of glove powder or pure starch gave rise to similar results ($p = 0.395$).

The median total peritoneal tumour load in rats treated with glove powder or pure starch, measured at peritoneal sites other than the uterus horns, differed significantly from the median total peritoneal tumour load in controls (Table 6.5, $p = 0.002$ and $p = 0.001$

respectively). These differences were mainly due to differences at the site of the subcutis and the omentum. Again treatment with glove powder or pure starch did not induce a significant different extent of total tumour load (p = 0.588).

Uterus horns	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
I^a. No powder	10	33 (10.5)	1-2			
I^b. Glove powder	10	84 (13.3)	2-3	<0.0001		
II^a. No starch	10	35 (9.9)	1-2			
II^b. Pure starch	10	81 (8.8)	2-3		<0.0001	ns

Table 6.2

Mean adhesion percentage (SD), and Zühlke classification of found adhesions, after inflicting minimal peritoneal trauma alone and after application of glove powder or pure starch following the infliction of minimal peritoneal trauma. N is the number of defected peritoneal sites (uterus horns) assessed. Statistics p1 (I^a *versus* I^b) and p2 (II^a *versus* II^b): *t* test for paired samples. Statistics p3: unpaired Student's *t* test (I^b *versus* II^b).

Uterus horns	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
III^a. Medipres rubbing	9	72 (10.4)	2-3			
III^b. Medipres rubbing & Glove powder	9	97 (5.5)	3	<0.0001		
IV^a. Medipres rubbing	10	71 (10.3)	2			
IV^b. Medipres rubbing & Pure starch	10	96 (6.08)	3		<0.0001	ns

Table 6.3

Mean adhesion percentage (SD), and Zühlke classification of found adhesions, after inflicting severe peritoneal trauma alone and after application of glove powder or pure starch following the infliction of severe peritoneal trauma. N is the number of defected peritoneal sites (uterus horns) assessed. Statistics p1 (III^a *versus* III^b) and p2 (IV^a *versus* IV^b): *t* test for paired samples. Statistics p3: unpaired Student's *t* test (III^b *versus* IV^b).

Uterus horns	n	Median tumour load (range)	p1	p2	p3
V. No powder	9	0 (0-1)	<0.0001		
VI. Glove powder	9	4 (3-5)		ns	
VII. Pure starch	9	4 (3-5)			<0.0001

Table 6.4

Median tumour load (range) at uterus horns which were only fixed to the lateral peritoneum (group V), at uterus horns which were treated with glove powder after fixation (group VI) and at uterus horns treated with pure starch after fixation (group VII). For each rat, the individual data concerning the 2 uterus horn sites were averaged; n is the number of data (= rats) per group used for analysis. Statistics p1 (V *versus* VI), p2 (VI *versus* VII) and p3 (V *versus* VII): Kruskall Wallis test with Mann-Whitney *U post hoc* test.

Abdominal sites	Tumour load V. No powder n = 9	Tumour load VI. Glove powder n = 9	Tumour load VII. Pure starch n = 9	p1	p2	p3
Subcutis	1 (0-1)	1 (1-4)	1 (1-3)	0.006	ns	0.006
Parietal peritoneum	1 (0-1)	1 (0-2)	1 (0-2)	ns	ns	0.039
Kidney	1 (0-2)	1 (1-2)	1 (0-2)	ns	ns	ns
Liver	0 (0-2)	1 (0-2)	1 (0-2)	ns	ns	ns
Retroperitoneum	1 (0-3)	1 (1-2)	1 (1-3)	ns	ns	ns
Omentum	1 (0-2)	2 (1-4)	2 (1-3)	0.037	ns	0.037
Total	0.8 (0-2)	1.3 (1-2)	1.3 (1-2)	0.002	ns	0.001

Table 6.5

Median tumour load (range) at different peritoneal sites in rats having undergone a laparotomy followed by fixation of the uterus only (group V), or laparotomy followed by fixation of the uterus horn and additive application of glove powder (group VI) or pure starch (group VII). N is number of treated rats. Statistics p1 (V *versus* VI), p2 (VI *versus* VII) and p3 (V *versus* VII): Kruskall Wallis test with Mann-Whitney *U post hoc* test.

DISCUSSION

Since the introduction of surgical gloves in 1896, several components and additives have been used in the glove industry to facilitate manufacturing and to lessen the hazards associated with glove use.[4] Modern gloves are made of two main components: rubber and glove lubricants. Glove lubricants have been associated with a number of iatrogenic problems in surgical patients.[4] Especially starch powder in surgical gloves can lead to serious complications such as granulomatous peritonitis, adhesion formation and infection potentiation.[18] These conditions themselves give rise to serious, sometimes even lethal, complications such as intestinal obstruction, infertility, chronic pelvic pain, complicated and technically difficult re-operations. The exact pathogenic mechanisms behind the undesirable reactions to starch granules are not clear and may be diverse.[19] A possible (host defence) cascade by which starch increases the propensity of tissues to form adhesions starts with irritation of the peritoneum by starch particles which leads to reduced fibrinolysis and activation of neutrophils and marophages.[20, 21] Activated macrophages produce oxygen free radicals, prostaglandin E_2, thromboxane B_2, and various cytokines which are part of the fibrotic process.[21] Our rat adhesion model previously showed that the degree of peritoneal trauma correlated with the extent of adhesion formation.[9] In the currently described experiments using the same rat model powder obtained from starch-powdered gloves as well as pure starch significantly increased adhesion formation. There was no difference in the adhesion inducing capacity of glove powder or pure starch. These results fortify the hypothesis that starch is the main component of powdered gloves that provokes adhesion formation due to a traumatising effect on the peritoneum.

We previously suggested that the peritoneal defence mechanism triggered by surgical trauma to the peritoneum not only promotes adhesion formation but also stimulates tumour recurrence.[14] Several theories speculate on the underlying mechanisms of the adhesion and growth of spilled tumour cells. According to the theory of metastatic efficiency, implantation of tumour cells onto raw tissue surfaces is an efficient process as opposed to inefficient implantation on intact surfaces.[11] The fibrin entrapment hypothesis proposes that tumour cells are trapped in fibrin at the resection site and abraded peritoneal surfaces, hereby providing protection from host defence mechanisms.[22] In peritoneal wound healing inflammatory and mesothelial cells produce an abundance of cytokines and growth factors which might also be beneficial for tumour cell adhesion and tumour growth.[23-25] Indeed a significant correlation between the intensity of surgical trauma to the peritoneum and the degree of tumour recurrence could be demonstrated in a rat tumour adhesion and growth model.[14] In the current experiments, using the same experimental model, the traumatising effect of glove powder and pure starch promoted adhesion and growth of free intra-

abdominal tumour cells. The most impressive tumour load was found at directly traumatised peritoneal sites (sites on which glove powder or starch was applied). However the tumour cell adhesion and growth promoting effects of glove powder and starch were not confined to the traumatised sites, as tumour load also was significantly higher at remote peritoneal sites. Despite the evidence in support of starch induced complications, a considerable number of general surgeons continues to wear starch powdered gloves.[26] It has been shown that careful washing of powdered gloves with saline fails to remove all the starch and even results in clumping of the residual starch granules.[7] Cleansing with povidone-iodine and sterile water did reduce the amount of starch particles,[27] but our previous study in rats showed that povidone-iodine itself induces adhesion formation.[28] This study shows that starch induced peritoneal trauma not only leads to adhesion formation but also to adhesion and growth of free intra-abdominally tumour cells. Providing substantial reason to avoid peroperative intra-abdominal contamination with starch from powdered gloves. Since good powder-free alternatives are available there is no longer any justification for the use of powdered gloves during intra-abdominal surgery.

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PART IV

Pathways of Adhesion Formation and Intra-Abdominal Tumour Recurrence after Surgical Peritoneal Trauma; Possible Methods for Prevention

Chapter VII

The Inflammatory Sequelae of Surgery Provoke Enhanced Tumour Recurrence; a Crucial Role for Neutrophils and Cytokines

Submitted for publication

After potentially curative resection of colorectal carcinoma, the most common site for recurrence is loco-regional. In rat studies we previously demonstrated that surgical peritoneal trauma is an important factor in the promotion of local tumour recurrence and that within a few hours after peritoneal trauma, surgical related factors in the abdominal cavity could be captivated in a lavage fluid and enhance tumour recurrence in naive recipients. The objective of this study was to evaluate the role of different individual inflammatory mediators, produced after abdominal surgery and captivated in a lavage fluid, in loco-regional tumour recurrence.

Using our reproducible rat model post trauma lavage fluid was collected and separated in a cellular and supernatant component, the latter containing soluble factors. Tumour recurrence was determined after injection of naive recipients with either component of the lavage fluid. The effect of soluble factors in the lavage fluid on tumour cell adhesion to a mesothelial monolayer was investigated in an in vitro model. In an additional experiment, different intensities of surgical peritoneal trauma were inflicted to identify the cell compilation in the traumatised abdominal cavity. Cytokine and growth factors concentrations were determined in the supernatant.

Intra-peritoneal injection of naive recipients with both components of the post trauma collected lavage fluid resulted in statistically significant more tumour recurrence than injection with RPMI ($p \leq 0.01$). The cellular component produced the highest tumour load. In vitro tumour cell adhesion to the mesothelium was not affected by soluble factors in the lavage fluid Analysis of the lavage fluid, gathered after minimal or severe peritoneal trauma, demonstrated a significant influx of neutrophils (PMN) after infliction of severe peritoneal trauma ($p < 0.0001$). The acute phase cytokines IL-1β, IL-6 and TNF-α were present irrespective of trauma-intensity. Statistically significant more IGF-I was detected in the lavage fluids of severely traumatised rats ($p < 0.0001$).

It seems in vivo tumour recurrence is mainly promoted by the cellular component of the post-surgical inflammatory process. Cytokines also enhance in vivo recurrence, but play an inferior role. IGF-I may facilitate the growth of lodged tumour cells.

INTRODUCTION

Surgical treatment of gastro-intestinal malignancies is often complicated by loco-regional recurrence.[1] Regardless of this detrimental adversity, surgery remains the best treatment option. In a clinical trial, Busch et al put forward an association between recurrent disease and the extent of surgical injury.[2] In experimental models, post-surgically produced factors have been shown to augment local and remote tumour growth. Experimental and clinical studies have demonstrated that enhanced tumour cell adherence and tumour growth are inevitable repercussions of surgical trauma.[1, 3-7] We previously demonstrated that within a few hours after infliction of peritoneal trauma, surgically related factors in the abdominal cavity could be captivated in a lavage fluid and enhance tumour recurrence in naive, non-operated recipients.[7]
Surgery is an inflammatory stimulus that activates the body's immune response. The ensuing influx of polymorph nuclear leukocytes (PMN) and mononuclear cells to the surgically traumatised site is mediated through chemotactic factors like IL-8 (CINC in rats) and pro-inflammatory cytokines such as IL-1β or TNF-α and is the first line of defence.[8-10]
Insights in the host defence mechanisms of the peritoneum have demonstrated that peritoneal lymphocytes, sub-mesothelial monocytes, PMN and mesothelial cells act in an orchestrated response under the control of locally expressed cytokines, chemokines and adhesion molecules. The peritoneal membrane and the mesothelium in particular, as a site for the production of mediators, play a pivotal role in the activation and control of inflammation.[11, 12]
Inflammatory products produced after abdominal surgery might participate directly or indirectly in effective tumour recurrence. In this study, we focus our attention on the individual capacity of the, by peritoneal trauma released, inflammatory peritoneal cells and soluble factors to ascertain which element is responsible for enhanced tumour recurrence. In an experimental model post surgically produced tumour enhancing factors are captivated in a lavage fluid, and passively transferred to naive recipients. The *in vivo* and *in vitro* studies presented in this paper investigate a possible correlation between the inflammatory cells and the cell free soluble factors present in the abdominal cavity after surgical trauma and postoperative tumour cell adhesion and growth.

MATERIALS AND METHODS

Animals

Female inbred rats of the WAG strain, weighing 155-200 g, were obtained from Harlan-CPB, Zeist, The Netherlands. The rats were bred under specific pathogen-free conditions, kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/ 12 hours dark) and fed with laboratory diet and water *ad libitum*. The experimental protocol

adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumour

Tumour CC531 is a moderately differentiated, weakly immunogenic colonic adenocarcinoma induced in the WAG/Rij rat by 1,2-dimethylhydrazine. It is transplantable in syngeneic WAG rats. A cell line was established from this carcinoma and maintained by serial passage after trypsinization in culture medium.[13] CC531 tumour cells were cultured in RPMI 1640 medium supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), L-glutamin (2 mM) and penicillin (10^5 U/L). Medium and all supplements were obtained from Life Technologicals BV, Breda, The Netherlands. Cells were passaged once a week using trypsin (0.05%) and EDTA (0.02%). Viability was measured by trypan blue exclusion and always exceeded 90%.

Mesothelial cell culture

Mesothelial cells (MC) were isolated from the small bowel mesentery of rats as described before.[14] Mesothelial monolayers were established in 96 well plates (Greiner, The Netherlands) precoated with collagen type I (15 μ/cm^2, Boehringer Mannheim, Mannheim, Germany). The plates were incubated at 37°C, in a humidified atmosphere of 5% CO_2 in air. Medium consisted of RPMI enriched with 10% FCS, glutamin (2 mM), penicillin (105 U/L) and fungizone (1.25 mg/L) and was replaced daily with fresh medium. Monolayers reached confluence in 2 days as determined by microscopic evaluation.

Tumour cell labelling

The dye solution, calcein-AM, used to quantify tumour cell adhesion was prepared by dissolving 50 μg calcein (Molecular Probes, Leiden, The Netherlands) in 5 μl anhydrous dimethyl sulphoxide and adding this solution to 5 ml of RPMI medium supplemented with 0.5% bovine serum albumin (RPMI/0.5%BSA). Trypsinized CC531 cells (1 x 10^6 cells/ml) were incubated in this solution at 37°C for 45 minutes with occasional mixing. Before adding to the mesothelial monolayers, the labelled cells were washed twice with RPMI/0.5%BSA to remove free dye.

Operative procedures

Under ether anaesthesia, of 14 rats the abdomen was shaved and cleansed with alcohol 70%. Laparotomy was performed using a lower midline incision of 5 cm; exposure and rubbing of both uterus horns and a 5 cm long part of the small intestine with surgical Medipres gauze inflicted subsequent trauma to the peritoneum. Rubbing was performed with a device enabling the application of a constant pressure of 120 gr/cm^2.[7] The uterus horn was rubbed 10 times over its

total length. In this way a standardised amount of peritoneal trauma can be inflicted. The abdomen was closed in one layer with silk 2-0 sutures (Braun, Melsungen AG, Germany). After 5 hours a second laparotomy was performed during which the abdominal cavity was lavaged with 5 ml RPMI 1640 medium. After massaging the abdomen the remaining fluid was aspired, pooled and kept on ice until further processing.

Experimental design

Effect of inflammatory cells and cell free soluble factors on intra-peritoneal tumour cell adhesion and growth; *in vivo* experiment

The collected post trauma lavage fluid was centrifuged, the cell pellet re-suspended to original volume with RPMI and thus divided into a "cellular" component containing the different cell types present in the abdominal cavity after surgical trauma and a "supernatant" containing soluble components produced after surgical peritoneal trauma.

Subsequently 24 rats were divided into three groups. Group I served as a control group receiving RPMI 1640 medium. Group II was acceptor for the cellular component of the post trauma lavage fluid and group III for the supernatant. Of all three components, 3 ml was injected intra-peritoneally together with 0.5 million CC531 cells (in 0.5 ml RPMI) without opening the abdominal cavity. In this way, the factors contained by the different components represented the mediators after surgical abdominal trauma, without inflicting additional trauma.

Evaluation of intra peritoneal tumour cell adhesion and growth

Tumour scoring took place three weeks after tumour injection. The rats were sacrificed and intra-peritoneal tumour load was scored semiquantitatively at the following peritoneal sites: parietal peritoneum, kidney, liver, retroperitoneum, omentum and mesentery. The scoring was performed by two independent observers using a tumour scoring system derived from the peritoneal cancer index described by Steller and ranging from 0 to 5 per abdominal site (Table 6.1, Chapter 6).[15] For each rat the score at all peritoneal sites was summarised, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is sometimes used to illustrate tumour load, which is the net result of tumour cell adhesion and tumour growth, because we presume that intra-peritoneal injecting of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Tumour cell adhesion assay; *in vitro* experiment

To demonstrate an effect of surgery related soluble factors on tumour cell adhesion to the mesothelial cells of the peritoneum, a standardised tumour cell adhesion model was used.[16]

After confluence, overnight pre-incubation of the mesothelial monolayers took place with 200 μl of the supernatant samples of lavage fluids collected directly after a midline laparotomy (minimal peritoneal trauma, n = 10), 5 hours after a midline laparotomy during which the peritoneum was traumatised by rubbing with surgical gauze (severe peritoneal trauma, n = 14) or RPMI 1640 medium enriched with 10% FCS. Non pre-incubated monolayers in mesothelial cell culture medium served as standardised control.

Medium was removed from the monolayers and 200 μl RPMI/0.5%BSA containing 30,000 calcein labelled tumour cells was added. Plates were centrifuged for 1 minute at 80 g on a Heraeus centrifuge and incubated for 1 hour at 37°C to allow cell adhesion. After this, the medium of each well was removed and washed twice with 200 μl RPMI/0.5%BSA. Fluorescence of adherent cells was measured on a Perkin Elmer plate reader using 485 excitation and 530 emission filters. On each plate a standard curve was prepared by adding different numbers of labelled tumour cells to the wells. The amount of tumour cells adhered was determined by calibrating the measured fluorescence of the experimental wells on the standard.

Cell differentiation and ELISA

Lavage fluid was collected directly after performing a midline laparotomy without handling of intra-abdominal structures (minimal peritoneal trauma, n = 10) and 5 hours after a midline laparotomy during which the peritoneum was traumatised by rubbing with surgical gauze (severe peritoneal trauma, n = 14). The collected lavage fluid samples were kept individually on ice and were separated in a cellular component and a supernatant.

The cellular component was re-suspended in RPMI medium, total cell amount was determined and HE stained cytocentrifuge slides were made for cell differentiation. Under a light microscope at a magnification of 100x, 100 cells were counted in duplicate and classified into granulocytes (eosinophils, neutrophils and mast cells) and lymphoid cells (mononuclear phagocytes and lymphocytes). Cell classification was done on duplicate slides.

The supernatants were filtered over a low binding 0.45 μm filter and stored at -80°C for further analysis. The presence of the acute phase cytokines IL-1β, IL-6 and TNF-α in addition to the growth factor IGF-I were determined by ELISA (Biomedical Diagnostics, Brugge, Belgium) and RIA (Biosource Europe, Fleurus, Belgium).

Statistical analysis

The median and range of intra-peritoneal tumour load at each site, of the total tumour load, of cell counts in lavage fluids and of different soluble factors in the supernatant of lavage fluids were calculated per group. Statistical analysis was performed using the non-parametric Kruskal-Wallis analysis of variance to determine overall differences If the latter overall test indicated

significance, comparisons between groups were made using the Mann-Whitney *U* test. The mean and standard deviation of in vitro tumour cell adhesion was calculated per group. Data were statistically analysed using analysis of variance (ANOVA) to determine overall differences, followed by the Newman-Keuls *post hoc* test to compare between groups. Statistical significance was defined as $p < 0.05$.

RESULTS

Effect of inflammatory cells and cell free soluble factors on intra-peritoneal tumour cell adhesion and growth; *in vivo* experiment

After intra-peritoneal injection of the lavage fluid samples collected after surgical trauma, diffuse peritoneal tumour load was found in all groups. However, injection with the cellular component of the lavage fluid resulted in the highest amount of recurrence. When compared to the control group (RPMI) both the cellular factors and the supernatant caused significantly enhanced tumour recurrence ($p \leq 0.01$). Injection of tumour cells with RPMI alone resulted in a total of 31% tumour load whereas injection with the cells or supernatant resulted in 79% and 67% total tumour load (Figure 7.1).

Impressive differences in peritoneal tumour deposits were observed between the groups receiving the cellular part and the supernatant of the lavage fluid. When taking the tumour size into account an obvious shift towards larger tumours is evident in the first group. Whereas no tumours larger than 2 cm were found in the control or supernatant injected group, 38% of the tumours in the cellular injected group were scored in this range (Figure 7.2). Table 7.1 shows the distribution of tumour deposits at the different peritoneal sites. Statistically significant differences in tumour load were scored in liver, retroperitoneum, omentum, and mesentery between supernatant and cellular fraction ($p \leq 0.01$).

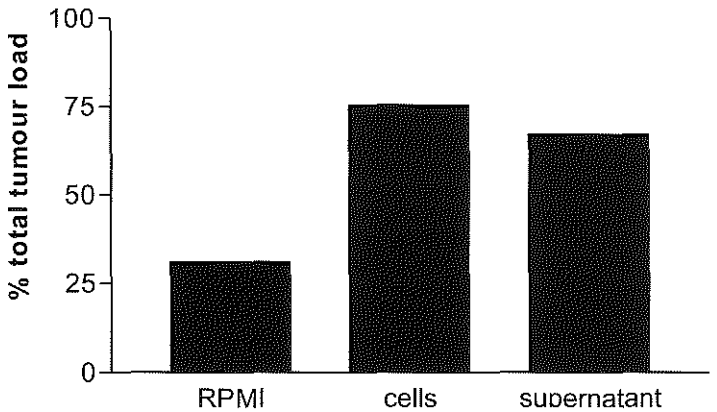

Figure 7.1

Differences in median percentages of total peritoneal tumour load (range) after passive transfer of RPMI only, the cellular fraction or the supernatant of lavage fluid collected after infliction of severe peritoneal trauma. The percentage total tumour load was defined after scoring the presence or absence of a tumour irrespective of tumour size. P1 (I *versus* II) = 0.001, p2 (II *versus* III) = 0.003 and p3 (I *versus* III) = 0.01. Statistics: Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

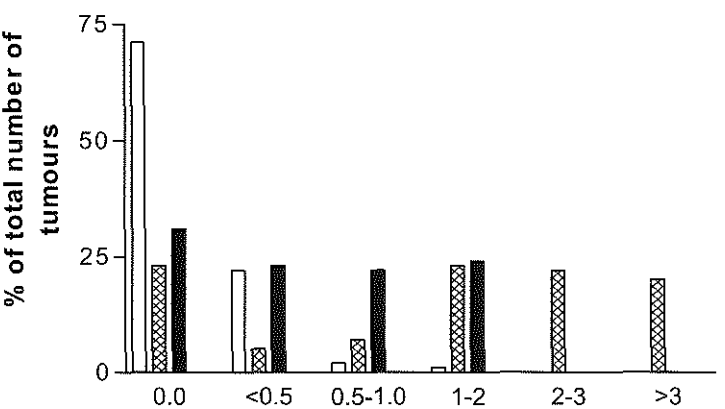

Figure 7.2

Comparison of median percentages of different tumour sizes (range) after passive transfer of RPMI only, the cellular fraction or the supernatant of lavage fluid collected after infliction of severe peritoneal trauma. Open bars represent RPMI, cross-hatched bars the cellular fraction and the filled bars the supernatant fraction. Statistics: Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

Abdominal sites	Tumour load I. RPMI n = 8	Tumour load II. Cells n = 8	Tumour load III. Supernatant n = 8	p1	p2	p3
Parietal peritoneum	0 (0-0)	0.0 (0-0)	0.0 (0-0)	ns	ns	ns
Kidney	0 (0-3)	2.5(0-4)	1.0 (0-2)	ns	ns	ns
Liver	1 (0-1)	4.0 (2-5)	2.5 (0-3)	0.001	0.01	0.01
Retroperitoneum	0 (0-2)	3.5 (3-5)	2.5 (0-3)	0.000	0.007	0.007
Omentum	0 (0-2)	4.5 (4-5)	3.0 (0-5)	0.000	0.005	0.004
Mesentery	0 (0-2)	2.0 (1-3)	0.5 (0-2)	0.007	0.03	ns
Total	0 0 (0-3)	3.0 (0-5)	1.0 (0-5)	0.001	0.003	0.01

Table 7.1

Median tumour load (range) at different abdominal sites in rats having been injected with RPMI medium (group I), the cellular fraction of the lavage fluid (group II), or the supernatant of the lavage fluid (group III). N is the number of treated rats. The p-value 1 represents differences between groups I and II; p-value 2 represents differences between group II and III; p-value 3 represents differences between groups I and III. Statistics: Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

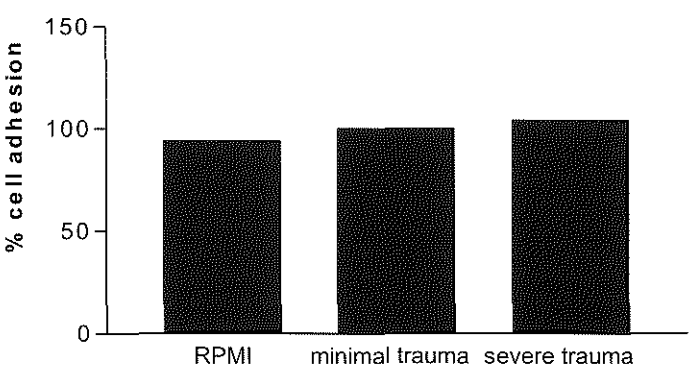

Figure 7.3

Percentage tumour cell adhesion *versus* control after pre-incubation of mesothelial cells with supernatant of lavage fluids gathered after infliction of different intensities of surgical peritoneal trauma. Mean values (SD) are shown (n = 6 per fluid sample). There was no difference in cell adhesion between groups. Statistics: ANOVA test with Newman-Keuls *post hoc* test.

Tumour cell adhesion assay; *in vitro* experiment

Figure 7.3 shows the percentage of tumour cell adhesion versus the standardised control of adhesion assay, consisting of mesothelial cell culture medium. Control cell adhesion consisted of 23% from the total amount of cells added (data not shown). RPMI did not affect tumour cell adhesion, nor did lavage fluid samples from both surgically traumatised groups.

Effect of surgical trauma on peritoneal cell compilation

There was no change in the total cell amount between minimally (laparotomy only) and severely (laparotomy and rubbing) traumatised rats (Figure 7.4). In the cell differentiation however, a significant reversal was seen regarding the ratio of granulocytes and lymphoid cells, with a 22-78% ratio of granulocyte-lymphoid cells in the minimally traumatised group *versus* a 78-22% ration in the severely traumatised group ($p < 0.0001$) (Figure 7.5). The granulocyte component consisted for 99.5% out of PMN cells.

Effect of surgical trauma on cytokine and growth factor production

IL-1β was detected in 7 out of 14 lavage fluid samples of the severely traumatised rats ranging from 20-72 pg/ml and in 2 out of 10 of the minimally traumatised rats ranging from 37 to 69 pg/ml. IL-6 was present in 13 samples of the severely traumatised (range 54-848 pg/ml) and all samples of the minimally traumatised group (range 72-1194 pg/ml), TNF-α in 2 and 5 samples of the minimally and severely traumatised rats (67 and 170 pg/ml and ranging from 35 to 110 pg/ml) respectively. IGF-I was present in all samples, however statistically significant more IGF-I was detected in lavage fluid from the severely traumatised rats ($p < 0.0001$) (Figure 7.5).

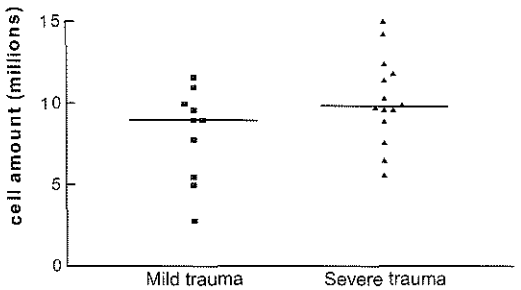

Figure 7.4

Differences in total cell amount in lavage fluids gathered from minimally and severely traumatised peritoneal cavities. Median and range are shown. Statistics: Mann-Whitney *U* test.

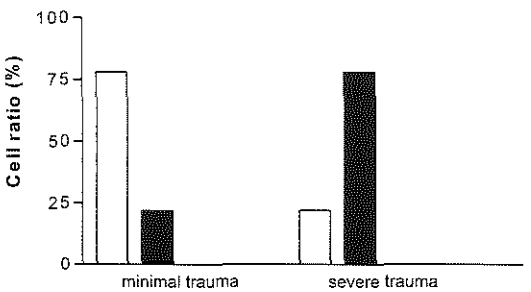

Figure 7.5

Differentiation of cellular fraction of lavage fluids taken from minimally and severely traumatised abdominal cavities. Open bars represent the median percentage lymphoid cells and filled bars the median percentage granulocytes. There was a significant shift towards granulocyte ration after severe trauma from 22 to 78%, p = 0.0001. Statistics: Mann-Whitney *U* test.

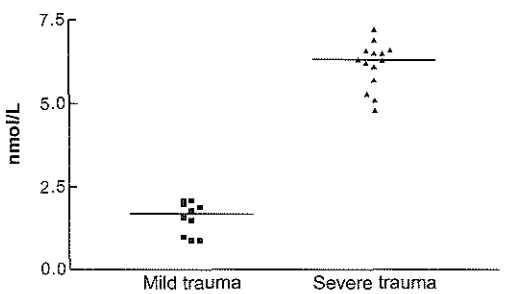

Figure 7.6

Median and range of IGF-I concentration in the supernatant fraction of lavage fluids of differently traumatised rats. Statistically significant more IGF-I was found in abdominal cavities of severely than in cavities of minimally traumatised rats, p < 0.0001. No IGF-I was found in RPMI. Statistics: Kruskal-Wallis test, with a Mann-Whitney *U post-hoc* test.

DISCUSSION

The success of surgical treatment in patients with gastro-intestinal cancer is often limited due to local recurrence or peritoneal carcinosis by peroperatively seeded tumour cells.[1] In this study, a cell seeding model was used to mimic the clinical situation of free intra-peritoneal tumour cells and associates the combination of cell adhesion and growth ultimately leading to manifest tumour recurrence. The presented results give evidence that the inflammatory sequelae of surgery enhance peritoneal tumour recurrence. In an experimental rat model, we previously demonstrated that components produced after surgical peritoneal trauma, which can be captivated in a lavage fluid collected from peritoneally traumatised abdominal cavities, could enhance tumour recurrence in naive recipients.[7] The current study demonstrates that separated components of lavage fluid collected after infliction of peritoneal trauma i.e. inflammatory cells and soluble factors, each lead to enhanced tumour recurrence.

The cellular fraction however, led to the greatest tumour load manifested by large tumours. More detailed analysis of the cellular fraction revealed a trauma related influx of granulocytes i.e. PMN into the abdominal cavity. Similar shifts in cell differentiation have also been shown in other animal models following peritoneal trauma.[9, 10, 17] The shift of primarily monocytes to PMN will evidently affect the homeostatic milieu of the peritoneum. PMN generate reactive oxygen metabolites and discharge contents of granular organelles into either phagocytic vacuoles or the local environment to ingest foreign particles or microorganisms. Both oxygen-dependent and oxygen-independent processes participate in the killing of bacteria and also in damage to host tissue.[10, 18, 19] In *vitro* increased adhesion of activated PMN to a mesothelial monolayer has been shown to induce retraction, gap formation and detachment ending with substantial mesothelial cell injury.[20] Mesothelial cell injury leads to exposure of underlying extra cellular matrix components. Experimental studies have demonstrated a preferential adhesion of tumour cells to these denuded areas.[21] Effective inhibition of tissue injury by PMN has been achieved by blocking of cell adhesion molecules used to enter the inflamed tissue[20, 22] and scavenging of reactive oxygen species.[23, 24]

A relation has been demonstrated between the extent of tissue trauma and tumour recurrence. In order to diminish tissue trauma and tumour recurrence, minimal invasive surgery is promoted.[3, 7, 21] Diminished tumour recurrence after laparoscopic surgery as compared to conventional surgery has attributed to this phenomenon.[3, 21] In addition, laparoscopic surgery appears to impact on the cellular components of the immune response less than laparotomy.[5, 25] Carbon dioxide pneumoperitoneum has been shown to impair peritoneal macrophage cytokine production (IL-1, TNT-α) and coincides with diminished neutrophil superoxide anion release and chemotaxis.[26]

In a peritonitis model, Pruimboom et al also showed a non-significant increase in cells in the peritoneal cavity. In the peritoneal cell compilation a pronounced increase of PMN was seen on the first day followed by an influx of macrophages from day 1 until seven days after induction of peritonitis. PMN produced inflammatory mediators, however the capacity of these cells was very low in comparison with peritoneal macrophages.[9, 10] In this study, the supernatant fraction of the lavage fluid, containing inflammatory factors produced after surgical trauma also enhanced tumour recurrence *in vivo*. *In vitro*, no difference was seen in the cell adhesion between lavage fluid taken from differently traumatised rats *versus* control.

The presence of the acute phase cytokines IL-1β, IL-6 and TNF-α was detected in the fluid although no significant differences in the concentration were observed between minimally and severely traumatised rats. IL-1β and TNF-α have been shown to upregulate cell adhesion molecules on mesothelial cells, which are used by PMN for adhesion.[20] In addition, cytokine activated mesothelial cells produce chemoattractant cytokines such as IL-8 (CINC in rats), required for PMN recruitment.[12, 27] In this way, the mesothelium and inflammatory cells in the abdominal cavity may perpetuate a cytokine loop, resulting in extreme activation of the inflammatory process. However, when omitting inflammatory cells, as done *in vitro*, the sole effect of cytokines present in the peritoneal fluid does not affect cell adhesion. The *in vivo* effect may therefore be direct, based on the additional trauma inflicted by inflammatory cells. High concentrations of IL-1β and TNF-α have been demonstrated to upregulate adhesion molecule expression and therefore enhance the possibility of cell adhesion.[12, 27] Taking this into account a direct cytokine effect may occur locally, at the site where cytokines are produced. In this study this effect may be missed as the absolute concentration of cytokines is diluted by lavaging with excess fluid.

Growth factors play a vital role in post surgical wound healing. Insulin like growth factor-I (IGF-I) is released during the first stage of wound healing.[28] Previous in vitro experiments demonstrated IGF-I does not affect tumour cell adhesion but was a potent growth stimulant for tumour and mesothelial cells.[29] Clinical and experimental studies have demonstrated that surgery is followed by a rapid decrease of IGF-I in serum.[30, 31] This apparent suppression of IGF-I system is thought to be caused by an increased efflux of IGF-I from the blood to the peripheral tissues.[30] In this study, significantly higher IGF-I concentrations were found in the abdominal lavage fluids from severely traumatised rats. The origin of IGF-I could be the proposed efflux from the blood or could be the result of local production by mesothelial cells.[14] IGF-I stimulation and over-production has been shown in several types of carcinoma and may support an autocrine growth loop of the tumour cells. IGF-I is also potently capable of priming PMN for an enhanced respiratory burst that may lead to additional peritoneal tissue damage.[32] In this way, tumour cells can use the hosts immune response in wound healing for their own benefit.

In conclusion, the studies brought forward in this paper provide evidence that the intra-abdominal release of inflammatory sequelae after surgery promote local tumour recurrence and that this effect is mainly based on the cellular component of the inflammatory process. Extrapolation of the presented results to the clinical situation is limited as the inflammatory response in animals deviates from humans. There is, however, limited species variation in the first line of defence after surgical peritoneal traumatisation when taking PMN influx and macrophage activation into account.[10] Preventing tissue damage by inflammatory cells therefore may provide a novel strategy to defeat progression and metastasis of cancer. Manipulation of the intricate cytokine network may cause unwanted side effects in the wound healing process. Tackling of reactive oxygen products, however, seems a feasible way of preventing cellular tissue damage ultimately resulting in diminished tumour recurrence.

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Chapter XIII

Preventing Intra-Abdominal Influx of Neutrophils after Surgical Peritoneal Trauma Reduces Adhesion Formation and Local Tumour Recurrence

Submitted for publication

Peritoneal trauma activates a cascade of peritoneal defence mechanisms responsible for postoperative adhesion formation. The same cascade is thought to play a key role in the process of intra-abdominal tumour recurrence. We previously demonstrated that within a few hours after peritoneal trauma, surgery related factors in the abdominal cavity could be captivated in a lavage fluid and enhance tumour recurrence in naive recipients. The inflammatory cells contained by the lavage fluid proved to have the strongest tumour recurrence promoting effect. FACS analysis of the inflammatory cells demonstrated a trauma related influx of neutrophils (PMN) in the abdominal cavity. We evaluated if intra-peritoneal injection of anti-neutrophil serum (ANS) could reduce post-traumatic intra-abdominal PMN influx, and if so, whether this influenced adhesion formation and tumour recurrence.

Total cell amount and differentiation of cellular parts of post-traumatic lavage fluids were estimated in four groups of rats. Reproducible rat models allowing semiquantitative scoring of adhesion formation and intra-abdominal tumour load were used to observe adhesions and tumour recurrence in the same four groups. In one group minimal peritoneal trauma was inflicted (day 0). The other groups underwent severe peritoneal trauma (day 0) without or with intra-peritoneal injections of 3 doses (day –1, 0, +1) or 1 dose ANS (day –1).

Severe peritoneal trauma provoked a significant intra-abdominal PMN influx, this influx could be prevented by ANS treatment (3 and 1 dose) ($p \leq 0.02$). Treatment with 3 doses ANS also significantly decreased blood lymphocyte, monocyte and PMN counts ($p \leq 0.02$) while treatment with 1 dose only affected blood PMN counts ($p = 0.009$). Treatment of severely traumatised rats with 3 doses ANS reduced adhesion formation ($p = 0.0001$) and induced tumour load significantly ($p \leq 0.002$), whereas treatment with 1 dose ANS caused statistically significant less tumour recurrence ($p < 0.0001$).

Intra-abdominal influx of PMN after surgical peritoneal trauma plays a crucial role in postoperative adhesion formation and in the process of adhesion and growth of spilled tumour cells. A well-balanced prevention of post-traumatic intra-abdominal PMN influx reduces adhesion formation and local tumour recurrence.

INTRODUCTION

Postoperative adhesion formation as well as loco-regional tumour recurrence of colorectal carcinomas remain important complications of potentially curative surgical intra-abdominal interventions. For neither problem clinically relevant curative treatment modalities are available yet. The pathogenesis of the processes responsible for postoperative adhesion formation and intra-abdominal tumour recurrence is only partly clarified. We previously suggested that the dynamic cascade of peritoneal healing, following peritoneal damage, not only plays an important role in postoperative adhesion formation but also in the process of intra-abdominal tumour recurrence,[1] which is in agreement with the tumour cell entrapment hypothesis.[2] A clinical trial performed at our institute indeed demonstrated an association between recurrent tumour disease and the extent of surgical injury.[3] It has also become evident from experimental studies that enhanced tumour cell adherence and tumour growth are inevitable repercussions of surgical peritoneal trauma.[1, 4-6] We demonstrated earlier that within a few hours after infliction of peritoneal trauma, factors in the abdominal cavity could be captivated in a lavage fluid and enhance tumour recurrence in naive, non-operated recipients.[1] Separated components of these lavage fluids i.e. inflammatory cells and soluble factors, could each enhance tumour recurrence, however the cellular fraction led to the greatest tumour load. More detailed analysis of the cellular fraction revealed a peritoneal trauma related influx of polymorph nuclear leucocytes (PMN) in the abdominal cavity.[7] Similar shifts in cell differentiation following peritoneal trauma have been demonstrated in other animal models.[8, 9, 10] A role of PMN in the pathophysiological cascade leading to adhesion formation has also been suggested earlier.[11, 12, 13]

Assuming that the observed post-traumatic intra-abdominal influx of PMN is an important factor in the communal dynamic cascade of peritoneal defence, responsible for both adhesion formation and local tumour recurrence, prevention of PMN influx might influence both processes and open up the way to novel therapeutic strategies. The present study was performed to evaluate whether post-traumatic intra-abdominal PMN influx could be reduced by treatment with anti-neutrophil serum (ANS), and if so, whether this reduction could influence postoperative adhesion formation and local tumour recurrence.

MATERIALS AND METHODS

Animals

Female inbred WAG rats of reproductive age weighing 140-180 g were obtained from Harlan-CPB, Austerlitz, The Netherlands. They were bred under specific pathogen-free conditions, kept under standard laboratory conditions (temperature 20-24°C, relative humidity

50-60%, 12 hours light and 12 hours dark cycles), and fed with standard rat food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam, The Netherlands.

Rabbit anti-rat neutrophil serum

Polyclonal rabbit anti-rat neutrophil serum (ANS) was purchased from Accurate, Westbury, NY, USA. ANS can deplete blood neutrophils by 99.9% when administered intra-peritoneally in a dose of 2 ml per kg bodyweight.[14, 15] The number of blood neutrophils remains at this low level until administration of ANS is stopped. In this dose ANS is not specific for neutrophils only, because the number of blood monocytes, lymphocytes and, to a lesser extent, the platelets decrease as well, i.e. by 100%, 80% and 25% respectively.[15]

In our experiments rats were either given a single dose of 1 ml ANS per kg bodyweight by intra-peritoneal injection 1 day before laparotomy (day –1) or received two additional intra-peritoneal injections of 0.5 ml ANS per kg for 2 consecutive days (day 0 and +1).

Tumour

Tumour CC531 is a moderately differentiated, weakly immunogenic colonic adenocarcinoma induced in WAG rats by 1,2-dimethylhydrazine.[16] It is transplantable in syngeneic WAG rats. The tumour is maintained as a cell culture in RPMI 1640 medium supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), 1% penicillin (5000 U/mL), 1% streptomycin (5000 U/mL), and 1% L-glutamine (200 mmol). Medium and all supplements were obtained from Life Technologicals BV, Breda, The Netherlands. Cells were passaged once a week using trypsin (0.05%) and EDTA (0.02%). Before use *in vivo*, tumour cells were harvested from stationary cultures by gentle trypsinisation (5 minutes, 37°C), centrifugation (5 minutes, 700 g), and re-suspension in RPMI 1640, providing cell suspensions with a viability greater than 90%. CC531 is relatively insensitive to chemotherapy but is sensitive to the effects of biologic response modifiers.

Operative procedures

To investigate whether treatment with ANS interferes with adhesion formation our previously described reproducible rat adhesion model was used.[17] Briefly, under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Three Surgilene 5-0 sutures (Braun, Melsungen, Germany) were applied to both lateral peritoneal sides 0.7 cm from each other and 1.5 cm downwards from the abdominal incision. All knots were double knots fastened tightly to ensure local ischaemia. Both uterus horns were exposed, not touched and sutured to the lateral peritoneum with Surgilene 6-0 (Braun)

proximally and distally from the three 5-0 sutures. In this way a standardised amount of minimal peritoneal trauma was inflicted. Severe peritoneal trauma was inflicted by additively rubbing the exposed uterus horns with surgical Medipres gauze (van Heek Medical, Losser, The Netherlands) before suturing them to the lateral abdominal wall. Rubbing was performed with a device enabling the application of a constant pressure of 120 g/cm^2. The uterus horns were rubbed 10 times over their total length. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures (Braun).

To study the effect of treatment with ANS on local tumour recurrence our reproducible tumour adhesion and growth model was used.[1] Under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Both uterus horns were exposed, not touched or rubbed with surgical Medipres gauze, and sutured to the lateral peritoneum both proximally and distally using Surgilene 6-0 sutures. In this way a standardised amount of minimal (only two sutures, no rubbing) or severe (rubbing and two sutures) peritoneal trauma was inflicted. Before closing the abdomen, 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures.

Experimental design

Effect of ANS treatment on cell content in peritoneal cavity and blood

To investigate the influence of ANS treatment on intra-abdominal neutrophil cell count and on the rat immune system the following procedures were performed. Under ether anaesthesia eighty-five rats underwent a laparotomy. In 10 rats (group A) standardised minimal peritoneal trauma was inflicted by exposing both uterus horns without rubbing them. In 25 rats (group B) standardised severe peritoneal trauma was inflicted by rubbing both exposed uterus horns and a 5 cm long part of the small intestine with surgical Medipres gauze. In 25 rats (group C) severe peritoneal trauma was inflicted in rats treated with 3 intra-peritoneal doses of ANS, on day -1, 0 and +1. In 25 rats (group D) severe peritoneal trauma was inflicted after a single intra-peritoneal injection of ANS on day -1. After 5 hours (t_1), 72 hours (t_2), 96 hours (t_3), 144 hours (t_4) and 192 hours (t_5) 5 rats of each group were operated for the second time. During this second laparotomy the abdominal cavity was lavaged with 5 ml RPMI 1640 medium. After massaging the abdomen the remaining fluid was aspirated and individually kept on ice until further processing.

Blood samples were obtained by cardiac puncture.

Adhesion formation after treatment with 3 doses of ANS

In 10 rats (group I) standardised minimal peritoneal trauma was inflicted to both lateral peritoneal sides. In 10 rats (group II) standardised severe peritoneal trauma was inflicted to both lateral peritoneal sides. In 10 rats (group III) severe peritoneal trauma was inflicted during treatment with intra-peritoneal injections of ANS on days –1, 0, and +1 perioperatively.

Intra-peritoneal tumour cell adhesion and growth after treatment with ANS

Nine rats (group IV) underwent minimal peritoneal trauma and nine (group V) severe peritoneal trauma. Nine rats (group VI) underwent severe peritoneal trauma and intra-peritoneal injection of ANS on days –1, 0 and +1 perioperatively. Before closing the abdomen 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally, 0.5 ml along the left and 0.5 ml along the right abdominal wall.

Minimal or severe peritoneal trauma was inflicted in 2 groups of 10 rats (group VII and VIII). In 10 rats (group IX) severe peritoneal trauma was inflicted after a single intra-peritoneal ANS injection on day -1. Before closing the abdomen 0.5 x 10^6 CC531 tumour cells were injected into the abdominal cavity.

Evaluation of cell content in peritoneal cavity and blood

The collected lavage fluid samples were separated in a supernatant and a cellular component by centrifugation (1500 rpm, 5 minutes). The cellular component was re-suspended in RPMI medium, total cell amount was determined and HE stained cytocentrifuge slides were made for cell differentiation. At a magnification of 100 x, 100 cells were counted in duplicate and classified into granulocytes (neutrophils, eosinophils, basophils and mast cells) and lymphoid cells (mononuclear phagocytes and lymphocytes). Total blood leukocyte counts were determined with a micro cell counter, and duplicate differential counts were carried out on May-Grünwald and Giemsa-stained blood smears.

Evaluation of adhesion formation

Two weeks after surgery, the rats were sacrificed for assessment of intra-abdominal adhesion formation. Macroscopically the adhesions were scored according to their extent (quantity) and type (quality) by two independent observers. The extent of adhesion formation was quantified by dividing the area to be scored into eight by means of the three 5-0 sutures in the lateral peritoneum (Figure 3.3, Chapter 3). The presence or absence of adhesions in the eight demarcated areas was scored. If there were adhesions in an area this accounted for 12.5% adhesions; a maximum of 100% adhesions could be scored. In each rat two lateral peritoneal sides were assessed. The type of adhesions formed was classified macroscopically using the Zühlke classification (Table 3.1, Chapter 3).[18]

Evaluation of intra-peritoneal tumour cell adhesion and growth

Three weeks after laparotomy all rats were sacrificed and intra-peritoneal tumour load was scored semiquantitatively at the following peritoneal sites: right uterus horn, left uterus horn, subcutaniously (at the site of the operative scar), parietal peritoneum (at the lateral abdominal wall sides where no uterus horns were fixed), kidney, liver, retroperitoneum, and omentum. The scoring was performed by two independent observers using a tumour scoring system derived from the peritoneal cancer index described by Steller and ranging from 0 to 5 per abdominal site (Table 6.1, Chapter 6).[19] For each rat the score at all peritoneal sites, except for the uterus horns, was summarised, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is sometimes used to illustrate tumour load, which is the net result of tumour cell adhesion and tumour growth, because we presume that intra-peritoneal injecting of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Statistical analysis

Median and range from different cell counts were estimated per group. Statistical analysis was performed using the non-parametric Kruskall Wallis test to determine overall differences followed by the non-parametric Mann-Whitney *U* test to compare differences between groups The mean adhesion percentage and standard deviation was calculated per group. Data were statistically analysed using the one-way ANOVA test to determine overall differences. If the ANOVA test was significant on a 5% level, the Student Newman Keuls *post hoc* test was carried out to make a comparison between groups.

The median and range of intra-peritoneal tumour load at each scored abdominal site and of the total tumour load were calculated per group. Statistical analysis was performed the Kruskall Wallis test followed by the Mann-Whitney *U post hoc* test. Statistical significance was defined as $p < 0.05$.

RESULTS

Effect of ANS treatment on cell content in peritoneal cavity and blood

There was a significantly higher total intra-abdominal cell count after infliction of severe peritoneal trauma than after infliction of minimal peritoneal trauma until 72 hours after the operation ($p = 0.009$) (Figure 8.1). Figure 8.1 also shows that intra-peritoneal administration of 3 doses of ANS as well as 1 dose of ANS significantly decreased the total intra-abdominal cell count after infliction of severe peritoneal trauma for at least 96 hours postoperatively ($p < 0.05$). After treatment with 1 dose of ANS the total cell count seemed to increase earlier and faster than after treatment with 3 doses, but differences were not significant at any time point ($p \geq 0.386$) (Figure 8.1). A reversal was seen in the differential cell counts of lavage fluids regarding the granulocyte-lymphocyte ratio, with a 43-57% ratio in the mildly traumatised group (A) *versus* an 81-19% ratio in the severely traumatised group (B) ($p = 0.009$). This reversal was seen till 96 hours postoperatively (Figure 8.2). Treating the rats with 3 doses (group C) as well as 1 dose (group D) of ANS did annul this reversal: a 23-77% ratio was found in both groups (t_1-t_3, $p \leq 0.01$) (Figure 8.2) at all time points. The total number of PMN's found in abdominal lavage fluids at different time points are depicted in Figure 8.3. Up till 96 hours after laparotomy severely traumatised peritoneal cavities contained significantly higher numbers of neutrophils than mildly traumatised cavities or severely traumatised peritoneal cavities treated with of ANS ($p \leq 0.02$).

Figure 8.4 summarises the results of blood differential cell counts in the 4 groups at different time points. Lymphocyte, monocyte and neutrophil counts did not differ significantly in severely or mildly peritoneally traumatised rats. Treatment with 3 doses of ANS significantly decreased these cell counts for a period of at least 96 hours ($p \leq 0.02$). This effect was not seen after treatment with 1 dose of ANS.

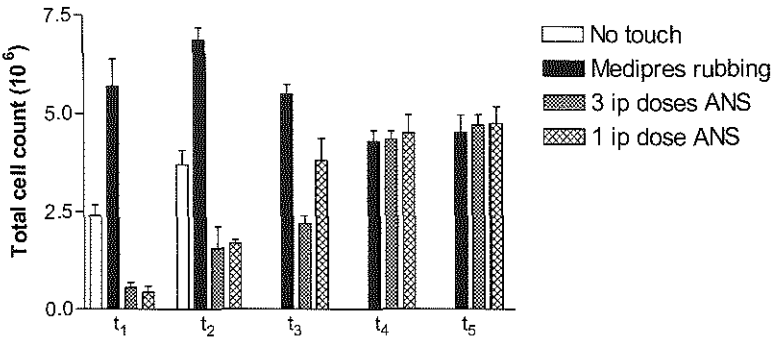

Figure 8.1

Median total cell count (range) in abdominal lavage fluids after infliction of minimal (group A, open bars) or severe (group B, filled bars) peritoneal trauma and after infliction of severe peritoneal trauma in rats treated with 3 (group C, chequered bars) or 1 (group D, cross-hatched bars) intra-peritoneal doses of ANS. Fluids were collected 5 (t_1), 72 (t_2), 96 (t_3), 144 (t_4), and 192 hours (t_5) postoperatively. Statistics p1 (A *versus* B), p2 (A *versus* C), p3 (A *versus* D), p4 (B *versus* C), p5 (B *versus* D), p6 (C *versus* D): Kruskall Wallis test with Mann-Whitney *U post hoc* test. T_1; p1-p5 = 0.009, p6 ns. T_2; p1, p4, p5 = 0.009, p2, p3 = 0.014, p6 ns. T_3; p4 = 0.021, p5 = 0.048, p6 ns. T_4; p4-p6 ns. T_5; p4-p6 ns.

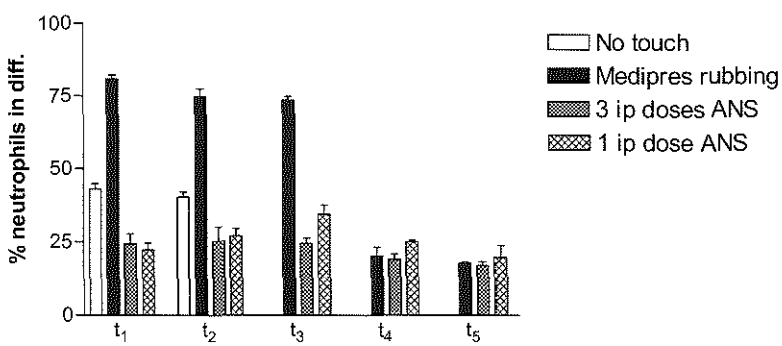

Figure 8.2

Median percentage of neutrophils (range) in lavage fluids taken from abdominal cavities after minimal (group A, open bars) or severe (group B, filled bars) peritoneal trauma and after severe peritoneal trauma in rats treated with 3 (group C, chequered bars) or 1 (group D, cross-hatched bars) intra-peritoneal doses of ANS. Fluids were collected 5 (t_1), 72 (t_2), 96 (t_3), 144 (t_4), and 192 hours (t_5) postoperatively. Statistics p1 (A *versus* B), p2 (A *versus* C), p3 (A *versus* D), p4 (B *versus* C), p5 (B *versus* D), p6 (C *versus* D): Kruskall Wallis test with Mann-Whitney *U post hoc* test. T_1; p1-p5 = 0.009, p6 ns. T_2; p1, p4, p5 = 0.009, p2 = 0.03 , p3 = 0.004, p6 ns. T_3; p4, p5 = 0.01, p6 ns. T_4; p4-p6 ns. T_5; p4-p6 ns.

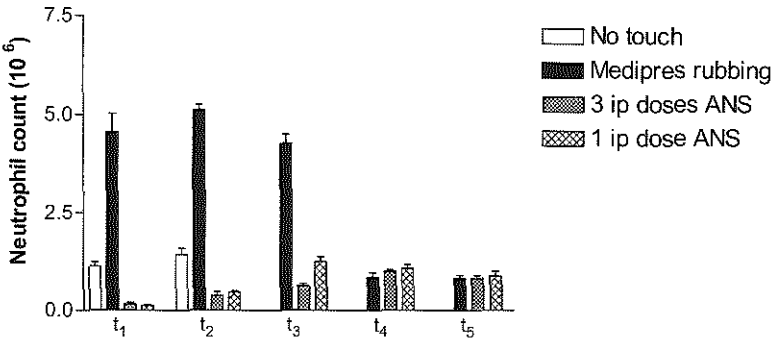

Figure 8.3

Median neutrophil granulocyt count (range) in abdominal lavage fluids after infliction of minimal (group A, open bars) or severe (group B, filled bars) peritoneal trauma and after infliction of severe peritoneal trauma in rats treated with 3 (group C, chequered bars) or 1 (group D, cross-hatched bars) intra-peritoneal doses of ANS. Fluids were collected 5 (t_1), 72 (t_2), 96 (t_3), 144 (t_4), and 192 hours (t_5) postoperatively. Statistics p1 (A *versus* B), p2 (A *versus* C), p3 (A *versus* D), p4 (B *versus* C), p5 (B *versus* D), p6 (C *versus* D): Kruskall Wallis test with Mann-Whitney *U post hoc* test. T_1; p1, p4, p5 = 0.009, p2 = 0.02. p3 = 0.014, p6 ns. T_2; p1-p5 = 0.009, p6 ns. T_3; p4, p5 = 0.021, p6 ns. T_4; p4-p6 ns. T_5; p4-p6 ns.

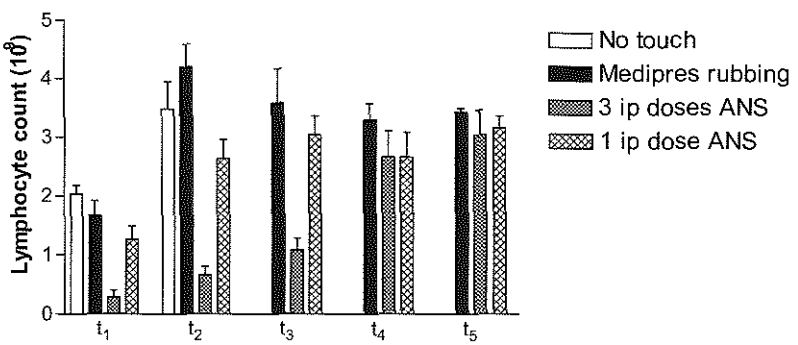

Figure 8.4

Median blood lymphocyte count after infliction of minimal (group A, open bars) or severe (group B, filled bars) peritoneal trauma and after infliction of severe peritoneal trauma in rats treated with 3 (group C, chequered bars) or 1 (group D, cross-hatched bars) intra-peritoneal doses of ANS. Blood samples were obtained 5 (t_1), 72 (t_2), 96 (t_3), 144 (t_4), and 192 hours (t_5) postoperatively. Statistics p1 (A *versus* B), p2 (A *versus* C), p3 (A *versus* D), p4 (B *versus* C), p5 (B *versus* D), p6 (C *versus* D): Kruskall Wallis test with Mann-Whitney *U post hoc* test. T_1; p1, p3, p5 ns, p2, p4 = 0.009. p6 = 0.018. T_2; p1, p3, p5 ns, p2 = 0.014, p4 = 0.009, p6 = 0.027. T_3; p4 = 0.021, p5 ns, p6 = 0.043. T_4; p4-p6 ns. T_5; p4-p6 ns.

Adhesion formation after treatment with 3 doses of ANS

Part of table 8.1 shows that the infliction of severe peritoneal trauma induced significantly more adhesions than infliction of minimal peritoneal trauma ($p = 0.0001$). It also shows that injection of 3 doses of ANS significantly reduced adhesion formation after infliction of severe peritoneal trauma ($p = 0.0001$), although the mean adhesion percentage was still significantly higher than after infliction of minimal trauma ($p = 0.0001$).

Intra-peritoneal tumour cell adhesion and growth after treatment with 3 doses of ANS

Table 8.2 shows that the median tumour load at severely traumatised peritoneal sites was significantly higher than the median tumour load at minimally traumatised peritoneal sites ($p < 0.0001$). Surprisingly, intra-peritoneal injection of 3 doses of ANS induced significantly more tumour load at severely traumatised peritoneal sites ($p = 0.002$), but also at all other not directly traumatised sites of the peritoneum ($p = 0.001$), than in controls (Table 8.2). The median total tumour load after infliction of minimal peritoneal trauma was always significantly lower than after infliction of severe peritoneal trauma irrespective of ANS treatment or not ($p < 0.0001$). These differences were due to significant differences at subcutis, kidney and omentum ($p \leq 0.037$) (Table 8.2).

Intra-peritoneal tumour cell adhesion and growth after treatment with 1 dose of ANS

Table 8.3 shows that, treatment with a single dose of ANS significantly reduced median tumour load at severely traumatised peritoneal sites ($p < 0.001$), and at remote peritoneal sites in severely traumatised abdominal cavities ($p < 0.0001$) when compared to median tumour load found at these sites in severely traumatised rats not treated with ANS.

Uterus horns	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
I. No touch	10	22.5 (7.9)	1	0.0001		
II. Medipres rubbing	10	76.3 (7.1)	2-3		0.0001	
III. Medipres rubbing & 3x ANS ip	10	46.9 (10.6)	1-2			0.0001

Table 8.1

Mean adhesion percentages (SD), and Zühlke classification of found adhesions, after infliction of minimal peritoneal trauma (group I), severe peritoneal trauma (group II) and after infliction of severe peritoneal trauma and treatment with 3 intra-peritoneal doses of ANS (group III). For each rat, the individual data concerning the 2 lateral abdominal wall sites (uterus horns) were averaged; n is the number of data (= rats) per group used for analysis. Statistics p1 (I *versus* II), p2 (II *versus* III) and p3 (I *versus* III): one-way ANOVA test, with Student Newman Keuls *post hoc* test.

Abdominal sites	Tumour load IV. No touch n = 9	Tumour load V. Medipres n = 9	Tumour load VI. 3x ANS n = 9	p1	p2	p3
Uterus horns	0 (0-0)	4.2 (4-5)	5 (5-5)	<0.0001	0.002	<0.0001
Subcutis	1 (1-2)	3 (1-4)	5 (4-5)	0.01	<0.0001	<0.0001
Parietal peritoneum	0 (0-0)	0.5 (0-1)	3 (3-4)	ns	<0.0001	<0.0001
Kidney	1 (0-2)	2.5 (2-3)	4 (3-4)	0.001	<0.0001	<0.0001
Liver	0.5 (0-2)	1 (0-2)	3 (2-4)	ns	0.001	0.001
Retroperitoneum	0 (0-0)	0 (0-1)	2 (1-3)	ns	<0.0001	<0.0001
Omentum	1 (0-2)	2 (1-3)	5 (4-5)	0.037	<0.0001	<0.0001
Total	0.8 (0-2)	1.8 (1-2)	3.7 (3-5)	<0.0001	0.001	<0.0001

Table 8.2

Median tumour load (range) at none traumatised uterus horns (group VI), at uterus horns severely traumatised by rubbing with surgical Medipres gauze (group V) and at uterus horns severely traumatised by rubbing with surgical gauze during treatment with 3 intra-peritoneal doses of ANS (group VI). For each rat, the individual data concerning the 2 uterus horn sites were averaged. Median tumour load (range) at different not directly traumatised peritoneal sites and median total tumour load following laparotomy (group IV), laparotomy and severe

peritoneal traumatasation (group V) and laparotomy, severe traumatisation and treatment with 3 intra-peritoneal doses of ANS (group VI). N is the number of treated rats. Statistics p1 (IV *versus* V), p2 (V *versus* VI) and p3 (IV *versus* VI): Kruskall Wallis test with Mann-Whitney *U post hoc* test.

Abdominal sites	Tumour load VII. No touch n = 10	Tumour load VIII. Medipres n = 10	Tumour load IX. 1x ANS n = 10	p1	p2	p3
Uterus horns	0 (0-2)	5 (4-5)	2.2 (1-4)	<0.0001	<0.0001	<0.0001
Subcutis	1 (1-2)	3 (2-5)	2 (1-3)	<0.0001	0.002	0.007
Parietal peritoneum	0 (0-0)	0 (0-1)	0 (0-1)	ns	ns	ns
Kidney	1 (0-2)	2 (0-3)	1 (0-2)	0.002	0.002	ns
Liver	0 (0-2)	0.5 (0-2)	0 (0-1)	ns	ns	ns
Retroperitoneum	0 (0-0)	0 (0-1)	0 (0-1)	ns	ns	ns
Omentum	1 (1-3)	3 (2-4)	1.5 (1-3)	0.001	0.001	ns
Total	0.6 (0-2)	1.6 (1-2)	0.8 (0-2)	<0.0001	<0.0001	ns

Table 8.3

Median tumour load (range) at none traumatised uterus horns (group VII), at uterus horns severely traumatised by rubbing with surgical Medipres gauze (group VIII) and at uterus horns severely traumatised by rubbing with surgical gauze after treatment with 1 intra-peritoneal dose of ANS (group IX). For each rat, the individual data concerning the 2 uterus horn sites were averaged. Median tumour load (range) at different not directly traumatised peritoneal sites and median total tumour load following laparotomy (group VII), laparotomy and severe peritoneal traumatisation (group VIII) and laparotomy, severe traumatisation and treatment with 1 intra-peritoneal dose of ANS (group IX). N is the number of treated rats. Statistics p1 (VII *versus* VIII), p2 (VIII *versus* IX) and p3 (VII *versus* IX): Kruskall Wallis test with Mann-Whitney *U post hoc* test.

DISCUSSION

We and others showed earlier that peritoneal trauma promotes postoperative adhesion formation, induces local tumour recurrence at traumatised peritoneal sites and even increases tumour growth at extra-peritoneal sites.[1, 3, 4, 5, 6, 17, 20, 21]

The pathogenesis of the processes leading to postoperative adhesion formation and intra-abdominal tumour recurrence is multifactorial and only partly clarified. We previously suggested that one common cascade of reactions following peritoneal damage, is responsible for both pathological conditions.[1] Following peritoneal trauma, a variety of cytokines and other inflammatory mediators are produced by activated mesothelial cells and by stampeded inflammatory cells.[22, 23] The production of mesothelial and inflammatory cell derived chemokines such as IL-8 (CINC in rats), MCP-1 and IL-1β will cause post-traumatic migration of PMN and monocytes to the injured peritoneal cavity in order to promote the peritoneal healing process.[24-28] However, these mediators and recruited cells not only serve peritoneal healing, but are believed to be responsible for adhesion formation and tumour cell adhesion and growth as well.[7, 12, 29] We showed that lavage fluids collected after infliction of peritoneal trauma could enhance tumour recurrence in naïve, non-operated recipients.[1] Detailed analysis of the cellular fraction of these lavage fluids revealed a trauma related influx of PMN in the abdominal cavity.[7] PMN's generate reactive oxygen metabolites and discharge contents of granular organelles into either phagocytic vacuoles or the local environment to ingest foreign particles or microorganisms. Both oxygen-dependent and oxygen-independent processes participate in the killing of bacteria but also may (further) damage surrounding host tissue.[8, 30, 31] The coincidence of post-traumatic intra-abdominal PMN influx with adhesion formation and tumour cell adhesion and growth is no solid prove for the role of PMN in these pathogenetic processes. Effective inhibition of tissue injury by PMN has been achieved by neutralising PMN chemoattractants,[32] blocking of PMN adhesion molecules[33, 34] and scavenging of reactive oxygen species,[35, 36] but whether this affected adhesion formation or tumour recurrence has not been investigated. For pathogenetic disorders of other organ systems, the role of PMN could be demonstrated by blocking of PMN adhesion molecules which resulted in attenuated inflammatory tissue injury and decreased organ failure.[37-40]

The present study shows that infliction of severe peritoneal trauma provokes a significant intra-abdominal PMN influx during a period of at least 96 hours. This influx could be prevented by treatment with 3 doses of ANS. Averting the post-traumatic intra-abdominal PMN influx by intra-peritoneal injection of 3 doses of ANS significantly reduced adhesion formation which indicates that neutrophils play a crucial role in the dynamic cascade of adhesion formation. However, intra-peritoneal injection of 3 doses of ANS did not decrease

local total tumour load but significantly increased tumour recurrence. Treatment with 3 doses of ANS also significantly decreased blood lymphocyte, monocyte and PMN counts, thereby seriously compromising the rat immune system. It is conceivable that this immunosuppression does not have an effect on adhesion formation but does promote tumour growth. The effect of ANS on growth of the weakly immunogenic CC531 tumour may be twofold: inhibitory by reducing PMN influx and stimulatory by the additional immunosuppression. We observed earlier that immunosuppression leads to enhanced growth of the tumour used in the current experiments.[41] Treatment of severely peritoneally traumatised rats with 1 dose of ANS did still annul the post-traumatic PMN influx, but more selectively than 3 doses, because it did not influence blood lymphocyte and monocyte count. The last experiments showed that a more selective reduction of post-traumatic PMN influx, without causing immunosuppression, was possible and indeed significantly lowered tumour cell adhesion and growth.

In conclusion, these studies demonstrated the adhesion and tumour promoting effect of PMN's. Preventing tissue damage by reduction of inflammatory cells like PMN's or by prohibiting their intra-abdominal influx might provide a novel strategy in averting adhesion formation and tumour recurrence. Wound healing processes rely on the same biological mechanisms as adhesion formation and possibly, tumour recurrence, thus manipulation of PMN has to be done selectively and in moderation to prevent unwanted side effects. Our rat models may be helpful in further unravelling the cascade of both processes to find the perfect balance between wanted and unwanted effects of manipulation of the post-traumatic inflammatory response.

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Chapter IX

Hyaluronic-Based Coating Solution for Prevention of Surgical Adhesions Has no Major Effect on Adhesion and Growth of Intra-Peritoneal Tumour Cells

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We recently demonstrated that surgical peritoneal trauma induces a cascade of defensive events that can lead to adhesion formation and adhesion and growth of peroperatively spilled tumour cells. Placement of an absorbable barrier of hyaluronic acid/carboxymethylcellulose (Seprafilm™) or low viscosity 0.4% HA (Sepracoat™) between injured peritoneal sites during abdominal surgery can reduce postoperative adhesion formation. The objective of our study was to find out whether the use of the HA containing solutions might effect the adhesion and growth of free intra-abdominal tumour cells.

Rat mesothelial cells were cultured in monolayers and adhesion of CC531 tumour cells was measured after pre-incubation of the monolayers with Sepracoat™ or PBS. Reproducible rat models allowing semiquantitative scoring of tumour load were used to observe the effect of Sepracoat™ on local tumour cell adhesion and growth at minimally and severely traumatised and remote, not directly traumatised, peritoneal sites.

Sepracoat™ had a small but significant inhibitory effect on the adhesion of CC531 tumour cells to mesothelial cell monolayers in vitro ($p < 0.05$). However, we were unable to repeat these effects in our in vivo rat models, the peroperative inoculation of Sepracoat™ did not reduce postoperative tumour load at directly minimally or severely, or indirectly traumatised peritoneal surfaces.

Sepracoat™ may inhibit adhesion of tumour cells to the mesothelium in culture but in the currently used dose it had no appreciable effect on intra-abdominal tumour cell adhesion and growth in rats.

INTRODUCTION

Peritoneal trauma is, as we recently demonstrated, not only is a causal factor for adhesion formation, but also for the enhanced adhesion and growth of tumour cells.[1, 2] Using a reproducible rat model, a significant correlation was found between the amount of peritoneal trauma and the degree of tumour recurrence. Remarkably, this was not only observed at the traumatised site, but also distantly at non-abraded peritoneal surfaces.

A new development in the field of adhesion prevention is the use of bioabsorbable formulations based on hyaluronic acid (HA). Seprafilm™ is a membrane composed of hyaluronic acid and carboxymethylcellulose with proven efficacy against adhesion formation in animals and humans.[3-5] Sepracoat™ is a more manageable coating solution, that contains 0.4% of sodium hyaluronate in phosphate buffered saline (PBS). Precursor formulations of the latter product have been demonstrated to prevent adhesion formation in various animal models.[6, 7]

Hyaluronic acid (HA) is a major component of the extra-cellular matrix (ECM) and is also secreted by mesothelial cells. ECM and mesothelial cells, either exposed or activated by surgery, play major roles in the dynamic process of tumour cell adhesion. It has been demonstrated that the presence of a HA-containing coat on mesothelial cells interferes with the adhesion of tumour cells *in vitro*.[8]

The aim of the present study was to investigate whether Sepracoat™ might interfere with intra-peritoneal tumour cell adhesion. We investigated this *in vitro* by assessing the adherence of tumour cells to cultured rat mesothelial cell monolayers, and *in vivo* in a well-defined reproducible tumour adhesion and growth model.[1]

MATERIALS AND METHODS

Animals

Female inbred WAG rats of reproductive age weighing 200-250 g were obtained from Harlan, Zeist, The Netherlands. They were bred under specific pathogen-free conditions and kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light and 12 hours dark cycles). The rats were given standard food and water *ad libitum*. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Sepracoat™

Sepracoat™ coating solution is a sterile-filtered, non-pyrogenic 0.4% (w/w) solution of sodium hyaluronate in phosphate buffered saline (PBS). It was provided by the manufacturer

(Genzyme Corporation, Boston, Massachussets, USA).

Tumour

Tumour CC531 is a 1,2 dimethylhydrazine-induced weakly immunogenic, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats.[9] The tumour is maintained in cell culture in RPMI-1640 medium supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), 1% penicillin (5000 U/mL), 1% streptomycin (5000 U/mL), and 1% L-glutammine (200 mM). All supplements were obtained from Gibco, Paisley, UK. Cells were passaged once a week using trypsin (0.05%) and EDTA (0.02%). Before their use, cells were trypsinised (5 minutes, 37°C), centrifuged (5 minutes, 700g), re-suspended in RPMI-1640 and counted. Viability was measured by trypan blue exclusion and always exceeded 95%. Tumour cells were injected within 4 hours after being obtained. CC531 is relatively insensitive to chemotherapy but is sensitive to the effect of biologic response modifiers.

Mesothelial cell culture

Mesothelial cells were isolated from the small bowel mesentery of male WAG rats as described previously.[10] In brief, during a laparotomy under ether anaesthesia window like transparent triangular sheets of mesentery were isolated and collected in Hank's Balanced Salt Solution (HBSS) containing 5% human serum albumin (CLB, Amsterdam, The Netherlands), penicillin (5000 U/mL), 1% L-glutammine (200 mM) and fungizone (1.25 mg/L). After washing the sheets twice in this medium, they were incubated in a mixture of collagenase (1g/L) and dispase (2.4 x 10^3 U/L) (Boehringer, Mannheim, Germany). Following incubation during 15 minutes at 37°C and continuous gentle shaking, the detached mesothelial cells were pelleted by centrifugation at 300 g for 5 minutes. Cell viability was determined by trypan blue and always exceeded 95%. The isolated mesothelial cells were re-suspended in culture medium consisting of RPMI 1640 supplemented with 10% foetal calf serum, penicillin (5000 U/mL), 1% L-glutamin (200 mM) and fungizone (1.25 mg/L). Medium and al supplements were obtained from Life Technologicals BV, Breda, The Netherlands. The mesentery derived cells grew forming a mesothelial monolayer in 48 well plates (Greiner, The Netherlands), pre-coated with collagen type I (15 µg/cm^2 collagen S) (cell biology Roche Diagnostics, Almere, The Netherlands). The identity of mesothelial cells was illustrated by positive immunohistochemical staining for vimentin and keratin, and the absence of von Willebrand factor-staining, as reported previously.[10] Cells for staining were harvested from confluent mesothelial cell monolayers with the typical cobblestone appearance. For all experiments, primary cell cultures were used. Isolated mesenteries from at least three different rats were used.

Operative procedures

To study the effect of Sepracoat™ on tumour cell adhesion and growth in minimally traumatised abdominal cavities a laparotomy model allowing semiquantitative scoring of tumour load was used. Under ether anaesthesia the abdomen was opened using a lower midline incision of 5 cm. Both uterus horns were left untouched, thus not damaged. Laparotomy was the only minimal surgical injury inflicted to the peritoneum. Sepracoat™ or PBS was instilled into the abdominal cavity. Half a million CC531 tumour cells, in 1 ml RPMI 1640, were left behind in the abdominal cavity and the abdomen was closed in two layers with Dexon 5-0 and silk 2-0 sutures (Braun, Melsungen, Germany).

To study the effect of Sepracoat™ on tumour cell adhesion and growth at minimally and severely traumatised peritoneal sites our reproducible tumour adhesion and growth model was used.[2] Briefly, under ether anaesthesia a laparotomy was performed using a lower midline incision of 5 cm. Sepracoat™ or PBS was instilled into the abdominal cavity. One uterus horn was exposed, not touched and sutured to the lateral peritoneum both proximally and distally with Surgilene 6-0 (Braun). In this way a standardised amount of minimal peritoneal trauma was inflicted. The other uterus horn was exposed and rubbed with severely traumatising surgical Medipres gauze (van Heek Medical, Losser The Netherlands) before suturing it to the lateral abdominal wall. Rubbing was performed with a device enabling the application of a constant pressure of 120 g/cm^2. The uterus horn was rubbed 10 times over its total length. A standardised amount of severe peritoneal trauma was inflicted this way. Half a million CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally and the abdomen was closed in two layers with Dexon 5-0 and silk 2-0 sutures (Braun).

Experimental design

Effect of Sepracoat™ on tumour cell adhesion to cultured mesothelial cell monolayers (*in vitro*)

After mesothelial monolayers, established in 48 well plates pre-coated with collagen, reached confluence (usually after 2 days as determined by light microscopy), culture medium was aspired and an arbitrarily chosen amount of 200 µl of Sepracoat™ per well was added. This was left to settle for 3 minutes after which 5 x 10^4 CC531 cells in 1 ml of culture medium were added. Non-treated mesothelial monolayers served as controls. Tumour cell adhesion to the monolayer was assessed at 1, 2, 4 and 8 hours by measuring the DNA content of the cells per well, after washing away superfluous non-adherent cells, as described previously.[11] Specific adherence was calculated by substraction of the DNA content of control monolayers. Mesothelial monolayers and cultures of CC531 were also cultured seperately with Sepracoat™

to investigate its possible interference with normal cell growth in both circumstances.

Effect of Sepracoat™ on tumour cell adhesion and growth in minimally traumatised abdominal cavities (*in vivo*)

Eighteen rats underwent a laparotomy during which both uterus horns were left untouched. In 9 rats (group I) 3 ml of Sepracoat™ and in 9 rats (group II) 3 ml of PBS was instilled in the abdominal cavity. Before closing the abdomen, 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally (0.5 ml along the left and 0.5 ml along the right abdominal wall).

Effect of Sepracoat™ on tumour cell adhesion and growth at minimally and severely traumatised peritoneal sites (*in vivo*)

In 5 rats (group III) 3 ml of Sepracoat™ and in 4 rats (group IV) 3 ml of PBS was inoculated after opening the abdominal cavity. Subsequently, in all 9 rats both uterus horns were exposed and sutured to the lateral peritoneum. All right uterus horns were rubbed with severely traumatising Medipres gauze before fixation and all left uterus horns remained untouched. Before closing the abdomen, 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were inoculated intra-peritoneally (0.5 ml along the left and 0.5 ml along the right abdominal wall).

Evaluation of intra-peritoneal tumour cell adhesion and growth

Three weeks postoperatively the rats were sacrificed and tumour load was scored semiquantitatively at the following sites: right uterus horn, left uterus horn, subcutaneously (at the site of the operative scar), parietal peritoneum (at the lateral abdominal wall sides where no uterus horns were fixated), kidney, liver, retroperitoneum and omentum. The scoring was performed by 2 independent observers using a tumour scoring system derived from the peritoneal cancer index described by Steller and ranging from 0 to 5 per abdominal site (Table 6.1, Chapter 6).[12] For each rat the score at all peritoneal sites, except for the uterus horns, were summarised, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is sometimes used to illustrate tumour load, which is the assumed *in vivo* net result of tumour cell adhesion and tumour growth, because we presume intra-peritoneal injecting of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Statistical analysis

The mean and standard deviation of in vitro tumour cell adhesion was calculated per group. Data were statistically analysed using analysis of variance (ANOVA) to determine overall

differences, followed by the Newman-Keuls *post hoc* test to compare between groups. Statistical significance was defined as $p < 0.05$. The median and range of intra-peritoneal tumour load at each site and of the total tumour load were calculated per group. Statistical analysis was performed using the non-parametric Mann-Whitney *U* test. Statistical significance was defined as $p < 0.05$.

RESULTS

Effect of Sepracoat™ on tumour cell adhesion to cultured mesothelial cell monolayers (*in vitro*)

Sepracoat™ had no effect on growth or survival of mesothelial cells and CC531 tumour cells (data not shown). The effect of Sepracoat™ on the adhesion of tumour cells to mesothelial cell monolayers is shown in figure 9.1. Although the differences were small, tumour cell adhesion in the presence of Sepracoat™ was significantly less than in controls at all time points ($p < 0.05$)

Effect of Sepracoat™ on tumour cell adhesion and growth in minimally traumatised abdominal cavities (*in vivo*)

Table 9.1 shows that intra-abdominal injection of Sepracoat™ after infliction of minimal peritoneal trauma did not significantly decrease the median total tumour load in comparison to the median total tumour load found in abdominal cavities injected with PBS ($p = 0.6$). Only at one peritoneal site (the parietal peritoneum) the median tumour load was significantly lower in the group injected with Sepracoat™ than in the control group injected with PBS ($p = 0.03$).

Effect of Sepracoat™ on tumour cell adhesion and growth at minimally and severely traumatised peritoneal sites (*in vivo*)

Table 9.2 shows that neither at the severely nor at the minimally traumatised peritoneum of the uterus horn the median tumour load could be significantly impaired by intra-abdominal inoculation of Sepracoat™ before peritoneal traumatisation ($p \geq 0.8$).

The median tumour load at not directly traumatised peritoneal sites and the median total tumour load did not differ significantly in groups intra-abdominally treated with Sepracoat™ or PBS ($p \geq 0.05$) (Table 9.3).

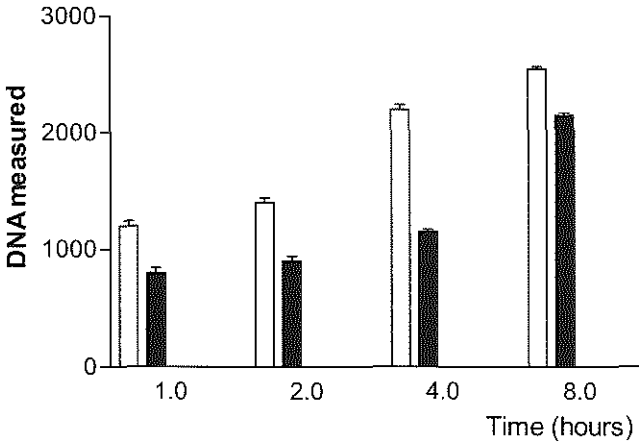

Figure 9.1

Time dependent adhesion of CC531 tumour cells to mesothelial monolayers in the presence (filled bars) or absence (open bars) of Sepracoat™. $P < 0.05$ at all time points. The number of cells adhering was estimated by DNA measurements (ng of DNA per well). Data were expressed as the mean and SD. Statistics: ANOVA test with Newman-Keuls *post hoc* test.

Abdominal sites	Tumour load I. Sepracoat™ n= 9	Tumour load II. PBS n = 9	p
Uterus horns	0 (0-0)	0 (0-0)	ns
Subcutis	3 (0-4)	3 (1-3)	ns
Parietal peritoneum	0 (0-1)	1 (0-3)	0.03
Kidney	2 (0-4)	2 (0-3)	ns
Liver	1 (1-3)	1 (0-3)	ns
Retroperitoneum	1 (0-3)	1 (0-3)	ns
Omentum	2 (1-4)	2 (1-4)	ns
Total	2 (0-4)	2 (0-4)	ns

Table 9.1

Median tumour load (range) at different not directly traumatised peritoneal sites in rats having undergone a laparotomy followed by injection of 3 ml Sepracoat™ (group I) or 3 ml PBS (group II). For each rat, the individual data concerning the 2 uterus horn sites were averaged; n is the number of data (= rats) per group used for analysis. Statistics: Mann-Whitney *U* test.

Abdominal sites	Tumour load III. Sepracoat™ n = 5	Tumour load IV. PBS n = 4	p
Medipres rubbing	4 (2-4)	4 (2-4)	ns
No touch	4 (2-4)	3 (2-4)	ns

Table 9.2

Median tumour load (range) at uterus horns severely traumatised by rubbing with Medipres gauze and at not directly traumatised uterus horns in rats inoculated with 3 ml Sepracoat™ (group III) or 3 ml PBS (group IV). N is the number of data (uterus horns) per group used for analysis. Statistics: Mann-Whitney *U* test.

Abdominal sites	Tumour load III. Sepracoat™ n = 5	Tumour load IV. PBS n = 4	p
Subcutis	3 (0-4)	3 (1-3)	ns
Parietal peritoneum	0 (0-0)	0 (0-0)	ns
Kidney	1 (0-2)	1 (0-2)	ns
Liver	1 (0-2)	1 (0-3)	ns
Retroperitoneum	1 (0-3)	1 (0-3)	ns
Omentum	2 (1-4)	2 (2-4)	ns
Total	2 (0-4)	1.5 (0-4)	ns

Table 9.3

Median tumour load (range) at different not directly traumatised peritoneal sites in rats having undergone a laparotomy followed by inoculation of 3 ml Sepracoat™ (group III) or 3 ml PBS (group IV). In all rats at least one uterus horn was rubbed with severely traumatising Medipres gauze. N is the number of data (= rats) per group used for analysis. Statistics: Mann-Whitney *U* test.

DISCUSSION

Sheets and solutions containing HA have clinically and experimentally shown to be effective in preventing surgery-induced adhesion formation.[3-8] HA is an important component of the extra cellular matrix (ECM) and mesothelial cells, both of which play pivotal roles in tumour cell adhesion. HA is a principal ligand for CD44 which is an ubiquitous, multistructural and multifunctional surface adhesion molecule regulating cell-cell and cell-matrix interactions.[13] CD44 and its variants are present on many cancer cell types and have found to be involved in local tumour progression and metastasis.[14, 15] CD44 is also present on tumour CC531.[16] Therefore, a major objective of the current study was to investigate whether peroperative application of a HA containing solution, i.e. Sepracoat™, might affect the local adhesion and growth of "spilled" tumour cells. We anticipated that pre-treatment with Sepracoat™ might reduce the severity of peritoneal trauma by protecting the mesothelial cell lining, and thus prevent activation of the mesothelium and exposure of ECM. Consequently, tumour cell adhesion might have been decreased, especially at traumatised sites. The results obtained in the tumour adhesion and growth model indicate that this was not the case. If anything, Sepracoat™ seemed to enhance tumour cell adhesion to traumatised peritoneal sites, resulting in a higher score at the uterine horns and subcutis, however the differences were not statistically significant. It is noteworthy that there was no difference between the tumour load scores at the rubbed and unabraided uterus horns. A difference might have been expected because of the recently demonstrated relationship between degree of trauma and tumour load.[2] The explanation for this seemingly discrepancy is that local intra-peritoneal trauma does affect the whole peritoneal cavity. The existence of this emanation phenomenon has currently been demonstrated by van den Tol et al.[2] In the laparotomy model the inflicted surgical trauma was relatively mild and consisted of laparotomy only. We previously demonstrated that this procedure suffices to enhance the intra-peritoneal adhesion and growth of CC531 tumour cells considerably, as compared to the minimal outgrowth occurring in rats that did not undergo surgery.[17] Sepracoat™ applied in this mild surgical setting may, theoretically, act in two opposite directions. Firstly, it may decrease mesothelial activation and consequently tumour cell adhesion by preventing dissication. On the other hand, Sepracoat™ applied on an intact mesothelial surface may improve tumour cell adhesion by providing numerous sites for HA-CD44 interaction. The results in our *in vitro* studies do not support the hypothesis that Sepracoat™ may improve tumour cell adhesion; on the contrary the adhesion of tumour cells to mesothelial monolayers was inhibited. Our *in vivo* results in the laparotomy model were obvious; except for one site, there were no significant differences in tumour load scores between the two groups. Possibly this was the net result of the two opposing mechanisms

described above.

In conclusion, although the *in vitro* results indicate that Sepracoat™ may inhibit the adhesion of tumour cells to mesothelial surfaces, this effect had no impact *in vivo*, when it had neither a beneficial nor a detrimental effect on intra-abdominal tumour cell adhesion and growth in the models used.

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Chapter X

Icodextrin® Is a Potent Inhibitor of Postoperative Adhesion Formation in Rats but Does not Affect Intra-Peritoneal Tumour Cell Adhesion and Growth

Submitted for publication

Postoperative adhesion formation and intra-abdominal tumour recurrence are significant clinical problems causing severe morbidity and mortality. Peroperative peritoneal trauma activates a cascade of peritoneal defence mechanisms responsible for postoperative adhesion formation. The same cascade may, according to our previous studies, play a key role in the process of intra-abdominal tumour recurrence after "curative" resection of colorectal carcinomas. The purpose of the present study was to evaluate the influence of a new glucose polymer solution, which physically separates peroperatively injured peritoneal surfaces (icodextrin®), on postoperative intra-abdominal adhesion formation and tumour recurrence.

Reproducible rat models allowing semiquantitative scoring of adhesion formation or tumour load were used in three different groups of rats. After the infliction of severe peritoneal trauma one group was treated by peroperative intra-abdominal instillation of a 7.5% icodextrin® solution, one by instillation of RPMI (placebo) and in one group there was no instillate (controls).

Treatment of severely peritoneally traumatised rats with icodextrin® caused a more than 60% reduction in postoperative adhesion formation ($p < 0.0001$), while RPMI treatment had no significant effect. Peroperative intra-abdominal treatment with icodextrin® did not affect intra-peritoneal tumour cell adhesion and growth of free intra-abdominal tumour cells in severely traumatised peritoneal cavities.

Icodextrin® was found to be a well tolerated, safe and easy to use solution that effectively reduces extent and severity of postoperative adhesion formation in rats without promoting or inhibiting tumour recurrence.

INTRODUCTION

Postoperative adhesion formation is a major, up till now unavoidable complication of any kind of abdominal surgery. Postoperative adhesions occur in 55-100% of patients undergoing surgery, with an average of approximately 85%.[1-3] These adhesions can result in bowel, fertility and abdominal syndromes. Adhesions are the cause of 30% of all bowel obstructions,[4-6] of 15-20% of infertility in women[7, 8] and of 13-26% of chronic pelvic pain in women.[9, 10] Adhesions also increase the technical difficulty and the risk of intraoperative complications at subsequent surgery.[11] Clearly, in addition to morbidity and mortality adhesion formation also has financial consequences.[12-14] A simple cost effective method to reduce or prevent adhesion formation is therefore needed.

Different animal and clinical studies have indicated that placement of absorbable barriers like expanded polytetraflouroethylene (Preclude®), polaxamer 407, oxidized regenerated cellulose (INTERCEED®), or hyaluronic acid/carboxymethylcellulose (Seprafilm™) between injured peritoneal sites, or the peroperative intra-abdominal application of a viscous solution such as ionically cross-linked 0.5% hyaluronic acid (Intergel™), 32% dextran 70 (Hyskon®) or low viscosity 0.04% hyaluronic acid (Sepracoat™), can reduce postoperative adhesion formation.[15-23] A major disadvantage of the site-specific adjuvants is that the surgeon must augur adhesion formation sites to determine barrier placement. Non site-specific adjuvants show doubtful adhesion preventive qualities and are associated with undesirable local and systemic side effects like oedema, body weight and central venous pressure increase, and transient liver function disturbances.[24-26] To overcome these problems, a glucose polymer solution already successfully and safely used in peritoneal dialysis was recently further developed into a fluid that is absorbed only slowly, allowing prolonged "hydroflotation" of the peritoneal cavity.[27, 28] One aim of this study was to further evaluate the adhesion reducing effect of this new glucose polymer solution, icodextrin®, in a well-defined rat adhesion model.

Despite intentionally curative resection for gastro-intestinal carcinoma, local recurrence and peritoneal dissemination are a common cause for post-surgical tumour recurrence.[29] Distribution patterns of first peritoneal recurrence show that the resection site is preferential, and combined recurrence on peritoneal surfaces and resection site is common.[30] We and others previously suggested that the dynamic cascade of peritoneal healing, induced by peritoneal damage, leading to adhesion formation also seems to be important in the process of intra-peritoneal adhesion and growth of tumour cells.[30, 31] Indeed previously described clinical and experimental studies showed that surgical trauma may promote intra-abdominal tumour recurrence.[31-33] The degree of inflicted peritoneal trauma correlated with the extent of intra-abdominal tumour load.[31] The second aim of this study was to analyse the effect of icodextrin® on tumour recurrence. Using a reproducible rat tumour adhesion and growth

model we analysed whether icodextrin® might reduce or possibly promote the adhesion and growth of intra-peritoneally injected tumour cells.

MATERIALS AND METHODS

Animals

Female inbred WAG rats of reproductive age weighing 145-190 g were obtained from Harlan-CPB, Austerlitz, The Netherlands. The rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light and 12 hours dark cycles), fed with standard rat food and water *ad libitum* and quarantined in our University facilities for at least two days prior to use. The experimental protocol adhered to rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus University Rotterdam, The Netherlands.

Icodextrin®

We used the 7.5 % solution of icodextrin, which is regularly used for peritoneal dialysis (Extraneal™, Baxter Healthcare Inc.). Icodextrin is a biodegradable, biocompatible, α-1,4 linked glucose polymer. The large icodextrin molecule is not digested intra-abdominally but is, via the lymphatic system, gradually absorbed into the bloodstream where it is partitioned sequentially by the enzymes α-amylase and maltase to maltose and glucose. Previous experimental and clinical studies assessed the safety, tolerability and preliminary effectiveness with regard to reducing postoperative adhesion formation. A volume of 20 ml per kg bodyweight has been indicated as the optimal applicable volume.[27, 28]

Tumour

Tumour CC531 is a 1,2-dimethylhydrazine-induced, moderately differentiated, weakly immunogenic colonic adenocarcinoma transplantable in syngeneic WAG rats.[34] The tumour is maintained as a cell culture in RPMI 1640 medium supplemented with 5% foetal calf serum (virus- and *Mycoplasma*-screened), 1% penicillin (5000 U/mL), 1% streptomycin (5000 U/mL) and 1% L-glutamine (200 mmol). Medium and all supplements were obtained from Life Technologicals BV, Breda, The Netherlands. Cells were passaged once a week using trypsin (0.05%) and EDTA (0.02%). Before use *in vivo*, tumour cells were harvested from stationary cultures by gentle trypsinisation (5 minutes, 37°C), centrifugation (5 minutes, 700 g) and re-suspension in RPMI 1640, providing cell suspensions with a viability greater than 90%. CC531 is relatively insensitive to chemotherapy but is sensitive to the effects of biologic response modifiers.

Operative procedures

To study the effect of icodextrin® on adhesion formation our previously described reproducible rat adhesion model was used.[35] Briefly, under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Three Surgilene 5-0 sutures (Braun, Melsungen, Germany) were applied to both lateral peritoneal sides 0.7 cm from each other and 1.5 cm downwards from the abdominal incision. All knots were double knots fastened tightly to ensure local ischemia. Both uterus horns were exposed, rubbed with surgical Medipres gauze (van Heek Medical, Losser, The Netherlands) and sutured to the lateral peritoneum with Surgilene 6-0 (Braun) proximally and distally from the three 5-0 sutures. Rubbing was performed with a device enabling the application of a constant pressure of 120 g/cm^2. The uterus horns were rubbed 10 times over their total length. In this way a standardised amount of severe peritoneal trauma was inflicted. Before closing the abdominal cavity the rats were treated by intra-abdominal instillation of icodextrin®, RPMI (placebo) or received no further treatment (controls). The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures (Braun).

To study the effect of icodextrin® on local tumour recurrence our previously designed reproducible tumour adhesion and growth model was used.[31] Under ether anaesthesia and aseptic conditions a laparotomy was performed using a lower midline incision of 5 cm. Both uterus horns were exposed, rubbed 10 times with surgical Medipres gauze and sutured to the lateral peritoneum both proximally and distally, using Surgilene 6-0 sutures. In this way a standardised amount of severe peritoneal trauma was inflicted. After traumatising the peritoneum icodextrin®, RPMI (placebo) or nothing (control) was administered intra-abdominally. Before closing the abdomen, 0.5 x 10^6 CC531 tumour cells, in 1 ml RPMI 1640, were injected intra-peritoneally. The abdomen was closed in two layers with 5-0 polyglycolic acid and 2-0 silk sutures.

Experimental design

Effect of icodextrin® on adhesion formation

In 30 rats standardised severe peritoneal trauma was inflicted to both lateral peritoneal sides by applying 3 sutures in, and fixating the rubbed uterus horn to the lateral peritoneum. At the end of surgery in 10 rats (group I) 3 ml icodextrin® and in 10 rats (group II) 3 ml RPMI (placebo) was instilled intra-abdominally prior to closure of the abdomen. In 10 rats (group III) there was no instillate (controls).

Effect of icodextrin® on tumour cell adhesion and growth

In 30 rats standardised severe peritoneal trauma was inflicted by rubbing the exposed uterus horns and fixating them to the lateral peritoneum. In 10 rats (group IV) 3 ml icodextrin® and in 10 rats (group V) 3 ml RPMI (placebo) was administered intra-abdominally after traumatising the peritoneum. Ten rats (group VI) received no treatment (controls). Before closing he abdomen the CC531 tumour cells were injected intra-peritoneally in all groups.

Evaluation of adhesion formation

Fourteen days after laparotomy all rats were sacrificed for assessment of intra-abdominal adhesion formation. Macroscopically the adhesions were scored according to their extent (quantity) and type (quality) by two independent observers. The extent of adhesion formation was quantified by dividing the area to be scored into eight segments marked by the three 5-0 sutures in the lateral peritoneum (Figure 3.3, Chapter 3). The presence or absence of adhesions in the eight demarcated areas was scored. If there were adhesions in an area this accounted for 12.5% adhesions; a maximum of 100% adhesions could be scored. In each rat two lateral peritoneal sides were assessed. The type of adhesions formed was classified macroscopically using the Zühlke classification (Table 3.1, Chapter 3).[36]

Evaluation of intra-peritoneal tumour cell adhesion and growth

Twenty-one days after surgery all rats were sacrificed and intra-peritoneal tumour load was scored semiquantitatively at the following peritoneal sites: right uterus horn, left uterus horn, subcutaniously (at the site of the operative scar), parietal peritoneum (at the lateral abdominal wall sides where no uterus horns were fixated), kidney, liver, retroperitoneum, and omentum. The scoring was performed by two independent observers using a tumour scoring system derived from the peritoneal cancer index described by Steller and ranging from 0 to 5 per abdominal site (Table 6.1, Chapter 6).[37] For each rat the score at all peritoneal sites, except for the uterus horns, was summarised, from which a mean total tumour load per rat could be estimated. In the present study the term tumour recurrence is sometimes used to illustrate tumour load, which is the net result of tumour cell adhesion and tumour growth, because we presume that intra-peritoneal injection of tumour cells resembles the clinical situation of tumour cell spill during tumour resection.

Statistical analysis

The mean adhesion percentage and standard deviation was calculated per group. Data were statistically analysed using the one-way ANOVA test to determine overall differences. If the ANOVA test was significant on a 5% level, the Student Newman Keuls *post hoc* test was carried out to make a comparison between groups.

The median and range of intra-peritoneal tumour load at each scored abdominal site and of the total tumour load were calculated per group. Statistical analysis was performed using the non-parametric Kruskall Wallis test to determine overall differences followed by the Mann-Whitney *U* test to compare differences between groups. Statistical significance was defined as $p < 0.05$.

RESULTS

None of the rats were found to have adhesions at the initial operation. There were no postoperative complications e.g. bowel obstructions, peritonitis or tumour overgrowth. No leaking of fluids from the abdominal wounds and no postoperative bulging of abdomens was observed. No remnant fluids were found at necropsy at day 14 or day 21.

Effect of icodextrin® on adhesion formation

Table 10.1 shows that icodextrin® significantly reduced postoperative adhesion formation after severe peritoneal trauma ($p < 0.0001$). The mean adhesion percentage found after peroperative instillation of RPMI did not differ significantly from that of controls. The adhesions formed in the icodextrin® group were filmy (Zühlke type 1-2) involving only pelvic fat. Adhesions found after administration of RPMI and in controls were stronger (Zühlke type 2-3), involving pelvic fat, uterine horn and small bowel.

Effect of icodextrin® on tumour cell adhesion and growth

Table 10.2 shows the tumour scores at directly traumatised peritoneal sites, at remote non-traumatised peritoneal sites and also depicts the total tumour load. Treatment with icodextrin® or RPMI did not significantly affect tumour load on severely traumatised uterus horns. The median total tumour scores (at not directly traumatised peritoneal sites) were similar in the three experimental groups. At one peritoneal site (retroperitoneum) the tumour score in the icodextrin® group was significantly lower than in the control group ($p = 0.007$).

Uterus horns	n	Percentage adhesion formation (SD)	Zühlke score	p1	p2	p3
I. Medipres rubbing & 3 ml icodextrin®	10	19.3 (8.0)	1-2	0.0001		
II. Medipres rubbing & 3 ml RPMI	10	65.0 (7.3)	2-3		ns	
III. Medipres rubbing	10	69.9 (14.9)	2-3			0.0001

Table 10.1

Mean adhesion percentages (SD), and Zühlke classification of found adhesions, after infliction of severe peritoneal trauma and intra-abdominal instillation of icodextrin® (group I) or RPMI (group II) and in non-treated controls (group III). For each rat, the individual data concerning the 2 lateral abdominal wall sites (uterus horns) were averaged; n is the number of data (= rats) per group used for analysis. Statistics p1 (I *versus* II), p2 (II *versus* III) and p3 (I *versus* III): one-way ANOVA test, with Student Newman Keuls *post hoc* test.

Abdominal sites	Tumour load IV. Icodextrin® n = 10	Tumour load V. RPMI n = 10	Tumour load VI. No treatment n = 10	p1	p2	p3
Uterus horns	5 (3.5-5)	5 (4-5)	5 (5-5)	ns	ns	ns
Subcutis	3 (2-3)	3 (2-4)	3 (2-4)	ns	ns	ns
Parietal peritoneum	0 (0-2)	0 (0-1)	0 (0-1)	ns	ns	ns
Kidney	2 (0-4)	2 (1-3)	2 (1-3)	ns	ns	ns
Liver	1 (1-1)	1 (0-2)	1 (1-2)	ns	ns	ns
Retroperitoneum	0 (0-0)	0 (0-1)	1 (0-1)	ns	ns	0.007
Omentum	2.5 (2-4)	3 (2-4)	3 (1-4)	ns	ns	ns
Total	1.6 (1-2)	1.7 (1-2)	1.8 (1-2)	ns	ns	ns

Table 10.2

Median tumour load (range) at severely traumatised uterus horns, at non-traumatised peritoneal sites, and the median total tumour load after intra-abdominal instillation of icodextrin® (group IV), intra-abdominal instillation of RPMI (group V) or in non-treated controls (group VI). For each rat, the individual data concerning the 2 uterus horn sites were averaged. N is the number of treated rats. Statistics p1 (IV *versus* V), p2 (V *versus* VI) and p3 (IV *versus* VI): Kruskall Wallis test with Mann-Whitney *U post hoc* test.

DISCUSSION

The aetiology of adhesion formation is only partly understood but the process is initiated by traumatising the peritoneal surface either by surgical action, by endometriosis, or by infection.[35, 38-40] The formation of an adhesion commences with injury to two layers of the peritoneum, one opposite the other, and the resulting exudation contributes to the deposit of fibrin and adhesion between the two membranes.[41, 42] Whether such adhesions are permanent or will eventually be lysed, is assumed to be dependent on the fibrinolytic capacity of the peritoneum.[42-44] A wide variety of therapeutic modalities affecting different levels in the cascade of fibinogenesis and fibrinolysis have been studied experimentally and clinically.[45-48] Results are inconsistent and associated with many side effects such as intra-abdominal haemorrhage and impaired wound healing. Avoiding per- and postoperative contact between traumatised peritoneal surfaces might bring us closer to reducing postoperative adhesion formation. Increasing the physiological liquid interface between two peritoneal surfaces is liable to reduce the initial adhesion phenomena at its origin. Indeed in a rat model, postoperative peritoneal dialysis significantly reduced adhesion formation, presumably due to a flotation effect and dilution of the fibrin exudates.[49] Obviously, postoperative peritoneal dialysis is not feasible in the clinical setting but these results stimulated further investigations. Various liquids have been installed peroperatively in the peritoneal cavity with the hope that "hydroflotation" would enable traumatised surfaces to be kept separate for a while.[21-23] Unfortunately these liquids were absorbed too quickly to bring about clinical successful adhesion prevention[50] or caused unwanted side effects.[25, 26] Separating peritoneal surfaces by means of slow absorption solutions, allowing prolonged postoperative "hydrofoliation" of the peritoneal cavity might have a more efficient anti-adhesive effect. Icodextrin is a large α-1,4 linked glucose polymer broken down sequentially by the enzymes α-amylase and maltase to maltose and glucose. Amylase is widely distributed throughout the body but is not or hardly present in the peritoneal cavity of humans and rats. When administered peroperatively, icodextrin is largely retained within the abdominal cavity for up to five days during which the polymer is gradually absorbed through the lymphatic system into the systemic circulation.[51] Icodextrin 7.5%, although iso-osmolar, induces ultrafiltration through colloid osmosis and the presence of the polymer is presumed to create a constant fluid layer between peritoneal surfaces.[52] Theoretically, the fluid acts to reduce the formation of adhesions by a 5-day period of "hydroflotation" of the total abdominal cavity, the time of maximum risk of adhesion formation.[53, 54] This study showed that after severe surgical peritoneal trauma icodextrin® significantly reduced the extent and severity of adhesion formation.

We have previously suggested that the peritoneal defence mechanism triggered by surgical trauma to the peritoneum not only promotes adhesion formation but also stimulates tumour recurrence.[31] Several theories speculate on the underlying mechanisms of the adhesion and growth of spilled tumour cells. Implantation of tumour cells onto raw tissue surfaces is an efficient process as opposed to implantation on intact surfaces.[30] The fibrin entrapment hypothesis proposes that tumour cells are trapped in fibrin at the resection site and abraded peritoneal surfaces, hereby providing protection from host defence mechanisms.[55] Whether postoperative "hydroflotation", could also keep free intra-abdominal tumour cells from adhering to damaged peritoneum was doubtful since icodextrin® does not prohibit disruption of the mesothelial cell lining and exposure of the underlying extracellular matrix playing a pivotal in adhesion of tumour cells. Results obtained in our tumour adhesion and growth model showed that tumour load at traumatised peritoneal surfaces indeed was not reduced by peroperative intra-abdominal icodextrin® treatment. This does not mean that there is no analogy in the processes of adhesion formation and tumour recurrence. Apparently it is not possible to keep free floating tumour cells from an injured peritoneal surface by a liquid barrier in which tumour cells suspend and stay alive. Physically, icodextrin® does not form a barrier between tumour cells and damaged peritoneal surfaces as it does between two injured peritoneal surfaces. Icodextrin® might be a medium in which tumour cells can proliferate and augment motility, increasing their metastatic ability. The results of this study did not support this hypothesis since total peritoneal tumour load at traumatised and remote peritoneal sites was not increased by icodextrin®, on the contrary tumour load at one remote peritoneal site was even significantly reduced in the icodextrin® treated group.

In conclusion the current study showed that the novel glucose polymer solution, icodextrin®, used as an intraoperative intra-abdominal instillate was well tolerated, safe, easy to use and significantly reduced postoperative adhesion formation without promoting or inhibiting tumour recurrence. Icodextrin® therefore seems to be a good modality to prevent intra-peritoneal adhesion formation and may be used safely in oncological surgery.

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PART V

General Discussion and Summary

Chapter XI

General Discussion

Surgical peritoneal trauma and postoperative adhesions

Peritoneal trauma causes a disturbance of the normal intra-abdominal inflammatory and fibrinogenetic defences leading to a disrupted equilibrium between mediators of the inflammatory response and between fibrinigenesis and fibrinolysis and thus induces post-surgical adhesions. Due to a lack of complete understanding of the dynamic process of peritoneal healing and its derailment, literature abounds on all kinds of methods to prevent adhesion formation. The results achieved with the different treatment modalities are inconsistent and associated with many side effects such as impaired wound healing, reduction of the immune system, intra-abdominal haemorrhage and gastrointestinal bleeding. Until today, only one technique is effective in reducing the occurrence of post-surgical adhesions to some extent. This technique aims to avoid manipulations which are known to provoke adhesion formation.[1-5] That includes preventing unnecessary desiccation, handling, clamping or suturing (ischaemia) of tissue and spilling of foreign material during operative intervention.[4, 5] Also, general agreement exists that it is unnecessary and even unwanted to close the peritoneum postoperatively.[6-17] However, although peritoneal trauma was proven to be a cause of adhesion formation, a link between the degree of peritoneal trauma and the extent of formed adhesions was never demonstrated.[6, 18, 19] Therefore, the first aim of the experiments described in this thesis was to analyse whether the extent and type of post-surgical adhesion formation correlates with the degree of peritoneal damage inflicted. In order to evaluate this, we developed a reproducible rat model, allowing semiquantitative and qualitative scoring of adhesions *in vivo* in which the clinical situation of post-surgical adhesion formation was imitated. Results of the first experiments showed that the extent of adhesion formation correlated significantly with the inflicted degree of peritoneal damage. Furthermore one of the aims of studies portrayed in this thesis was to identify whether agents like surgical gauze, lavage solutions and glove powder, not yet generally considered as traumatic for the peritoneum and commonly used in general surgical practice, indeed were traumatic and could consequently cause postoperative adhesions. We also investigated less

traumatic alternatives for these agents. Experimental results demonstrated that intra-abdominal manipulation with surgical Medipres gauze was very traumatic to the peritoneum and induced significantly more adhesion formation than manipulation of the peritoneum with less traumatic non-surgical Fastsorb textile. Moreover, there was no significant difference in the extent and type of adhesions formed after infliction of minimal peritoneal trauma alone or in combination with Fastsorb manipulation, suggesting Fastsorb is hardly traumatic for the peritoneum. Given these results and the fact that non-surgical Fastsorb textile meets important criteria for surgical abdominal swabs, this non-abrasive textile might be a suitable substitute for Medipres gauze in surgical practice. Further experiments showed that exposure of the abdominal cavity to lavage solutions, often used in the end stage of abdominal interventions, also traumatised the peritoneum and engendered significant adhesions formation. During surgery in non-contaminated abdominal cavities peritoneal lavage should not be performed and per-operative lavage of contaminated abdominal cavities should be viewed with caution. Last but not least, because powdered gloves are still universally used in abdominal surgery, we performed the studies demonstrating that local application of glove powder on minimally and severely traumatised peritoneum gave rise to a significantly higher percentage of adhesion formation than infliction of peritoneal trauma alone. This finding adds to the already existing evidence[20-25] that intra-abdominal contamination with starch by the use of starch powdered gloves should be avoided.

Surgical peritoneal trauma and tumour recurrence

Most attempts at treating intra-abdominal tumour recurrence, like local or regional chemo- and radiotherapy and photodynamic therapy,[27-30] are aimed at annulling manifest peritoneal tumour foci but do not focus on prohibiting the postoperative arising of local tumour recurrence. A different approach to diminishing post-surgical intra-abdominal tumour recurrence is to interfere with the primary implantation and growth process of intra-abdominally spilled free tumour cells. This necessitates a profound understanding of the underlying pathophysiologic mechanisms leading to local tumour recurrence. Subsequent studies described in this thesis were aimed at unravelling the links between surgical peritoneal trauma and the process of postoperative adhesion formation and intra-abdominal tumour recurrence and analysing the similarities amongst the latter.

The observed preference of tumours to recur at surgically traumatised sites[26, 31-35] and the theory of metastatic efficiency and the tumour cell entrapment hypothesis[26] brings up the question whether the dynamic peritoneal healing process, which when not in balance can lead to adhesion formation, also plays a role in the process of local tumour recurrence. This ascertainment and theoretic perceptions led us to investigate if a correlation existed between surgical peritoneal trauma and intra-abdominal tumour recurrence.

In order to meet this purpose and evaluate patterns of tumour recurrence *in vivo*, different rat models were used. We developed a reproducible rat tumour adhesion and growth model, analogous to the rat adhesion model, allowing semiquantitative scoring of tumour load in which the clinical situation of free intra-abdominal tumour cells was mimicked. This model associates the combination of cell adhesion and growth, ultimately leading to manifest tumour load. The amount of tumour recurrence at directly and not directly traumatised peritoneal sites appeared to be highly correlated with the degree of peritoneal trauma. The most impressive tumour load was observed at severely traumatised peritoneal sites. However, enhanced tumour recurrence was also seen at not directly traumatised peritoneal sites and the amount of tumour at these loco-regional sites also correlated with the severity of the trauma. This indicates that the sequelae of peritoneal trauma with regard to tumour recurrence are not confined to the inflicted site itself, but appear to have a generalised character. In a previously developed sub-renal capsule assay, the effect of surgical peritoneal trauma on the growth of extra-peritoneal tumours could be reproduced. Because promotion of adherence was irrelevant in this model it represents the systemic effects on tumour growth that may occur after surgical injury. After placing a solid tumour in the extra-peritoneal space, the intensity of surgical trauma correlated with the extra-peritoneal tumour growth, suggesting that systemic factors produced after surgical peritoneal trauma indeed play an important role in enhanced tumour growth. The apparent generalised character of tumour recurrence led to the hypothesis that factors produced after peritoneal trauma, may influence loco-regional and distant aspects of tumour recurrence. When these factors were captivated in a peritoneal lavage fluid and passively transferred to naive recipients, this hypothesis was confirmed. To study merely the effect of tumour cell adhesion an *in vitro* model was designed. Culturing a monolayer of mesothelial cells on a matrix coating mimicked the peritoneum. Tumour cell adhesion to mesothelial cells could be studied after pre-incubating the mesothelial monolayer with different substances. In experiments using this model, described by van Rossen et al,[36] cytokines and growth factors, produced by exposed endothelial cells, damaged mesothelial cells and inflammatory cells during peritoneal wound healing,[37-41] proved to be significant promoting factors in tumour cell adhesion to mesothelium. Supported by these results the initial impetus to separate the generalised term "tumour recurrence" into the more elementary mechanisms of tumour cell adhesion and growth was made. Both components may contribute evenly to successful intra-abdominal tumour recurrence and both are modified by surgical trauma.

As mentioned before, previous experiments showed that exposure of peritoneal cavity to surgical gauzes, lavage solutions and glove powder traumatised the peritoneum and promoted postoperative adhesion formation. Since subsequent data suggest that the factors responsible for the formation of post-surgical adhesions also play a role in the adhesion and

growth of tumour cells to the peritoneum we investigated whether surgical gauze and glove powder also could induce more tumour recurrence. The studies demonstrated that surgical Medipres gauze induced significantly more tumour load than non-surgical Fastsorb textile and peritoneal application of glove powder or pure starch induced significantly more intra-abdominal tumour load than when no powder was applied. Avoiding unnecessary peroperative peritoneal trauma by employing gentle surgical techniques and materials is therefore not only indicated to reduce postoperative adhesion formation but also to prevent intra-abdominal tumour recurrence. In accordance with this goal and given the fact that non-surgical Fastsorb textile meets important criteria for surgical purposes we again point out that this non-abrasive textile seems a suitable substitute for surgical gauze. Intra-peritoneal contamination with starch from starch-powdered gloves should also be avoided to prevent local tumour recurrence. Since good powder-free alternatives are available there is no longer any justification for the use of powdered gloves during intra-abdominal surgery.

Pathways of adhesion formation and intra-abdominal tumour recurrence

Since peroperative peritoneal trauma cannot entirely be prevented in practice, the need for supplementary prophylactic measures remains urgent. Better understanding of the underlying pathogenesis of postoperative intra-abdominal adhesion formation and peritoneal tumour cell adhesion and growth will undoubtedly help in the development of balanced and more effective therapeutic strategies. Cellular and soluble constituents of the peritoneal cavity are believed to both reflect the events arising during wound healing in the peritoneal cavity and have the potential to modulate the outcome of tissue response to injury.[18, 37-52] As cited before, preceding experiments showed that within a few hours after infliction of peritoneal trauma tumour cell adhesion and growth promoting factors could be captivated in a peritoneal lavage fluid and passively transferred to naive recipients. Further analysis of the fragments in the captured lavage fluid brought forward the presence of a cellular and soluble fraction. When these fractions were individually injected into pristine abdominal cavities, without any form of additional surgical trauma, both induced significantly more peritoneal tumour recurrence. However, the effect of the soluble factors was inferior to that of the cellular fraction. More detailed unravelling of the cellular fraction demonstrated that peritoneal trauma induces oscillation in intra-abdominal cells. Apart from an augmented absolute cell amount, the ration changes from primarily monocytes to mainly neutrophil granulocytes (PMN). This observation was made during the acute phase after surgery and most likely represents the PMN influx in response to chemoattractant factors. The coincidence of posttraumatic intra-abdominal PMN influx with adhesion formation and tumour cell adhesion and growth is no solid prove for the role of PMN in these pathogenetic processes. Therefore, studies were performed to investigate whether we were able to prohibit posttraumatic intra-abdominal PMN influx. Intra-

peritoneal injection of anti-neutrophil serum (ANS) indeed foiled this PMN influx in rats. Moreover a well-balanced prevention of posttraumatic intra-abdominal PMN influx by ANS reduces adhesion formation and local tumour recurrence significantly. Accordingly, intra-abdominal influx of PMN after surgical peritoneal trauma plays a crucial role in postoperative adhesion formation and in the process of adhesion and growth of spilled tumour cells. This knowledge opens up the way to develop of novel therapeutic strategies acting subtle, in order to prevent unwanted side effects, upon the (dis)balance of the peritoneal healing process.

The adhesion formation and tumour recurrence promoting effect of PMN's may be based on several characteristics of these cells. First, through PMN-cytokine production an upregulation of cell adhesion molecules may be induced,[53, 54] hereby facilitating adhesion of tumour cells. In addition, cytokine activated mesothelial cells produce chemoattractants like IL-8, required for PMN recruitment.[55, 56] In this way, the mesothelium and inflammatory cells in the abdominal cavity may perpetuate a cytokine loop, resulting in extreme activation of the inflammatory process. Secondly, mesothelial damage may be aggravated by the degranulation of organelles, but most likely through the production of reactive oxygen species (ROS).[57] Obviously, affecting one or more of these PMN involving steps in the cascade of derailed peritoneal healing may lead to the evolvement of new adhesion and tumour recurrence preventive formulas.

Adjuvant measures to prevent adhesion formation and tumour recurrence

Developing novel prophylactic measures through unravelling the pathophysiologic mechanisms of the peritoneal healing cascade is a time consuming process. In the mean time the need to treat today's patients as good as possible remains and on that account we analysed the effect of a known adhesion reducing fluid (Sepracoat™),[58-61] with also known trammels, on tumour recurrence *in vivo* and tumour cell adhesion *in vitro*. Hyaluronic acid is a principal ligand for CD44 which is an ubiquitous, multistructural and multifunctional surface adhesion molecule regulating cell-cell and cell-matrix interactions.[62] CD44 and its variants are present on many cancer cell types and have found to be involved in local tumour progression and metastasis.[63, 64] Therefore, hyaluronic acid containing solutions theoretically may affect local adhesion and growth of free inrta-abdominal tumour cells. The results of our experiments demonstrate that Sepracoat™ had a small but significant inhibitory effect on the adhesion of tumour cells to mesothelial cell monolayers *in vitro*. However, we were unable to repeat these effects in our *in vivo* rat models; the peroperative inoculation of Sepracoat™ did not reduce nor promote postoperative tumour recurrence.

To overcome a number of major disadvantages of issued absorbable barriers and viscous barrier solutions[60, 61, 65-74] a glucose polymer solution already successfully and safely used in

peritoneal dialysis was recently developed into a fluid that is absorbed only slowly, allowing prolonged "hydroflotation" of the peritoneal cavity.[75, 76] With this "water" barrier technique two damaged peritoneal surfaces are mechanically separated during the period of mesothelial regeneration, thereby in theory enabling the prevention of adhesion formation. In our hands icodextrin® was found to be a well tolerated, safe and easy to use solution that effectively reduces extent and severity of postoperative adhesion formation in rats without promoting or inhibiting intra-abdominal tumour recurrence. Apparently it is not possible to keep free floating tumour cells from an injured peritoneal surface by a liquid barrier in which tumour cells suspend and stay alive. Physically, icodextrin® does not form a barrier between tumour cells and damaged peritoneal surfaces as it does between two injured peritoneal surfaces during the peritoneal healing process. Icodextrin® seems a very promising development in the avenue of future resaerch because next to working as an adhesion reducing adjuvant by itself, it could also function as a long lasting carrier for other adhesion and tumour inhibiting substances. Whether this treatment modality will proof to be effective in human remains to be investigated but experimental studies look promising.

Considerations for clinical applications.

Any form of peritoneal trauma is likely to cause postoperative adhesion formation and enhance intra-abdominal tumour recurrence. The operating skill of the surgeon therefore is a relevant factor in adhesion and tumour prognosis. Avoiding of unnecessary desiccation, handling, clamping or suturing (ischaemia) of tissue and leaving behind as little foreign material as possible during operative intervention are generally accepted measures for performing surgery in a non-traumatising fashion.[1-5] In addition, we advice surgeons to prohibit unnecessary peroperative intra-abdominal use of surgical gauzes and if frequent or profound use is inevitable to choose a non-abrasive textile as a substitute. Furthermore during surgery in non-contaminated abdominal cavities peritoneal lavage should not be performed. And moreover since good powder-free alternatives are available powdered gloves should not be used during intra-abdominal surgery. Minimal invasive surgery may also diminish the extent of peroperative peritoneal injury.

Still intra-abdominal surgery will always bring about peroperative peritoneal trauma in some quantity at some point during operation. Adjuvant measures associated with surgical intervention seem a logical step not only to prevent postoperative adhesions but also to avert loco-regional tumour recurrence. At the present time, despite the great variety of agents employed, none of them seems to be overwhelmingly suitable for effective and save general use in the humans. Although it is difficult to manoeuvre, presently the clinically most successful and save anti-adhesive measure seems to be hyaluronic acid /carboxymethylcellulose. Since it effectively reduces the severity of postoperative adhesions

in humans and, as this thesis elaborates by animal studies, did not influence tumour recurrence it seems recommendable to use Sepreafilm™ at least during abdominal interventions which will be followed by planned relaparotomy or second look operations. Experiments described in this thesis showed that an easy to handle new glucose polymer solution, icodextrin®, could markedly reduce the extent and severity of postoperative adhesion formation in rats, (unfortunately) without affecting intra-abdominal tumour recurrence. It is too early to know whether these results can be repeated in humans but further research, in the form of clinical trials, has to be executed to estimate the clinical value of this barrier solution.

In this thesis, one major step in the pathway of adhesion formation and peritoneal tumour recurrence has been brought into perspective. Post-traumatic intra-abdominal PMN influx proved to play a major role in the processes of postoperative adhesion formation and intra-abdominal tumour recurrence. Efforts to dim the inflammatory reaction by mastering the mediators of this process has already been suggested as adhesion and tumour recurrence abating alternatives and these experimental results contribute to this idea. In the development of such substrates one always has to keep in mind that the peritoneal healing processes rely on the same biological mechanisms as adhesion formation and tumour recurrence, thus manipulation of steps in the derailed cascade has to be done selectively and in moderation to prevent unwanted side effects.

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Chapter XII

Summary and Conclusions

Postoperative adhesion formation as well as loco-regional tumour recurrence remain serious complications of potentially curative surgical intra-abdominal interventions. For neither problem clinically relevant curative treatment modalities are available yet. Although understanding of the pathogenesis of adhesions and local tumour recurrence has improved recently, the molecular mechanisms involved still need to be delineated. Adhesion formation and adhesion-free reepithelialisation are alternative pathways of the cascade of the peritoneal defence after peritoneal trauma and we assume the same cascade plays a role in the process of tumour recurrence. The studies described in this thesis were aimed at unravelling the correlation between surgical peritoneal trauma and the process of postoperative adhesion formation and intra-abdominal tumour recurrence.

The only consensus concerning the prevention of postoperative adhesions is the avoidance of peroperative damage to the peritoneum through the use of meticulous surgical techniques. Subsequently, we also tried to identify whether, besides generally accepted traumatic elements and actions, agents like surgical gauze, lavage solutions and glove powder, not yet generally considered as traumatic for the peritoneum and commonly used in surgical practice, were traumatic and could consequently cause adhesion formation and local tumour recurrence. Better understanding of the underlying pathogenesis of both processes is a prerequisite to rational prophylaxis and therapy of both surgical complications.

Further experiments laid down in this thesis aimed at studying which surgery related factors affected adhesion formation, tumour cell adhesion and/or growth and consequently tumour recurrence. One aspect of the peritoneal response to surgical trauma was highlighted with the intention to discover whether this part of the cascade could be used for specific therapeutical alternatives. The last experiments appointed in this thesis aimed at evaluating the capacity of a new glucose polymer solution, icodextrin®, in reducing adhesion formation and the capacity of Sepracoat™ and icodextrin® to modify adhesion and growth of free intra-abdominal tumour cells.

Chapter 1 describes postoperative adhesions as a long term and unpredictable problem causing impressive morbidity and even mortality, with great impact in surgical workload and hospital resources, resulting in considerable health care expenditures. An overview is given of the pathophysiology of the local peritoneal defence cascade following peritoneal trauma, sometimes leading to adhesion formation, and of the literature on adhesion preventive measures interfering with one or several steps in this dynamic process of peritoneal healing. Peritoneal dissemination is a common cause of post-surgical tumour recurrence after potentially curative resection of gastro-intestinal adenocarcinomas. The theoretical similarities in the pathogenesis of the process of intra-peritoneal tumour recurrence and the process of adhesion formation are discussed.

In **Chapter 2**, the aims of this dissertation are presented.

Chapter 3 describes the development of a reproducible rat model allowing semiquantitative and qualitative scoring of adhesions. Experiments using this model demonstrated that the infliction of minimal peritoneal trauma resulted in a significantly lower adhesion percentage than the infliction of moderate or severe peritoneal trauma. Adhesions formed after severe and moderate trauma of the peritoneum were dense and thick and could be classified as Zühlke type 2-3, while adhesions formed after minimal trauma of the peritoneum were filmy and could be classified as Zühlke type 1-2. Rubbing of the peritoneum with surgical gauze after infliction of minimal peritoneal trauma did induce significantly extra adhesion formation. After infliction of minimal peritoneal trauma, rubbing with surgical gauze did produce significantly more adhesions than rubbing with non-surgical textile. Moreover, rubbing the peritoneum with non-surgical textile after infliction of minimal peritoneal trauma did not cause additional adhesion formation at all.

Chapter 4 presents the results of studies evaluating the influence of peroperative lavage of the abdominal cavity with RPMI medium, NaCl 0.9%, polyvinylpyrrolidone iodine 1% (PVP-I), Viaspan and chlorhexidine 0.02% on postoperative adhesion formation. They show that peritoneal lavage of areas with ischaemic injury was associated with significantly increased adhesion formation. Aggressive (chlorhexidine 0.02%) as well as non-aggressive (RPMI, NaCl 0.9%, PVP-I and Viaspan) solutions engendered adhesions. Peritoneal lavage with aggressive solutions promoted adhesion formation significantly more than lavage with non-aggressive solutions.

Chapter 5 portrays the development of a reproducible rat tumour adhesion and growth model, analogous to the rat adhesion model, allowing semiquantitative scoring of tumour

load. This model was employed to test the metastatic efficiency and the tumour cell entrapment hypothesis. The displayed results clearly point out that a similar correlation exists between local tumour recurrence and peritoneal trauma as between adhesion formation and peritoneal trauma. Tumour load at directly and not directly traumatised remote peritoneal sites was significantly higher after severe trauma than following moderate or minimal trauma of the peritoneum. In addition, a significant correlation between the degree of peritoneal trauma and the growth of "ectopic" tumours, situated under the renal capsule, was observed during experiments using a previously developed sub-renal capsule assay. Moreover, within a few hours after infliction of peritoneal trauma tumour cell adhesion and growth promoting effects could be captivated in a lavage fluid and be passively transferred to naïve recipients. In these experiments severe peritoneal trauma was inflicted by rubbing the peritoneal surface with surgical Medipres gauze.

The experiments laid down in **Chapter 6** investigate whether the well-known adhesion provoking effect of glove powder could be reproduced in our rat adhesion model and whether glove powder also promotes intra-abdominal tumour recurrence in our rat tumour cell adhesion and growth model. Local application of glove powder or pure starch on minimally and severely traumatised peritoneum gave rise to a significantly higher percentage of adhesion formation than infliction of peritoneal trauma alone. Peritoneal application of glove powder or pure starch induced significantly more intra-abdominal tumour load than when no powder was applied.

The studies in **Chapter 7** focussed on further analysing the surgery related factors in the abdominal cavity that could be captivated in a lavage fluid and enhance tumour recurrence in naive recipients. Lavage fluids collected after trauma could be separated in a cellular and supernatant component, the latter containing soluble factors. Intra-peritoneal injection of naive recipients with both components resulted in statistically significant more tumour recurrence than injection with RPMI. The cellular component of the post-surgical inflammatory process was the most potent stimulator of tumour recurrence. Cytokines and growth factors also enhanced tumour load but played an inferior role. In vitro tumour cell adhesion to the mesothelium was not affected by soluble factors in the lavage fluid. Analysis of the cellular part of the lavage fluid, gathered after minimal or severe peritoneal trauma, demonstrated a significant influx of neutrophils (PMN) after infliction of severe peritoneal trauma. No differences in intra-abdominal cytokine concentrations were detected in mildly or severely traumatised rats. However, elevated concentrations of IGF-I were detected in the abdomens of severely traumatised animals.

In **Chapter 8** the question is addressed whether the observed coincidence of post-traumatic intra-abdominal PMN influx and the processes of adhesion formation and local tumour recurrence are causally related. Experimental results again exposed that infliction of peritoneal trauma provokes a significant intra-abdominal PMN influx during a period of at least 96 hours post-trauma. This influx could be prevented by treatment with anti-neutrophil serum (ANS, 3 and 1 dose). Averting the post-traumatic intra-abdominal PMN influx by injection of 3 doses of ANS significantly reduced adhesion formation. Yet, treatment with 3 doses of ANS did not decrease local tumour recurrence, on the contrary it significantly increased tumour recurrence. This was probably the consequence of immunosuppression, induced by 3 doses of ANS decreasing the blood lymphocyte, monocyte and PMN counts, which does not have an effect on adhesion formation but conceivably does promote tumour growth. Therapy with 1 dose of ANS did annul the post-traumatic PMN influx more selectively than 3 doses since it only affected blood PMN counts and did not influence blood lymphocyte and monocyte count. More selective prevention of post-traumatic PMN influx significantly lowered tumour cell adhesion and growth.

Chapter 9 elaborates on the issue of Sepracoat™ and postoperative intra-abdominal adhesion and growth of free intra-peritoneal tumour cells. Sepracoat™ had a small but significant inhibitory effect on the adhesion of CC531 tumour cells to mesothelial cell monolayers *in vitro*. However, we were unable to repeat these results in our *in vivo* rat models, the peroperative inoculation of Sepracoat™ did not reduce postoperative tumour load at directly or indirectly traumatised peritoneal surfaces. If anything, Sepracoat™ seemed to enhance tumour cell adhesion to traumatised peritoneal sites, however these differences were not statistically significant.

Chapter 10 deals with the way a new glucose polymer solution, icodextrin®, sways the processes of postoperative adhesion formation and local tumour recurrence. Peroperative intra-abdominal administration of icodextrin® after infliction of surgical peritoneal trauma significantly decreased the extent and severity of found adhesions. Treatment with icodextrin® did not prevent nor promote intra-peritoneal tumour cell adhesion and growth of free intra-abdominal tumour cells.

Chapter 11 includes the general discussion of this thesis. The results of the presented studies and the putative explanations for the findings are debated and possible considerations for clinical implications are postulated.

Conclusions

* The degree of peroperative peritoneal trauma correlates with the extent of postoperative adhesion formation. (Chapter 3)
* The degree of peroperative peritoneal trauma correlates with the extent of intra-abdominal tumour cell adhesion and growth. (Chapter 5)
* Standard surgical gauze is traumatic to the peritoneum and promotes adhesion formation and local tumour cell adhesion and growth. (Chapter 3 and 5)
* The non-abrasive textile Fastsorb meets important criteria for surgical swabs, is hardly traumatic to the peritoneum and consequently induces less adhesions and tumour cell adhesion and growth than standard surgical gauze. (Chapter 3 and 5)
* Lavage solutions are traumatic to the peritoneum and promote adhesion formation. (Chapter 4)
* Glove powder is traumatic to the peritoneum and promotes adhesion formation and tumour cell adhesion and growth. (Chapter 6)
* Inflammatory cells (mainly PMN), present in the abdominal cavity after surgical peritoneal trauma, promote tumour cell adhesion and growth. (Chapter 7)
* Cytokines and growth factors, present in the abdominal cavity after surgical peritoneal trauma, promote tumour cell adhesion and growth. (Chapter 7)
* Post-traumatic intra-abdominal neutrophil (PMN) influx is an important factor in the derailment of the dynamic cascade of peritoneal defence and preventing this influx reduces postoperative adhesion formation and tumour cell adhesion and growth. (Chapter 8)
* The hyaluronic acid solution Sepracoat™ reduces tumour cell adhesion to mesothelium in culture but has no effect on intra-abdominal tumour cell adhesion and growth. (Chapter 9)
* A new glucose polymer, icodextrin®, reduces postoperative adhesion formation very effectively but does not affect intra-abdominal tumour cell adhesion and growth. (Chapter 10)

Chapter XIII

Nederlandse Samenvatting

In dit proefschrift wordt nader ingegaan op het verschijnsel van postoperatieve adhesievorming en het lokaal in de buik (intra-abdominaal) terugkomen (recidiveren) van verwijderde kwaadaardige maagdarm tumoren. Adhesies zijn verklevingen tussen twee buikvlies oppervlakken welke normaliter niet met elkaar verbonden zijn. Adhesievorming ontstaat na bijna iedere buikoperatie en veroorzaakt meestal geen klachten. Toch kunnen adhesies leiden tot een aantal ernstige complicaties zoals darmobstructies, welke vaak acuut opnieuw operatief ingrijpen vereisen, onvruchtbaarheid bij vrouwen en chronische buikpijnklachten. Deze complicaties hebben niet alleen klinische- maar ook grote financiële gevolgen.

Postoperatieve adhesievorming is een dynamisch proces, een cascade van opeenvolgende gebeurtenissen, wat optreedt na beschadiging (trauma) van het buikvlies (peritoneum). Alhoewel we deze cascade nog niet volledig begrijpen, is wel bekend dat het een ontregeling betreft van het normale verdedigingsmechanisme dat zorgt voor wondheling van beschadigde peritoneale oppervlakken. Een groot aantal therapeutische mogelijkheden, dat aangrijpt op verschillende niveaus in de cascade van adhesievorming, heeft zijn klinische relevantie tot nu toe nog niet bewezen. Er bestaat dus nog geen algemeen geaccepteerde behandeling ter voorkoming van adhesies en hun complicaties.

Operatieve behandeling van tumoren in het maagdarmkanaal wordt vaak gecompliceerd door het terugkomen van kanker in de buik (loco-regionale tumor recidivering). De "tumor cell entrapment hypothese" zou het patroon van falen van de chirurgische therapie, leidend tot deze locale recidieven, kunnen verklaren. Bij deze hypothese zouden tijdens de operatie tumorcellen in de buikholte lekken uit de door de chirurg doorgesneden lymfebanen van het uitgenomen weefsel. De vrijgekomen tumorcellen zouden vervolgens op het peritoneum aanhechten en uitgroeien. De aanhechting (adhesie) van tumorcellen zou gemakkelijker plaatsvinden op beschadigde peritoneale oppervlakken. Theoretisch zou het proces van aanhechting van gespilde tumorcellen kunnen worden gestimuleerd door dezelfde cascade als het hierboven beschreven proces van adhesievorming van tijdens de operatie (peroperatief) beschadigde peritoneale oppervlakken. De studies beschreven in dit

proefschrift hebben het doel de mogelijke correlatie tussen chirurgisch peritoneaal trauma en de processen van adhesievorming en tumor recidivering te analyseren.
De enige effectieve therapeutische mogelijkheid ter voorkoming c.q. vermindering van postoperatieve adhesievorming is het zoveel mogelijk voorkomen van peroperatief peritoneaal trauma. Het laten uitdrogen van het peritoneum, onnodig plaatsen van klemmen en hechtingen en het achterlaten van vreemd lichaamsmateriaal wordt algemeen beschouwd als traumatisch voor het peritoneum. Dit dient dus zoveel mogelijk te worden vermeden tijdens buikoperaties. Voor dit proefschrift zijn experimenten opgezet die onderzoeken of veel bij operatie gebruikte en niet algemeen als traumatisch voor het peritoneum geachte materialen zoals chirurgische gazen, spoelvloeistoffen en handschoenenpoeder wellicht ook adhesievorming en tumorcel adhesie en groei kunnen bevorderen.
In dit proefschrift wordt verder bestudeerd welke factoren in de buik geproduceerd worden na beschadiging van het peritoneum, en welke van deze factoren een belangrijke rol spelen bij het ontstaan van postoperative adhesies en tumor recidivering. Op één facet van deze peritoneale respons op trauma wordt dieper ingegaan. Het is de bedoeling om te ontdekken of door ingrijpen op dit niveau van de helingscascade, adhesievorming en tumor recidivering kunnnen worden voorkomen. Dit ingrijpen dient dan wel te gebeuren zonder het peritoneale genezingsproces te beïnvloeden omdat anders ernstige bijwerkingen zouden kunnen optreden.
De in dit proefschrift als laatste beschreven experimenten evalueren de preventieve capaciteit van een nieuwe glucose polymeer oplossing, icodextrin® ten aanzien van adhesievorming. Voorts worden de mogelijkheden bestudeerd om met Seprecoat™ en icodextrin® de adhesie en groei van vrije in de buikholte aanwezige tumorcellen te voorkomen.

Hoofdstuk 1 geeft een samenvatting van literatuur gegevens over de incidentie, de klinische- en financiële consequenties, de eathiologie, de pathophysiologie, de behandeling en de preventie van postoperatieve adhesievorming en locoregionale tumor recidivering.

In **Hoofdstuk 2** worden de doelstellingen van dit proefschrift gepresenteerd.

Hoofdstuk 3 beschrijft de ontwikkeling van een reproduceerbaar proefdier model waarin semi-kwantitatieve en kwalitatieve beoordeling van adhesievorming mogelijk is. Met behulp van dit model kon worden aangetoond dat er een relatie bestaat tussen de intensiteit van peritoneaal trauma en de mate van adhesievorming; aanbrengen van ernstig trauma resulteerde in een significant hoger adhesie percentage dan aanrichten van minimaal trauma. Adhesies die ontstaan na ernstig trauma waren dikker en steviger dan adhesies die

ontstaan na minimaal trauma. In hetzelfde proefdier model werd vervolgens ook aangetoond dat intra-abdominaal gebruik van ruw chirurgisch gaas schadelijker is voor het peritoneum en tot significant meer adhesievorming leidt dan gebruik van een zachter gaas afkomstig uit de elektronica industrie. Het bleek zelfs dat minimaal peritoneaal trauma dat werd veroorzaakt door schuren met het zachtere gaas, helemaal niet leidde tot additionele adhesievorming.

Hoofdstuk 4 laat de resultaten zien van experimenten die het effect van verschillende spoelvloeistoffen op het peritoneum onderzoeken. Peroperatief spoelen van de buikholte met RPMI, NaCl 0.9%, polyvinylpyrrolidone iodine 1% (PVP-I), Viaspan en chloorhexidine 0.02% beschadigt het peritoneum zodanig dat het postoperatieve adhesievorming bevordert. Peritoneale spoeling van minimaal getraumatiseerde buikholtes met agressieve oplossingen bracht significant meer, dikkere en steviger adhesies teweeg dan lavage van minimaal getraumatiseerde buikholtes met minder agressieve oplossingen.

Hoofdstuk 5 beschrijft de ontwikkeling van een tweede reproduceerbaar proefdier model, analoog aan het ratten adhesiemodel. In dit model is een semi-kwantitatieve beoordeling van intra-abdominale tumor deposities mogelijk. Hiermee werd de correlatie tussen de intensiteit van peritoneaal trauma en de mate van tumor recidivering gedemonstreerd; op de plaatsen waar het peritoneum het meest werd beschadigd, werd een significant grotere tumor depositie aangetroffen dan op peritoneale oppervlakken die minder ernstig werden beschadigd. Nog opmerkelijker was dat er ook op de niet direct beschadigde peritoneale oppervlakken meer tumor depositie werd gevonden in ernstig getraumatiseerde buikholtes dan in minimaal getraumatiseerde buikholtes.
Met behulp van een eerder ontwikkeld reproduceerbaar proefdier model, waarin kwantitatieve beoordeling van de groei van buiten de buikholte gelegen tumoren mogelijk is, kon worden aangetoond dat de mate van groei van deze tumoren correleert met de intensiteit van aangebracht peritoneaal trauma. Het lijkt dan ook dat factoren die geproduceerd worden na een chirurgisch trauma van het peritoneum niet alleen locaal (in de buik), maar ook op afstand een tumor stimulerend effect hebben. Resultaten uit volgende experimenten toonden aan dat deze in de buikholte vrijkomende factoren konden worden gevangen in een vloeistof waarmee men de buik na het aanbrengen van peritoneaal trauma spoelde. Wanneer deze vloeistof nu samen met tumorcellen in buiken van niet geopereerde naïeve proefdieren werd ingespoten, stimuleerde dit tumorcel adhesie en groei op de niet beschadigde peritoneale oppervlakken. De mate van tumor depositie in deze niet geopereerde proefdieren hing zelfs samen met de intensiteit waarmee het peritoneum van het "donordier" werd beschadigd.

In studies vastgelegd in **Hoofdstuk 6** is onderzocht of het "bekende" adhesie provocerende effect van handschoenpoeder kon worden gereproduceerd in ons proefdier adhesiemodel en of handschoenpoeder ook recidivering van intra-abdominale tumoren veroorzaakt. Locale applicatie van handschoenpoeder of puur zetmeel op minimaal- en ernstig getraumatiseerd peritoneum deed een significant hoger percentage adhesies ontstaan dan aanbrengen van peritoneaal trauma alleen. Het aanbrengen van handschoenpoeder of puur zetmeel op het peritoneum induceerde ook een significant hogere tumor recidivering in de buikholte dan wanneer geen poeder was achtergelaten.

Experimenten beschreven in **Hoofdstuk 7** zijn gericht op verdere analyse van chirugie-gerelateerde factoren die na peritoneaal trauma in de buikholte geproduceerd worden. Het bleek dat niet alleen de spoel-oplossing in zijn geheel maar ook zijn gescheiden componenten tumor recidivering kon bevorderen. Oplosbare factoren en ontstekings (inflammatoire) cellen konden ieder individueel, wanneer zij samen met tumorcellen in de buikholtes van niet geopereerde ontvangers werden ingespoten, significant meer intra-abdominale tumor deposities veroorzaken dan wanneer alleen tumorcellen werden geïnjecteerd. De cellulaire component stimuleerde de tumorcel adhesie en groei significant meer dan de vloeistof die alleen oplosbare factoren bevat. Analyse van de cellulaire component liet zien dat peritoneaal trauma een significante influx van neutrofielen granulocyten (PMN) in de buikholte veroorzaakt. In het oplosbare deel van de spoel-oplossing waren acute fase pro-inflammatoire cytokines, chemokines en groeifactoren waarneembaar.

In **Hoofdstuk 8** wordt de vraag gesteld of de geobserveerde coïncidentie van post-traumatische influx van neutrofielen in de buikholte en post-traumatische adhesievorming en tumor recidivering ook betekent dat neutrofile granulocyten een hoofdrol spelen in het proces van adhesievorming en van adhesie en groei van tumorcellen. Experimentele resultaten lieten opnieuw zien dat peritoneale schade, gedurende minstens 96 uur na het trauma, een hoog aantal intra-abdominale neutrofielen bewerkstelligt. Deze post-traumatische PMN influx kon worden voorkomen door het intra-abdominaal toedienen van selectieve antilichamen tegen PMN (ANS). Verhindering van post-traumatische PMN influx door inspuiten van 3 doses ANS verminderde postoperatieve adhesievorming significant. Daarentegen reduceerde een behandeling met 3 doses ANS de tumor recidivering niet. Het bleek zelfs dat de behandeling met 3 doses ANS de tumor recidivering significant induceerde. Dit was waarschijnlijk de consequentie van immuunsuppressie omdat injectie van 3 doses ANS gepaard ging met een significante daling van het aantal lymfocyten, monocyten en neutrofielen in het bloed. Behandeling met één dosis ANS voorkwam de post traumatische

PMN influx meer selectief. Hierdoor daalden alleen de neutrofielen in het bloed nog licht. Deze meer selectieve preventie van post-traumatische PMN influx ging gepaard met een significante vermindering van post-traumatische tumorcel adhesie en groei.

In **Hoofdstuk 9** wordt de adhesiepreventieve gel Sepracoat™ getest op haar vermogen om tumorcel adhesie en groei na operatief ingrijpen te beperken. Sepracoat™ had een minimaal maar significant remmend effect op de aanhechting van tumorcellen op mesotheelcellen (buikvliescellen) in kweek. Echter, dit effect van Sepracoat™ kon niet worden gestaafd met resultaten verkregen uit *in vivo* studies; wanneer Sepracoat™ peroperatief in de buikholte werd aangebracht had dit geen invloed op postoperatieve tumor depositie op direct en indirect getraumatiseerd peritoneum.

In **Hoofdstuk 10** worden de resultaten gepresenteerd van experimenten die het effect van een nieuwe glucose polymeer oplossing op adhesievorming en tumor recidivering evalueren. Na aanbrengen van ernstig peritoneaal trauma reduceerde het in de buikholte achterlaten van icodextrin® de omvang en de ernst van postoperatieve adhesies significant. Behandeling van ernstig getraumatiseerde buikholtes met icodextrin® had noch een remmend noch een stimulerend effect op tumorcel adhesie en groei van vrij in de buikholte aanwezige tumorcellen.

Met de algemene discussie in **Hoofdstuk 11** wordt dit proefschrift besloten. Het geeft een overzicht van de resultaten van de in deze dissertatie gepresenteerde studies, bediscussieert deze resultaten tegen het licht van andere in de literatuur beschreven data over dit onderwerp, bespreekt de mogelijke klinische gevolgen en toepasbaarheid van de resultaten en geeft suggesties voor verder onderzoek. Het is belangrijk dat, zolang nog geen klinisch toepasbare preventieve mogelijkheden zonder bijwerkingen bestaan voor postoperatieve adhesievorming en tumor recidivering, het experimenteel onderzoek wordt voortgezet en de resultaten hiervan getest worden in prospectief gerandomiseerde klinische trials.

Conclusies

* De intensiteit van peroperatief aangericht peritoneaal trauma correleert met de mate en ernst van postoperatieve adhesievorming.
* De intensiteit van peroperatief aangericht peritoneaal trauma correleert met de mate van intra-abdominale tumorcel adhesie en groei en dus met de mate van locale tumor recidivering.

* Standaard chirurgisch buikgaas is traumatisch voor het peritoneum en stimuleert postoperatieve adhesievorming en intra-abdominale tumor recidivering.
* Zacht textiel afkomstig uit de elektronica industrie voldoet aan belangrijke eisen voor chirurgisch buikgaas, is nauwelijks traumatisch voor het peritoneum en induceert dus minder adhesievorming en tumor recidivering dan standaard chirurgisch gaas.
* Spoelvloeistoffen zijn traumatisch voor het peritoneum en stimuleren postoperatieve adhesievorming en intra-abdominale tumor recidivering.
* Handschoenpoeder is traumatisch voor het peritoneum en stimuleert postoperatieve adhesievorming en intra-abdominale tumor recidivering.
* Neutrofiele granulocyten, geproduceerd in de buikholte na peritoneaal trauma, stimuleren tumorcel adhesie en groei.
* Cytokines en groei factoren geproduceerd in de buikholte na peritoneaal trauma, stimuleren tumorcel adhesie en groei.
* Post-traumatische intra-abdominale neutrofiele granulocyten influx speelt een belangrijke rol bij de ontsporing van de dynamische peritoneale verdedigings cascade en het verijdelen van deze influx reduceert postoperatieve adhesievorming en tumorcel adhesie en groei.
* De hyaluronzuur oplossing Sepracoat™ reduceert tumorcel adhesie op mesotheelcellen in kweek, maar heeft geen invloed op intra-abdominale tumorcel adhesie en groei.
* Een nieuwe glucose polymeer oplossing, icodextrin®, reduceert postoperatieve adhesievorming maar heeft geen invloed op intra-abdominale tumorcel adhesie en groei.

APPENDICES

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ANS	anti-neutrophil serum
bFGF	basic fibroblast growth factor
BSA	bovine serum albumin
CAPD	continuous ambulatory peritoneal dialysis
CD	cluster of differentiation
ECM	extra cellular matrix
EDTA	ethylenediaminetetraacetic
EGF	epidermal growth factor
ELISA	enzyme linked immuno sorbent assay
FCS	foetal calf serum
HA	hyaluronic acid
HE	hematoxilin eosin
ICAM	inter cellular adhesion molecule
Ig	immunoglobulin
IGF	insulin-like growth factor
IL	interleukin
LDH	lactate dehydrogenase
MCP	monocyte chemotactic protein
NSAID's	nonsteroidal anti-inflammatory drugs
PAA	plasminogen activator activity
PAI	plasminogen activator inhibitor
PBS	phophate buffered saline
PDGF	platelet derived growth factor
PMN	polymorph neuclear leucocytes
PG	prostaglandins
PVP	polyvinylpyrolidine iodine
ROS	reactive oxygen species
RPMI	Rosewall Park Memorial Institute medium
SD	standard deviation
TGF	transforming growth factor
TNF	tumour necrosis factor
t-PA	tissue-type plasminogen activator
rt-PA	recombinant tissue-type plasminogen activator
u-PA	urokinase-type plasminogen activator

PUBLICATIONS AND AWARDS

Publications related to this dissertation

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