Peritoneal adhesions are found in up to 95% of patients after intraabdominal surgery and are the leading cause of intestinal obstruction with mortality rates of up to 15% [1–6]. Adhesions can also cause female infertility, chronic pelvic pain and they may complicate subsequent surgical procedures [1,7,8]. Peritoneal adhesions are the leading cause of intestinal obstruction with mortality rates of up to 15% [2,4–7]. The incidence of readmissions owing to adhesions varies from 5 to 20% [4,5,8,9]. Manipulation of tissues, ischemia, infection, inadequate hemostasis and foreign materials (e.g. sutures, glove powder) cause peritoneal damage [6]. Peritoneal damage leads to an inflammatory response, where inflammatory cells release cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 and -6 (IL-1β, IL-6) [10,11]. These cytokines induce release of plasminogen activator inhibitor-1 and -2 (PAI-1 and -2) from mesothelial cells, resulting in reduced activity of plasminogen activators (PAs) [12–16]. PAI inhibit fibrinolysis and the fibrin deposits are infiltrated by granulocytes, monocytes and fibroblasts, followed by ingrowth of capillaries, deposition of collagen and formation of adhesions [17]. Platelet derived growth factor (PDGF), transforming growth factor beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF) are growth factors promoting the healing of the injured peritoneum and subsequent adhesion formation [17–19].

Despite the morbidity caused by peritoneal adhesions, there is at present no effective prevention available. Knowledge of the complex processes causing peritoneal adhesions is necessary if a clinically applicable prevention is to be designed. One step on this path is a relevant and reproducible experimental adhesion model. Our model enables studies on both adhesion formation and the healing process at the same time. The latter, by measuring anastomotic bursting pressure is crucial when designing an antiadhesion treatment that should reduce adhesions without impaired anastomotic healing.

The aim of this study was to investigate the concentrations of certain cytokines, proteins and growth factors promoting peritoneal adhesions in a controlled experimental model.

1. Materials and methods

1.1. Animals and methods

Male outbred Sprague-Dawley rats were purchased from Taconic (Bomholt, Denmark). They were housed for a week in the animal
1.2. Surgical procedures

Anaesthesia was induced in a sealed chamber with inhaled 4% isoflurane and maintained using a facemask delivering 2.5% isoflurane. All procedures were performed under clean but nonsterile conditions by one surgeon (FF). Firstly, the abdominal fur was moistened with 70% ethanol and a 3 cm midline abdominal incision was performed. Secondly, the cecum was delivered and abraded over a mould (area 2 cm²) with surgical gauze for 2 min. The abraded cecum with punctuate bleedings was then left outside the abdominal cavity to desiccate. Thirdly, a 1 cm small bowel resection, −15 cm from the ileocecal junction was made and a single-layer end-to-end anastomosis was constructed with 8 stitches, using interrupted resorbable 6-0 Monosyn® sutures (B. Braun Medical AB, Danderyd, Sweden). Anastomotic patency was confirmed by feeding small bowel content through it. Fourthly, the cecum and the small bowel were returned to the abdominal cavity in their proper anatomic positions. Finally, the laparotomy incision was closed in 2 layers with continuous resorbable 5-0 Vicryl® sutures (Johnson & Johnson AB, Sollentuna, Sweden) for the fascia, and continuous nonresorbable 4-0 Ethilon® sutures (Johnson & Johnson AB) for the skin. The surgical procedure took ~30 min. Postoperatively the rats received 0.03 mg (0.1 mg/kg) of buprenorphine s.c. every eight hours, for 3 days.

1.3. Experimental design

The animals were randomly designated to one of eight groups to be examined at various time points (T) after incision (T = 0, 0.5 h, 6 h, 12 h, 24 h, 72 h, 7 d, and 21 d with 7 rats each, except T = 0 and 0.5 h with 5 animals). At T = 0, only the laparotomy was performed before collecting samples of plasma and peritoneal fluid. The same anaesthetic procedures were used at examination.

1.4. Plasma and peritoneal fluid collection

Blood samples were obtained by intracardiac puncture using a 22 G sterile needle and 5 ml syringes. Samples were transferred to chilled centrifuge vials coated with heparin and spun within 30 min at 4,000 × g for 10 min at 4 °C. The plasma was aliquoted and stored at −80 °C until analysis. Peritoneal fluid samples were collected by peritoneal lavage with 40 ml of Ringer’s acetate. Ten milliliters of aspirated fluid was stored at −80 °C until analysis.

1.5. Laboratory assays

The cytokines IL-1β, IL-6 and TNF-α concentrations were measured in plasma and peritoneal fluid by electrochemoluminescence using the Multi Spot® 4 Spot Cytokine Plate Rat 3 plex (Meso Scale Discovery, Rockville, MD) according to the manufacturer’s instructions. The detection limits were 2.4 pg/ml for IL-1β, 16.7 pg/ml for IL-6 and 3.1 pg/ml for TNF-α in plasma and 3.1 pg/ml, 0.5 pg/ml and 2.8 pg/ml respectively, in peritoneal fluid. PDGF-BB, TGF-β1 and VEGF concentrations were measured in peritoneal fluid by quantitative sandwich enzyme immunoassay technique according to the manufacturer’s instructions. Quantikine® ELISA Rat PDGF-BB, Quantikine® ELISA Rat TGF-β1 and Quantikine® ELISA Rat VEGF were all obtained from R&D Systems (Abingdon, UK). The detection limit was 7.7 pg/ml for PDGF-BB, 4.6 pg/ml for TGF-β1 and 8.4 pg/ml for VEGF. The Rat Tissue-type Plasminogen Activator (tPA) Active ELISA Assay kit (Eagle Biosciences Inc., Nashua, NH) was used according to the manufacturer’s instructions to measure active tPA in peritoneal fluid samples. The detection limit was 50 pg/ml.

Active PAI-1 was determined in peritoneal fluid by Zymutest PAI-1 Activity ELISA (Aniara Diagnostica, West Chester, OH) according to the manufacturer’s instructions. The detection limit was 100 pg/ml.

1.6. Scoring of adhesions

An inverted U-shaped abdominal incision was performed to avoid disturbing any adhesions between viscera and the abdominal wall. Adhesions were quantified in a blinded fashion using three different scoring systems [21–23] (Table 1). Animals were then euthanized by desanguination.

1.7. Anastomotic bursting pressure

The bursting pressures of the anastomoses were tested at T = 72 h (n = 4), 7 d (n = 5) and 21 d (n = 5) by continuous infusion of Ringer’s acetate in a ~4 cm ileal segment containing the anastomosis and ligated at both ends. Bursting pressure was defined as the maximal pressure reached (in mmHg) until leakage from or burst of the anastomosis occurred.

2. Statistical analyses

Cytokine samples and standards were assayed in duplicates and are expressed as means ± standard error of the mean (SEM). Cytokines, adhesion scores, anastomotic bursting pressures measured at designated time points were compared, respectively using the nonparametric Kruskal-Wallis test and Dunn’s Multiple Comparison Test in GraphPad Prism™ version 5.0c. Differences in which p < 0.05 were considered statistically significant; * denotes p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001.

3. Results

Forty-nine of the 52 rats completed the study. Two rats, one in each group T = 12 h and T = 21 d, died during anaesthesia and one rat (T = 21 d) died postoperative day 14 owing to small bowel obstruction, confirmed by autopsy. Cytokine concentrations in plasma and peritoneal fluid at different time points are shown in Figs. 1–4.

Table 1

<table>
<thead>
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<th>Adhesion scoring systems.</th>
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<td><strong>Menkes</strong></td>
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<td>1 = separated with gravity</td>
</tr>
<tr>
<td>2 = separated by blunt dissection</td>
</tr>
<tr>
<td>3 = separated by sharp dissection</td>
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<tr>
<td>4 = difficult sharp dissection</td>
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| **Kennedy**               |
| 0 = no adhesions          |
| 1 = thin, filmy adhesions |
| 2 = > one thin adhesion   |
| 3 = thick adhesion with focal point |
| 4 = thick adhesion with planar attachment |
| 5 = very thick vascularized adhesions/≥1 planar adhesion |

| **Nair**                  |
| 0 = complete absence of adhesions |
| 1 = single band of adhesions between viscera/abdominal wall |
| 2 = two bands between viscera/abdominal wall |
| 3 = ≥2 bands between viscera/abdominal wall or whole intestines forming a mass without being adherent to abdominal wall |
| 4 = viscera adherent to abdominal wall irrespective of number or extent of bands |
Plasma concentrations of cytokines IL-1β and TNF-α were not increased (data not shown). Plasma and peritoneal fluid concentrations of IL-6 peaked at T = 6 h (138.9 ± 25.4 pg/ml, 6772 ± 1894 pg/ml, respectively). Concentrations of IL-6 decreased significantly at T = 7 d (p < 0.05) in plasma, Fig. 1 and at T = 72 h (p < 0.01) in peritoneal fluid, Fig. 2. Peritoneal fluid concentrations of IL-1β peaked at T = 6 h (393.4 ± 202.8 pg/ml) and decreased at T = 21 d (p < 0.05), Fig. 3. Peritoneal fluid concentrations of TNF-α peaked at T = 6 h (21.4 ± 5.8 pg/ml) and remained increased to T = 7 d (p < 0.05), Fig. 4. Peritoneal fluid concentrations of PDGF-BB, TGF-β1, VEGF, tPA and PAI-1 were below the detection levels (data not shown).

There were no congenital adhesions at the initial laparotomy. The animals at T = 0 h and 0.5 h did not have adhesions. There were fewer rats with adhesions at T = 6 h (2/7, 29%) and T = 12 h (6/7, 86%) than at later time points (26/26, 100%). Examples of peritoneal adhesions in experimental group T = 7d are demonstrated in Fig. 5. Menzies scoring system (Fig. 6a) revealed significantly higher scores at T = 7 d (2.9 ± 0.4) and T = 21 d (4.0 ± 0.0) compared to earlier time points. Kennedy scoring system (Fig. 6b) revealed significantly higher scores at T = 24 h (3.9 ± 0.1) and at T = 21 d (5.0 ± 0.0) compared to earlier time points. Nair scoring system (Fig. 6c) revealed higher scores at T = 24 h (3.1 ± 0.1) and at T = 7 d (3.1 ± 0.4) compared to earlier time points.

Anastomotic bursting pressures (Fig. 7) were significantly increased at T = 21 d (292 ± 48.7) compared to T = 72 h (52.5 ± 5.0).

4. Discussion

Adhesion induction by abrasion of the cecal surface and construction of a small bowel anastomosis has previously been described in rabbits [24]. By using the more convenient laboratory rat, it is a relevant and reproducible experimental model designed for dissection of molecular mechanisms as well as interventional procedures. Peritoneal adhesion formation occurs as a response to trauma and can be considered as a failure in the normal wound healing process. Thus, there is a risk that antiadhesion treatment can interfere with the healing process. Antiadhesion treatment should reduce adhesions without impaired anastomotic healing. By combining adhesion induction with construction of an intestinal anastomosis, the balance between anastomotic healing and the inhibition of adhesions after antiadhesion treatment can be studied.

We found that IL-1β concentrations were increased early after surgery. Early elevation and high concentrations of IL-1 in peritoneal fluid after surgery are regarded as a biological marker for postoperative adhesions in humans [25,26]. IL-1 is secreted by macrophages, and following caspase activation it promotes inflammation and coagulation and reduces fibrin degradation locally [10]. Intraperitoneal injection of IL-1 in rats increased adhesion scores after peritoneal injury [27]. Interference with IL-1β signalling may reduce formation of adhesions, and can be assayed in detail in an experimental model such as ours.
We also found increased IL-6 concentrations in peritoneal fluid and plasma at 6 h after adhesion induction. Elevated IL-6 concentrations at 12 h and 24 h were also seen, as have been reported previously in humans after major abdominal surgery [26]. IL-6 is produced by T-lymphocytes, macrophages and fibroblasts at sites of tissue damage [28]. It triggers the acute phase response and stimulates mesothelial release of PAI-1 in vitro [15], which may explain the decrease in peritoneal fibrinolytic activity reported at 48–72 h after surgery in humans [29]. Elevated concentrations of IL-6 in peritoneal fluid were found in patients with pelvic adhesions [30]. Preoperative administration of IL-6 into the peritoneal cavity of rats enhanced adhesion formation, caused more vascular adhesions, with a higher number of inflammatory cells and fibroblast deposits. Concentrations of IL-6 in both plasma and peritoneal fluid correlated with adhesions scores following a defined bowel injury in rabbits [24]. In our study, plasma concentrations of IL-6 peaked at 6 h. This is in accordance with a report of IL-6 increase in serum in six patients within 1.5 h after abdominal incision, reaching a maximum between 1.5–4 h. Data suggest that the tissue damage may be proportional to concentrations of IL-6 [28]. In summary, our and other’s data suggest that also interference with IL-6 signalling may be an antiadhesion treatment.

TNF-α concentrations were elevated in peritoneal fluid in our study at 6 h after adhesion induction. TNF-α is a cytokine secreted by several different cell types. It stimulates adhesion formation by promoting the inflammatory response and coagulation, and impairs fibrinolysis. Significant correlations between the severity of adhesions and concentrations of TNF-α in plasma and peritoneal fluid after surgery have been reported in both rats and humans [24,25,31]. Our data confirm that early elevations of TNF-α concentrations in peritoneal fluid correlate to adhesion formation in rats, and it may be a therapeutical target.

Surprisingly, peritoneal fluid concentrations of PDGF-BB, TGF-β1, VEGF, tPA and PAI-1 were below detection levels. This may reflect either that they do not participate, or are not secreted to the peritoneal fluid compartment, or are active at later time points, or that the detection limit of our assay was too high (peritoneal fluid was diluted with 40 ml Ringer’s acetate in order to retrieve a sufficient

![Image](https://example.com/fig5.png)

**Fig. 5.** Example of peritoneal adhesion in group T = 7 d. Xiphoid process top, reflected abdominal wall below. The forceps elevates a broad and thick adhesion between the cecum and the midline incision. Score 4 in all three scoring systems.

![Image](https://example.com/fig6.png)

**Fig. 6.** a: Menzies scoring system at different time points after incision. b: Kennedy scoring system at different time points after incision. c: Nair scoring system at different time points after incision.
volume for analysis). A recent study in patients demonstrated increased PAI-1 activity in peritoneal fluid compared with plasma samples, suggesting that peritoneal PAI-1 activity is more important for adhesion formation than systemic activity [32]. Our data cannot confirm participation of PDGF-BB, TGF-β1, VEGF, tPA or PAI-1 in adhesion formation in the rat.

Adhesion quantification is mandatory for the development of antiadhesion treatment. The lack of a gold standard forced us to employ three different scoring systems with the intention to identify the most relevant and reproducible system. Interestingly, all three scoring systems suggest a biphasic development of adhesions with a notch at 72 h, which might reflect a transition from fibrinous to collagogenous adhesions. Our data confirm that the three scoring systems have similar properties, but the most relevant and reproducible system remains to be determined. The increase in anastomotic bursting pressure coincided with high adhesion scores day 7 and 21.

In conclusion, our data confirm that early elevations of IL-6, IL-1β and TNF-α concentrations in peritoneal fluid but not in plasma correlate to adhesion formation in this adhesion rodent model. The model is designed for antiadhesion treatment, and our data indicate that antiadhesion treatments should be early, local and not systemic. In the next series of experiments using this animal model we want to test the hypothesis that early local antiadhesion therapy can prevent adhesions without impaired wound and anastomotic healing by injection of an antiadhesion substance (anti-inflammatory substance) in the peritoneal incision and in the intestinal wall before performing abrasion of cecum.

References