Original research

Effects of tamoxifen citrate on postoperative intra-abdominal adhesion in a rat model

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**A R T I C L E   I N F O**

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**A B S T R A C T**

**Background/aims:** The aim of this study was to evaluate the effects of oral tamoxifen citrate on postoperative intra-abdominal adhesions.

**Materials and methods:** Forty-five rats were randomly separated into 3 groups. Group 1: Control group (15 rats), Group 2: tmx 1 group (15 rats) and Group 3: tmx 10 group (15 rats). The cecum was abraded with a sterile gauze until subserosal hemorrhage had developed. Full-thickness 4–0 silk sutures were also placed in the traumatized anterior cecal wall to increase the adhesive reaction. In Group 1 (control group), adhesion induction was performed and no treatment was given. In Group 2 (tmx 1 group), after adhesion induction, 1 mg/kg/day tamoxifen citrate was given by orogastric gavage. In Group 3 (tmx 10 group), adhesion induction was performed and 10 mg/kg/day tamoxifen citrate was given by orogastric gavage. Rats were sacrificed on postoperative day 30. At the time of second surgery, after the abdominal fascia had been opened blood samples were collected to evaluate serum TGFβ-1 levels and following the macroscopic adhesion scoring, tissue specimens of the bowel and adhesions were subjected to histopathological investigation.

**Results:** In group 1 and group 2 we detected higher scores for the macroscopic classification (2.25 ± 1.13 vs. 1.53 ± 0.77) and histopathological scores (2.72 ± 0.64 vs. 2.53 ± 0.87) for fibrosis and serum TGFβ-1 levels (42800 ± 2935 vs. 32988 ± 10804). In group 3 we have detected decreased scores for macroscopic classification (0.91 ± 0.51) and histopathological scores (1.58 ± 0.90) for fibrosis and serum TGFβ-1 levels (22847 ± 4976). There were no significant differences between group 1 and group 2 according to the macroscopic classification and pathologocic scores for fibrosis. There were statistically significant difference between tamoxifen 10 mg/kg group and the other groups according to macroscopic classification (P: G1-3: 0.004; G2-3: 0.046), pathological scores for fibrosis (P: G1-3: 0.004; G2-3: 0.011) and serum TGFβ-1 levels (P: G1-3::<0.001).

**Conclusions:** In conclusion tamoxifen citrate seems to be useful for preventing postoperative intra-abdominal adhesions. Its effects are in a dose and time dependent manner. Further studies must be carried out to use tamoxifen for preventing intra-abdominal postoperative adhesions in clinical practice.

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**1. Introduction**

Intraabdominal adhesions may be postoperative, inflammatory or rarely congenital. Mechanical injury, foreign bodies, ischemia and infection in abdominal operations can increase the severity of adhesions. Postoperative intraabdominal adhesions are the major causes of morbidity after laparotomy.1−3 Postoperative intraabdominal adhesions are fibrotic bands that can be form between an organ and peritoneum, omentum and intestine or intestine and intestine.

Intra-abdominal adhesions causes complications such as postoperative pain, bowel obstruction and infertility. Fibroblasts isolated from normal peritoneum and from adhesions have been showed to have significant molecular differences.4 These differences include phenotypic alterations in adhesion fibroblasts such as a reduction in both the ratio of tissue plasminogen activator/plasminogen activator inhibitor-1 and in the rate of apoptosis under hypoxic conditions, and greater ability to produce the inflammatory cytokine transforming growth factor-β (TGF β) and extracellular matrix molecules, than normal peritoneal fibroblasts.5,6 TGFβ-1 is a growth factor with a wide variety of tissue-specific effects. TGFβ-1 stimulates fibroblasts to produce many proteins, including collagen, fibronectin, and integrins. It also decreases the...
production of proteins whose function is to degrade the extracellular matrix, such as collagenase and heparinase. TGF β is an extremely potent chemoattractant for macrophages, mononuclear leukocytes and fibroblasts and it acts as a potent stimulant for collagen and fibronectin synthesis and inhibits epithelial cell growth. Of the isoforms of TGF β, TGFβ-1 and TGF β-2 have been associated with fibrogenic conditions, whereas TGF β-3 tends to decrease fibrosis and scarring. TGFβ-1 has been shown to stimulate fibroblast proliferation and the production of extracellular matrix (ECM) components in vitro, making it a prime candidate as a causative factor.\(^\text{7,8}\)

Tamoxifen citrate is a synthetic nonsteroidal antiestrogen, used in the treatment of breast cancer. It has been shown to inhibit keloid fibroblast proliferation and decrease collagen production. Tamoxifen decreases the total (all 3 isomers) TGF β produced by keloid fibroblast in cell culture in a dose dependent manner.\(^\text{9}\)

2. Materials and methods

Forty-five Wistar-Albino female rats, weighing 250 ± 25 g, were housed individually in wire cages under constant temperature (21 ± 2 °C) with a 12 h light–dark cycle. The animals were allowed free access to water and standard rat chow. Twelve hours before anesthesia, animals were deprived of food, but had free access to water 2 h before anesthesia. No enteral or parenteral antibiotics were administered during the operation. All operations were performed by the same surgeon. Rats were allowed access to food and water after the operation. All operations were performed by the same surgeon. Rats were housed individually in wire cages under constant temperature (21 °C).

The animals were allowed free access to water and standard rat chow. Twelve

2 hours before anesthesia, animals were deprived of food, but had free access to water 2 h before anesthesia. No enteral or parenteral antibiotics were administered during the study. The procedures in this experimental study were performed in accordance with the National Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Ankara Research and Training Hospital.

Rats were randomly divided into three groups of 15 animals each. All animals were anesthetized by intramuscular injection of 30 mg/kg ketamine hydrochloride (Ketalar\(^\text{®}\), Parke-Davis, Istanbul) and 5 mg/kg xylazine (Rompun\(^\text{®}\), Bayer, Istanbul). Liposomal povidone-iodine hydrogel (Repithel\(^\text{®}\)) was purchased from Mundi-pharma GmbH (Limburg, Germany). After the rats were anesthetized, the abdomen was shaved and prepared with povidone-iodine. Under sterile conditions and with powder free gloves, 3 cm long midline incision was made up, and the anterior cecal wall was abraded with 20 strokes of a toothbrush. Also full thickness 4–0 silk sutures were placed in the traumatized anterior cecal wall to increase the adhesion reaction. The abdominal incision was closed in two layers with simple, continuous sutures of silk 3/0. The methods were described by Oncel M. et al.\(^\text{13}\)

In Group 1 (control group), adhesion induction was performed and no treatment was given. In Group 2 (tmx 1 group), after adhesion induction, 1 mg/kg/day tamoxifen citrate (Sigma Chemical Co, St Louis, USA) was given by orogastric gavage. In Group 3 (tmx 10 group), after adhesion induction, 10 mg/kg/day tamoxifen citrate was given by orogastric gavage. Animals were allowed access to food and water after the operation. All operations were performed by the same surgeon. Rats were sacrificed with high dose ethered on postoperative day 30. Blood samples were collected at the time of second surgery, after the abdominal fascia has been opened and immediately centrifuged at 30 000 rpm for 5 min at 4 °C. The serum was aspirated and stored at -80 °C until analyzed. Adhesions were scored using the macroscopic classification described by Zühle et al.\(^\text{14}\)

1 Filmy and easy to separate by blunt dissection
2 Blunt dissection possible, partly sharp dissection necessary, beginning vascularization
3 Lysis possible by sharp dissection only, clear vascularization
4 Lysis possible by sharp dissection only, organs strongly attached with severe adhesions, damage of organs hardly preventable.

The histopathological analyses were carried out in the Pathology Department of Koceren Research and Training Hospital. Histopathological examination was performed by using light microscopy. The samples obtained from the abraded cecal tissue and the adjacent peritoneal tissue was fixed in 10% neutral buffered formalin solution for 2 days. Tissues were washed in running water, and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96% and 100%). After dehydration, specimens were placed into xylene to obtain transparency and embedded in paraffin. Embedded tissues were cut into 5 µm-thick sections and were stained with hematoxylin/eosin and trichrome. Histopathological examinations were performed by a pathologist blinded to the study groups. Histological classification was carried out according to the criteria mentioned by Yılmaz et al.\(^\text{15}\)

| No fibrosis | 1 Thin bunches of a cellular fibrosis | 2 Wide areas of fibrosis with reduced vascularization | 3 Areas of fibrosis formed by thick bunch of collagen |

0.64 vs. 2.53

0.77 vs. 0.91

1.13 vs. 1.53

0.87 vs. 1.58

Histopathology

<table>
<thead>
<tr>
<th>Group 1 (n:12) mean score ± s.d.</th>
<th>Group 2 (n:13) mean score ± s.d.</th>
<th>Group 3 (n:12) mean score ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic classification 0.004</td>
<td>2.25 ± 1.13</td>
<td>1.53 ± 0.77</td>
</tr>
<tr>
<td>TGFβ-1 -0.001</td>
<td></td>
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<tr>
<td>Histopathology 0.003</td>
<td>2.72 ± 0.64</td>
<td>2.53 ± 0.87</td>
</tr>
</tbody>
</table>

The mean adhesion scores for macroscopic classification, histopathological examination and the mean serum TGFβ-1 levels are summarized in Table 1.

3. Results

A total of 45 rats sacrificed on postoperative 30th days. Group 1: Control group (15 rats), Group 2: tmx 1 group (15 rats) and Group 3: tmx 10 group (15 rats).

The mean adhesion scores for macroscopic classification, histopathological examination and the mean serum TGFβ-1 levels are summarized in Table 1.

3.1. Intra-abdominal adhesion scores

In group 1 and group 2 we detected higher scores for the macroscopic classification (2.25 ± 1.13 vs. 1.53 ± 0.77). In group 3 we have detected decreased scores for the for macroscopic classification (0.91 ± 0.51). There were no significant difference between group 1 and group 2 according to the macroscopic classification.

3.2. Histopathological scores

In group 1 and group 2 we detected higher scores for histopathological scores (mean scores: 2.72 ± 0.64 vs. 2.53 ± 0.87) for...
fibrosis. But in group 3 we have detected decreased histopathological scores (mean score: 1.58 ± 0.90) for fibrosis. There were no significant difference between group 1 and group 2 according to histopathological scores for fibrosis. There were statistically significant difference between tamoxifen 10 mg/kg group and the other groups according to histopathological scores for fibrosis (P: G1-3: 0.004; G2-3: 0.011).

3.3. Serum TGFβ-1 levels

For serum TGFβ-1 levels in group 1 and group 2 we detected higher scores (mean scores: 42000 ± 2935 vs. 32988 ± 10804). But in group 3 we have detected the lowest scores for serum TGFβ-1 levels (mean score: 22847 ± 4976). According to serum TGFβ-1 levels there was no significant difference between group 2 and group 3, but there were statistically significant difference between group1 and group 3 and between group 1 and group 2 (P: G1-3: <0.001 vs. G1-2: 0.04).

The images of adhesions and abrasion of cecum are shown in Figs. 1–3.

The histopathological images of the pathological scores are shown in Figs. 4–7.

4. Discussion

Despite improvements in surgical techniques and instruments, postoperative intra-abdominal adhesions are still a challenging problem and major cause of morbidity and mortality.

Postoperative adhesions can form after trauma to the peritoneal cavity, inflammatory conditions, intraperitoneal infection, congenital, tissue ischemia and intraabdominal presence of foreign material such as blood or bile. Peritoneal adhesions are defined as fibrous bands of tissue that join together organs that are normally separated.

Peritoneal mesothelial cells provide a natural protective barrier that prevents the organs from adhering to adjacent opposing surfaces. In normal peritoneum, fibrinolytic activators in the mesothelium convert plasminogen to plasmin which lyses fibrin. Usually the rates of fibrin deposition and fibrinolysis are equal. However, the factors that cause intraeritoneal adhesion impair the fibrinolytic activators and lead to excess fibrin deposition. If cellular or tissue injury is relatively extensive, leading to excess migration and proliferation of various wound cells, i.e. fibroblasts, this response initiates a cascade of events that often results in the development of peritoneal adhesions. These adhesions are known to be the major cause of bowel obstruction, pain, infertility and hospital readmission.

Peritoneum has an intrinsic fibrinolytic activity that breaks the peritoneal adhesions. Peritoneal injuries with ischemia interfere this fibrinolytic activity and cause adhesions. The fibrotic response also involves increased production of cytokines and growth factors, including TGF β1 and connective tissue growth factor (CTFG).

TGF β1 has been shown to stimulate fibroblast proliferation and the production of ECM, making it a prime candidate as a causative factor. Experimental data from peritoneal adhesions in human and animal models of surgically induced adhesions suggest that the local over-expression of TGF β is a key to development of adhesions. TGF-β1 induces many of the processes involved in fibrosis, including fibroblast to myofibroblast transdifferentiation, ECM deposition, although without inducing inflammation. Chegini et al. demonstrated that adhesions express a higher concentration of TGF-β1, with large bowel and omentum expressing the lowest
concentrations, while TGF-β3 expression was relatively less variable than TGF-β1 with uterine serosa expressing the highest concentration. Hobson et al. confirmed that human peritoneal adhesions contain high amounts of TGF-β1 compared with normal tissue and the increased TGF-β1 expression in scar tissue persists for several years after the initial injury.

Williams et al. were designed a study to measure the effect of exogenous TGF-β on postoperative peritoneal wound healing and they were demonstrated that TGF-β increases the severity of adhesion formation after surgical injury, but has no effect on uninjured peritoneum.

Tamoxifen citrate is a synthetic nonsteroidal antiestrogen agent mainly used in the treatment of breast cancer. It may also be effective in the treatment of abnormal proliferative disorders. In vitro studies have shown that tamoxifen inhibits the proliferation of keloid fibroblasts, decreases the rate of collagen synthesis, decreases the production of TGF-β, and decreases the ability to contract fibroblast-populated collagen lattices.

Mc Namara et al. showed that there were not significant difference in adhesion scores between placebo treated and tamoxifen treated rats on postoperative 3, 5 and 7 days, although Nilsson et al. showed that long term tamoxifen treatment decreased the secreted levels of TGF-β1 whereas estradiol increased these levels. They found that 24 h of tamoxifen treatment gave a significant increase in TGF-β1 protein levels in conditioned media. In contrast to the short term treatment, tamoxifen treatment for seven days significantly decreased TGF-β1 protein levels. In vivo experiments showed that tamoxifen treatment was significantly decreased angiogenesis and TGF-β1 protein levels in solid breast cancer.

The adhesion formation is related to the time of the second surgery because postoperative adhesions at the peritoneum most commonly form within 7–10 days and become persistent after 14 days. The time of second surgery at Mc Namara study seems to be early to evaluate postoperative adhesion. So that we sacrificed rats on postoperative 30 days.

In the present study, we utilized a rat model to compare the effect of tamoxifen 1 mg/kg and 10 mg/kg on postoperative adhesions. We found that treatment with tamoxifen 10 mg/kg significantly reduced postoperative adhesion formation. We found a statistically significant difference between control group and tamoxifen 10 mg/kg group.
Also the difference was statistically significant between tamoxifen 10 mg/kg and 1 mg/kg groups. The differences between control group and tamoxifen 1 mg/kg group were not statistically significant. TGFβ-1 levels were significantly reduced with tamoxifen 10 mg/kg treatment. There were statistically significant difference between tamoxifen 10 mg/kg group and control group.

The pathological scores for fibrosis were significantly different between tamoxifen 10 mg/kg group and the other groups.

We detected connective tissue maturation and being wide-spread of collagenation for fibrosis scoring. In Tamoxifen 10 mg/kg treatment group the production of collagen connective tissue was reduced while it was significantly induced in control group and tamoxifen 1 mg/kg group.

In tamoxifen 1 mg/kg group we also found that connective tissue were more extensive and spread about striated muscle and smooth muscle of bowel. It was also rich from inflammatory cells. Tamoxifen 1 mg/kg was not reduced enough TGFβ-1 levels. So that more extensive connective tissue and rich inflammatory cells were found.

We have detected the antifibrotic effects of tamoxifen citrate in supra-therapeutic doses, yet we don’t know the possible side effects of supra-therapeutic doses in human being especially in man. We used supra-therapeutic doses of tamoxifen citrate to see the antifibrotic effects in short term therapy, so a long term therapy should be scheduled with lesser doses of tamoxifen citrate to see the same effects. Further studies are needed to see the side effects of supra therapeutic doses in human being or to evaluate the choice of long term therapy with therapeutic doses. To our knowledge there are not any other inhibitors of TGFβ-1 used in vivo or in vitro studies as tamoxifen citrate.

5. Conclusion

In conclusion, the antifibrotic effects of tamoxifen are related with the reduced TGFβ-1 levels, so it reduces postoperative adhesions in a dose and time dependent manner. Our study indicates that tamoxifen may be used as a preventive agent for postoperative intra-abdominal adhesions. Further experimental and clinical studies are needed to use tamoxifen in clinical practice as a preventive agent for postoperative intra-abdominal adhesions.

Ethical approval

Animal Ethics committee of Ankara Research and Training Hospital, Ankara, Turkey.

Funding

None

Author contribution


Conflict of interest

None

References