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A randomized controlled study of the efficacy of misoprostol and hyaluronic acid in preventing adhesion formation after gynecological surgery: a rat uterine horn model

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ABSTRACT

Objective: To investigate the effect of misoprostol in the reduction of adhesion formation after gynecological surgery.
Study design: A double blind, randomized controlled experimental study was designed. Twenty-one female Wistar Hannover rats were divided into three groups as control, misoprostol and Hyalobarrier® groups. A uterine horn adhesion model was created. After anesthesia induction, 1.5–2 cm injuries were made to the each uterine horn by cautery. The control group received no special medications except for the standard surgical procedure. The misoprostol group received 10 μcg/kg misoprostol in addition to the standard surgical procedure, and the Hyalobarrier® group received 1 cm^3 ready-for-use Hyalobarrier® gel intraperitoneally in addition to the standard surgical procedure. After 14 days from the first surgical procedure, adhesion scores were evaluated.
Results: The extent ($p < 0.001$), severity ($p < 0.001$), degree ($p < 0.001$) and total adhesion score ($p < 0.001$) values of the control group were statistically higher than the values of misoprostol and Hyalobarrier® groups. The inflammation score value of misoprostol group was statistically lower than control and Hyalobarrier® groups ($p < 0.001$).
Conclusion: In this study, we have found a new therapeutic potential of misoprostol that may be useful in preventing pelvic adhesion and reducing inflammation scores.

1. Introduction

Postoperative intra-abdominal adhesions occur in 50–95% of women who undergo gynecological surgery [1]. The development of peritoneal adhesions following abdominal and pelvic surgery leads to clinical problems, including intestinal obstruction, infertility, and chronic pelvic pain [2]. Adhesions remain a potential problem for future surgical procedures, increasing health expenses and hospital readmission rates, and reducing the quality of life of the patient [3]. There are several described causes of adhesions, such as mechanical trauma, ischemia caused by sutures or electrocautery, presence of foreign bodies, tissue desiccation, and infection [4]. Furthermore, during peritoneal healing following surgery, the increase in peritoneal fluid, cytokines, proteins and fibrin formation can promote adhesion formation [5,6].

Currently, there are various methods of preventing adhesion formation such as the use of barrier materials, non-steroid anti-inflammatory drugs, calcium channel blockers, corticosteroids, vitamin E, antihistamines, metformin, melatonin, progesterone, estrogen, gonadotrophin-releasing hormone (GnRH)-agonists and antagonists, anticoagulants, fibrinolytic agents, and antibiotics [7–10].

Hyalobarrier® gel is a highly viscous gel derived from hyaluronan, obtained through an auto-crosslinking process, and used as an adhesion prevention agent in a variety of surgical procedures [11–13]. Hyalobarrier® gel can reduce the incidence and severity of postoperative adhesions in cases of severe uterine damage caused by laparotomy or laparoscopic myomectomies [14,15]. The gel can also be easily applied during laparoscopic and hysteroscopic procedures.

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Misoprostol, a synthetic analog of prostaglandin E1, is used as an anti-ulcer drug that increases gastric mucus secretion and reduces gastric acid secretion [16]. Misoprostol is also used off-label by obstetricians for the induction of first trimester abortion [17]. Misoprostol has been shown to alter enzymatic processes that cause degradation and remodeling of collagen fibers [18]. Nevertheless, no in vivo studies have demonstrated that misoprostol can reduce adhesion formation. Misoprostol is commercially available as Cytotec® (Ali Raif İlaç, İstanbul, Türkiye) in a pharmaceutical complex with hydroxypropyl methylcellulose, microcrystalline cellulose, sodium starch glycolate, and hydrogenated castor oil [19].

In this study, we aimed to investigate the effect of the intraperitoneal use of misoprostol in the prevention of adhesions by reducing collagen synthesis, which plays a major role in wound healing and scar formation, compared with that of Hyalobarrier® gel, which is known to prevent adhesions in clinical practice.

2. Materials and methods

The study was approved by the local ethical review board of the Bagcilar Training and Research Hospital in Istanbul, Turkey, for the use of laboratory animals, and was performed at the experimental surgery training and education center of the same hospital (approval number: 2013–17).

2.1. Animal maintenance and treatment

Rats weighing approximately 180–260 g were housed under the institutional review board's guidelines for animal care using a day cycle of 14 h of light with free access to food and water. A power analysis was performed to calculate the minimum sample size required for the study (alpha error = 0.05 and 1-beta = 0.8) and at least 12 uterine horns were required for each study group. As 10% of the animals are lost during the procedures, we included in total 14 horns. The rats were randomly assigned to three study groups, each consisting of seven rats, using computer-based randomization. Twenty-one mature, nonpregnant, female Wistar Hannover rats (aged 8–10 weeks) were used as models for the experimental induction of postoperative uterine horn adhesion, as defined by Kelekci et al. [20]. Animals were anesthetized using 50 mg/kg of 10% ketamine hydrochloride (Ketasol; Richter Pharma AG, Wels, Austria) intramuscularly (IM) and 5 mg/kg of 2% xylazine (Rompun; Bayer Health Care LCC, Kansas, KS) IM. Prior to the operation, the abdominal skin was shaved and disinfected using 10% povidone-iodine solution (Batticon; Adeka Laboratories, Istanbul, Turkey). A 3-cm midline incision was made, and the uterine horns were exposed. Seven standard lesions were inflicted in the antimesenteric surfaces of each uterine horn using a 10 W bipolar cautery (Bovie®, FL, USA). An area of 1.5 cm × 1.5 cm between the two main vascular branches of the lower side wall at the level of each uterine horn was cauterized to produce an opposing lesion. The first group of rats served as the control group and received no special medication. The second group (misoprostol group) received 10 μcg/kg misoprostol intraperitoneally (IP) using a diluted Cytotec® 200 μcg tablet (Ali Raif İlaç, İstanbul, Türkiye). The third group (Hyalobarrier® gel group) received 1 mL of ready-to-use Hyalobarrier® gel (Fidia Advanced Biopolymers S.r.I Abano Terme, Italy) IP. The abdominal incision was closed in two layers with a 4-0 polyglycolic acid suture (Vicryl™; Johnson and Johnson Medical Limited, Ethicon Limited, UK) for the peritoneum and 3-0 polyglactin suture for the skin.

After the animals recovered from surgery, they were housed separately under controlled temperatures of 22 ± 2 °C and a 14 h light cycle with food and water ad libitum. The surgery was limited to approximately 15 min for each rat to prevent tissue drying at room temperature. All the surgical procedures were performed by the same researchers.

2.2. Tissue sample collection and histopathology

Following the 14-day recovery period, the rats were sacrificed by cervical dislocation. The adhesion areas were evaluated and graded macroscopically by two observers who were blinded to the study groups. The extent and severity of the adhesions were evaluated using an established scoring system [21]. The extent of adhesion spread to the uterine horn defect was scored as follows: 0, no uterine adhesion; 1, 1–25% involvement, 2, 26–50% involvement, 3, 51–75% involvement, and 4, 76–100% involvement. Adhesions were further characterized on gross examination according to the severity of attachment between the uterine horns and other abdominal organs as follows: 0, no adhesion, 1, filmy avascular, 2, vascular or opaque, and 3, cohesive. The degree of adhesion formation was evaluated and scored using the following; 0, no adhesion; 1, the adhesion could be separated from the tissue with gentle traction, 2, the adhesion could be separated from the tissue with moderate traction, 3, the adhesion could be separated from the tissue by sharp dissection. The sum of the three parameters was used as the total score for each group.

Tissue samples were obtained from all serosal surfaces where adhesions had developed. One histologist evaluated all tissues and was blinded to the origin of the samples. Tissues were graded for levels of inflammation and fibrosis using previously published grading scales. For histological analyses, the excised tissues were fixed in 10% buffered formalin solution for 24 h. After fixation, a routine tissue-processing procedure was performed, and sample tissues were embedded in paraffin. Paraffin wax blocks were cut into 4-μm thick sections. Prepared sections were then stained with hematoxylin and eosin, and levels of inflammation were scored as follows: 0, no inflammation; 1, presence of giant cells, occasional lymphocytes, and plasma cells; 2, presence of giant cells, plasma cells, eosinophils, and neutrophils; and 3, presence of many inflammatory cells and microabscesses. The level of fibrosis was scored as follows: 0, no fibrosis; 1, minimal, loose; 2, moderate; 3, florid dense [22].

2.3. Immunohistochemistry

Slides were immunostained with 100 μg of transforming growth factor beta-1 (TGF-β1) rat polyclonal antibody (Acris Antibodies GmbH; Schillerstraße 5, D-32052 Herford, Germany).

The sections were incubated at 65 °C for 1 h overnight. Deparaffinization was performed using the following series of washes: two xylene washes (3 min each), two 100% ethanol rinses (3 min each), and one wash each of 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, and Tris-buffered saline with 0.05% Tween (TBST; 3 min each wash on a shaker).

For antigen retrieval, 500 mL of distilled water was added to a pressure cooker. The slides were immersed in a staining dish containing the antigen retrieval solution and then placed in a de-cloaking chamber. Sodium citrate buffer (10 mM Sodium citrate, 0.05% Tween 20, pH 6.0) was used as the antigen retrieval buffer. The sections were boiled for approximately 10 min and cooled at room temperature. The slides were then removed and rinsed in TBST.

For the staining procedure, the slides were washed with TBST and covered with 3% hydrogen peroxide to inactivate the endogenous peroxidase. The slides were washed three times with TBST for 3 min each on a shaker and blocked with a universal protein blocking solution for 1 h. The TGF-β1 rat antibody (AP06350PU-N) was diluted to 1:50. The primary antibody was applied to each section and incubated overnight in a humidified