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Preventing intraperitoneal adhesions with ethyl pyruvate and hyaluronic acid/carboxymethylcellulose: a comparative study in an experimental model

E. Kıyak Caglayan [a,*], K. Caglayan [b], N. Erdogan [c], H. Cinar [d], B. Güngör [e]

[a] Bozok University, Medical Faculty, Department of Obstetric and Gynecology, Adnan Menderes Bulvarı No:190, Yozgat, Turkey
[b] Bozok University, Medical Faculty, Department of Surgery, Yozgat, Turkey
[c] Taksim Training and Education Hospital, Department of Pathology, Istanbul, Turkey
[d] Siirt Kurtalan State Hospital, Department of Surgery, Siirt, Turkey
[e] Ondokuz Mayıs University, Medical Faculty, Department of Surgery, Samsun, Turkey

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ABSTRACT

Objective: To compare the effectiveness of ethyl pyruvate (EP) with that of hyaluronic acid + carboxymethyl cellulose (Seprafilm) for the prevention of intraperitoneal adhesions. Seprafilm has been shown to be effective in many experimental and clinical studies.
Study design: Thirty rats were divided into three groups at random, and uterine horn abrasion was performed by laparotomy. One group received no treatment (control group), one group received a single intraperitoneal dose of EP 50 mg/kg (EP group), and a 2 × 1-cm patch of Seprafilm was applied in the third group (Seprafilm group). All rats were killed 14 days after surgery. Macroscopic and histopathological evaluation were performed by a surgeon and a pathologist who were blinded to group allocation. Histopathologically, inflammation, fibroblastic activity, foreign body reaction, collagen proliferation, vascular proliferation, Masson-Trichrome score, matrix metalloproteinase-2 score and vascular endothelial growth factor score were studied.
Results: Median macroscopic intraperitoneal adhesion scores for the control, EP and Seprafilm groups were 2.8, 1.2 and 1.1, respectively. Multiple comparisons between groups showed a significant difference ($p < 0.05$). In binary comparisons, significant differences were found between the control group and the EP group, and between the control group and the Seprafilm group ($p < 0.05$). No significant difference was found between the adhesion scores for the EP group and the Seprafilm group ($p > 0.05$). After histopathological evaluation, significant differences in all parameters were found between the groups ($p < 0.05$). In the paired comparison, significant differences were found between the control group and the EP group, and between the control group and the Seprafilm group ($p < 0.0167$), but no significant difference was found between the EP group and the Seprafilm group ($p > 0.0167$).
Conclusions: In comparison with the untreated control group, EP and Seprafilm were found to reduce the formation of intraperitoneal adhesions. No significant difference was found between EP and Seprafilm.

Introduction

Intra-abdominal adhesions occur in 90% of patients who undergo laparotomy, and retreatment is required in 5–20% of cases. These patients face the risk of further surgical intervention, with associated increases in cost, morbidity and mortality [1,2]. Intra-abdominal adhesions may cause intestinal obstruction, chronic abdominal pain and infertility [3]. Understanding the pathogenesis of intra-abdominal adhesions at cellular and molecular level may facilitate the development of effective treatment options for their prevention. When the formation mechanisms are studied, initiation of a response at cellular level starts a cascade continuing with an interaction between the fibrinolytic system and extracellular matrix (ECM) being closely related with mainly pro-anti-inflammatory cytokines following the injury of blood vessels and that of mesothelial cells in the peritoneum [4].

In order to prevent intra-abdominal adhesions after surgery, mechanical barriers such as hyaluronic acid + carboxymethyl

* Corresponding author. Tel.: +90 354 2127949; fax: +90 354 2122789.
E-mail address: emelkiyak@hotmail.com (E.K. Caglayan).

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cellulose (Seprafilm; Genzyme Corp., Cambridge, MA, USA), oxidized regenerated cellulose (Interceed) and polylactic acid film, HMG-CoA reductase inhibitors (statin), melatonin and collagen have been used [5,6].

Ethyl pyruvate (EP; Sigma Aldrich Co, St Louis, MO, USA) is the lipophilic ester derivative of pyruvate [7]. Several experimental studies have shown that EP has a protective effect on various tissues in haemorrhagic-endotoxic shock and with ischaemia-reperfusion injury. EP has also been shown to have an anti-inflammatory effect [8–10]. It reduces the activation of pro-inflammatory substances such as nuclear factor-jB (NF-jB), and inhibits the release of pro-inflammatory substances [tumour necrosis factor (TNF), interleukin (IL)-6] and cyclo-oxygenase-2 (COX-2) in intestinal mucosa [11]. NF-jB activates many angiogenic genes such as inducible nitric oxide synthase, COX-2, plasminogen activator 1–2 and matrix metalloproteinases (MMPs) in the angiogenesis pathway [12]. As such, as EP inhibits the NF-jB pathway, it also has anti-angiogenic activity [10].

To the authors' knowledge, this is the first study on the prevention of intra-abdominal adhesions using EP. This study compared the anti-adhesive effects of EP with no treatment and Seprafilm; the efficacy of Seprafilm has been shown previously in experimental and clinical studies. For comparison of EP and Seprafilm, this study investigated the presence of MMP-2 and vascular endothelial growth factor (VEGF) in adhesion tissue playing role in the stages of occurrence of fibrosis, pro-inflammatory cytokines and angiogenesis which are key steps in the development of adhesions, and the effect of these medications on MMP-2 and VEGF. Histopathological assessment of adhesion tissue was also undertaken.

Materials and methods

Animals and pre-operative preparation

This study was approved by Ondokuz Mayıs University Local Ethics Committee for Animal Experiments (11/07/2012, Ref. No. 2012/49). Thirty female Wistar Albino rats weighing 250–300 g were used in the study. A sample size of 10 animals for each group was calculated using a mean difference of 1.5 units, standard deviation of 0.8 units, 95% confidence interval and at least 90% test power. All rats were fed ad libitum with standard rat chow and tap water. All subjects were kept in a 12-h dark and 12-h light cycle at a standard temperature (22 °C) before and after surgery. Subjects were observed for at least 48 h before inclusion in the study. After 12 h of starvation, subjects were weighed and divided at random into three groups: control group, EP group and Seprafilm group.

Operative technique

Ketamine 50 mg/kg (Ketalar, Pfizer, Turkey) and xylazine HCl 10 mg/kg (Rompun, Bayer, Turkey) were administered to all animals for anaesthesia. For each subject, the abdomen was shaved and painted with iodine povidone under general anaesthesia. All surgical procedures were performed under sterile conditions. Laparotomy was performed with a midline incision of approximately 2 cm. After locating the uterus and tuba, a researcher (KC) who was blinded to group allocation created abrasion using forceps on the tubal antimesenteric side in all subjects. In the control group, abrasion alone was performed. In the Seprafilm group, a 2 × 1-cm patch of Seprafilm was placed between the uterine horn and the abdominal wall after abrasion. In the EP group, a single intraperitoneal dose of EP 50 mg/kg (0.1 ml solution) was given after abrasion. Midline incisions of all subjects were closed with 3/0 silk suture after surgical procedures. All rats were returned to their cages, kept at an ambient temperature of 22 °C and fed a standard rat diet after surgery. None of the animals died or had clinical evidence of adverse events (e.g. signs of wound infection, anorexia, vomiting, diarrhoea or altered behaviour). All rats were killed with a high dose of anaesthetic 14 days after surgery. A repeat laparotomy was performed immediately through the same midline incision, and the uterine horn and abdominal sidewall were evaluated for adhesions.

Macroscopic evaluation

Macroscopic adhesions were evaluated by a surgeon (HC) who was blinded to group allocation. Postoperative intraperitoneal adhesions were classified in accordance with Mazuji et al. [13]. The adhesions were graded as follows: Grade 0, no adhesions; Grade 1, very tiny and irregular adhesions; Grade 2, easily separable, medium-intensity adhesions; Grade 3, intense, not easily separable regular adhesions; and Grade 4, very hard, not easily separable, homogeneous adhesions.

Histopathological, histochemical and immunohistochemical evaluations

Following macroscopic assessment, tissue samples from fibrous bands between the peritoneum and the uterine horn from the three groups were fixed in 10%-buffered formaldehyde solution for 24 h, and embedded into paraffin blocks after routine tissue follow-up. Sections with 4-μm thickness were plated on to polylysine-coated slides for histological examination, and for histochemical and immunohistochemical staining. Histological adhesion scores for preparations stained with haematoxylin and eosin were graded semi-quantitatively between 0 and 4, using an Olympus BX53F microscope, in terms of inflammation [inflammation score (IS), fibroblastic activity score (FAS), foreign body reaction score (FBRS), collagen proliferation score (CPS) and vascular proliferation score (VPS)] according to the method of Kanbour-Shakir et al. [14] (Table 1). Masson-Trichrome histochemical stain (Bio-optica) was applied to identify fibrosis and collagen proliferation. As angiogenesis, inflammation and fibrosis are of critical importance, primary antibodies such as VEGF Ab-7 Ab (Mouse Monoclonal Antibody, Cat # 1467-R7; Neomarkers, Fremont, CA, USA) and MMP-2 Ab (Mouse Monoclonal Antibody, Cat # MS-806-R7; Neomarkers) were applied to the sections using the Streptavidin-Biotin method. Sections stained with Masson-Trichrome were scored between 0 and 4 with respect to the severity of fibrosis (0, no fibrosis; 1, slight fibrosis; 2, moderate fibrosis; 3, significant fibrosis; 4, massive fibrosis) in parallel with histological assessment. Immunohistochemical staining results were scored by considering cytoplasmic staining patterns in the

Table 1
Histological adhesion score.

	İnflammation	Fibroblastic activity	Foreign body reaction	Collagen proliferation	Vascular proliferation
Grade 0	No	No	No	No	No
Grade 1	25% mix inflammation	Slight	Slight	Slight	Slight
Grade 2	50% mix inflammation	Moderate	Moderate	Moderate	Moderate
Grade 3	75% mix inflammation	Marked	Marked	Marked	Marked
Grade 4	Massive inflammation	Massive	Massive	Massive	Massive