Experimental Validation for the Use of a Film on the Basis of Modified Hyaluronic Acid for Prevention of Postoperative Peritoneal Adhesions

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 144, No. 8, pp. 238-240, August, 2007
Original article submitted January 30, 2007

A film coating based on hyaluronic acid and 5-aminosalicylic acid was developed for the creation of anti-adhesion barrier. Application of the protective film in Wistar rats with modeled adhesion process in the abdominal cavity reduced the formation of peritoneal adhesions.

Key Words: *postoperative adhesions; anti-adhesion barriers; hyaluronic acid*

Barrier methods for the prevention of postoperative adhesions are developed and introduced in clinical practice.

Anti-adhesion barriers are effective due to hydrofloating, sliding, and mechanical separation of surfaces. This trend is ethiopathogenetically valid, because these barriers prevent adhesion of damaged surfaces to the adjacent organs and creates favorable conditions for reparative regeneration of tissues [2,3,5].

Strong and flexible protective coating based on immunologically neutral hyaluronic acid (HA) are widely used [2,5]. Disinfecting and wound-healing effects of HA were proven [4]. Being an important component of the connective tissue matrix, HA plays an important role in the realization of the reparative functions of the connective tissue [1]. Chemically modified HA (in the form of gels, films, sponges) is characterized by high biocompatibility, low (compared to native HA) degradation rate, and is used for the prevention of adhesions and stimulation of fibrous tissue formation [5-7].

A protective film from chemically modified HA with 5-aminosalicylic acid (5-ASA) was designed at Laboratory for Construction of Bioactive Substances, Institute of Petroleum Chemistry and Catalysis. The film is characterized by a pronounced antiinflammatory effect, due to a nonsteroid anti-inflammatory agent in its composition (patent of the Russian Federation for invention No. 2233164 of 27.07.04).

The study was aimed at substantiation of the use of the film consisting of modified HA with 5-ASA as the anti-adhesion barrier.

MATERIALS AND METHODS

The study was carried out on adult Wistar rats (n=60; 180-200 g) divided into 2 groups, 30 per groups. The adhesion process in the abdominal cavity was surgically induced in all animals under ether narcosis. A flap (2.0×1.5 cm) of the parietal peritoneum (PP) was resected from the lateral abdominal walls on both sides, the serosa on the distal portion of the small intestine was removed at the length of 3 cm. In group 1 (control), the wound was not treated. The anterior abdominal wall was sutured layer-by-layer with catgut continuous sutures. In group 2, a film of HA with 5-ASA was applied to the intestine free from serous membrane and to damaged PP areas. The film adheres spontaneously under conditions of moderate humidity and sticks well to the surface of damaged peritoneum.

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The animals were sacrificed on days 1, 3, 5, 7, and 14 (6 rat from each group per term). Specimens of PP and small intestine were collected for histological study. Adhesions in the abdominal cavity were counted on days 3, 5, 7, and 14 postoperation, their incidence, type, and dissemination were evaluated [2].

RESULTS

In group 1, the peritoneum was characterized by significant edema and cyanosis. Small intestinal areas free from the serosa were colored cyanotic-purple with petechial hemorrhages. The intestinal loops and omentum were loosely connected by fibrinous adhesions to the PP in the wound zone. During the first 3 days, the intestinal loops and omentum were easily separated. On day 5 after surgery, the number and length of adhered zones in intestinal loops and omentum decreased. Morphologically, adhesions were accumulations of fine slightly twisted collagen and elastic fibers with high content of cell elements (Fig. 1). The adhered sites were difficult to separate from the wound surface. On day 7, edema and hyperemia around the PP wound decreased significantly, but petechial hemorrhages in the wound persisted. Adhesions in histological preparations were presented by connective tissue with high content of collagen and elastic fiber bundles (Fig. 2), the connective tissue was well vascularized due to numerous vessels of different diameters. Fibroblasts, fibrocytes, and solitary macrophages were seen between collagen and elastic fibers. Myocytes of the muscle layer formed groups, which limited the growth of the connective tissue. Pronounced lymphocytic infiltration was detected in the lamina propria. Intestinal loops and omentum were bound with well-formed cord-like dense adhesions. On day 14, PP was smooth, edema and hyperemia disappeared. The adhesive process presented mainly as cord-like compact adhesions; in 8 cases, intestinal loops and omentum together with the wound area of PP formed a solid conglomeration.

In group 2, the operation wound on day 1 was covered with HA film with 5-ASA. In some cases, intestinal loops and omentum loosely adhered to PP around the film. However, fibrinous adhesions were less extensive than in group 1. Mild edema and petechial hemorrhages were detected in the wound under the film. On day 3, the peritoneum was smooth, with its natural pinkish color. In 3 cases the omentum adhered to the PP in the wound above and below the film application area, but these sites were easily separated. No film fragments were detected. On day 5, the operation wound was completely

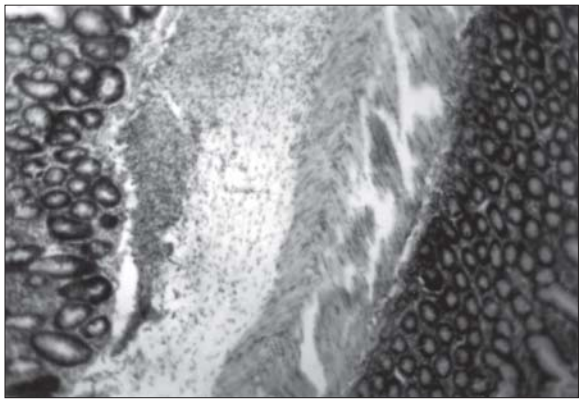

Fig. 1. Fine connective tissue adhesion of the small intestine on day 5 of the experiment in control group. Here and in Fig, 2: hematoxylin and eosin staining, ×200.

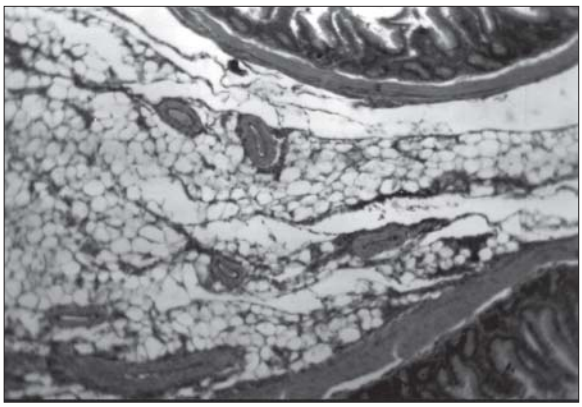

Fig. 2. Adhesion between two loops of the small intestine on day 7 of the experiment in control group.

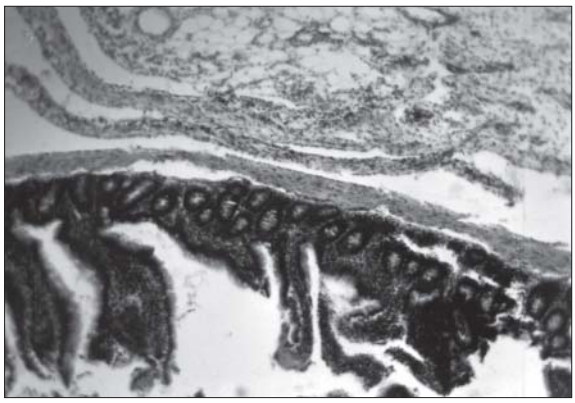

Fig. 3. A site of VP and PP 7 days after application of film consisting of modified HA. Hematoxylin and eosin staining, ×400.

epithelialized. In 2 cases, the omentum adhered above the site of the film application to PP. Complete recovery of the PP and visceral peritoneum (VP) was observed on day 7 after surgery (Fig. 3). VP was covered with squamous epithelium (mesothelium). The connective tissue basis consisted of oriented and twisted collagen and elastic fibers, elongated or polygonal. The muscle layer at the site

TABLE 1. Incidence of Formation, Extension, Type, and Compactness of Postoperative Adhesions in Wistar Rats, Depending on Operation Wound Protection ($M \pm m$)

Group	Number of cases	Number of adhesions, score	Type of adhesions, score	Area of adhesions, score	Total score
1	29	3.2±0.3	2.5±0.2	2.2±0.1	7.9±0.6
2	10	1.2±0.1	1.0±0.1	0.5±0.2	2.7±0.4

of intervention did not change. A moderate number of lymphoid tissue cells were diffusely scattered among fibroblasts, resident macrophages, and plasma cells in the mucosa and loose connective tissue. The surface of the PP was smooth, the restored mesothelium was presented by flat of cubical cells lying on the basal membrane. Numerous macrophages and fibrocytes were seen in the connective tissue base of recovering serosa. No visible changes were seen in capillaries. Muscular and mucous membranes were also unchanged except diffusely scattered lymphocytes in the villi. Solitary cord-like adhesions of the omentum to the median operation suture were seen. On day 14, the peritoneum was smooth, glossy, of pink shade.

In group 2, adhesions formed around the site of film application in 10 cases, which differs significantly from the control group (29 cases; $p<0.001$; Table 1).

Hence, application of the film consisting of modified HA with 5-ASA significantly prevented the formation of postoperative adhesions in the abdominal cavity. This effect was attained due to restoration of the peritoneum, temporary separation of the wound surface, and creation of the optimal conditions for rapid regeneration of mesothelial cells.

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