Effect of Hyaluronidase Electrophoresis from Actinohyal Solution on the Formation of Postoperative Adhesions

B. I. Kulakov, L. V. Adamyan, O. A. Mynbaev, and V. I. Strugatskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 12, pp. 589-591, December, 1995
Original article submitted February 17, 1995

Rats repeatedly administered hyaluronidase electrophoresis from a solution of actinohyal (a novel preparation containing the hyaluronidase produced by a pathogenic *Streptomyces actinocidus* strain) showed marked reductions in the incidence and density of postoperative adhesions.

Key Words: *adhesions; hyaluronidase; actinohyal; electrophoresis*

In surgical gynecology, the formation of adhesions and their recurrence after adhesiolysis are important determinants of surgical failure and dictate the need for finding effective means of preventing their development [2,3].

The present investigation was undertaken to study the antiadhesive efficacy of hyaluronidase (HR) electrophoresis from a solution of actinohyal - a novel Russian-made preparation containing the HR produced by the pathogenic *Streptomyces actinocidus* strain 77. This enzyme catalyzes the hydrolysis of hyaluronic acid and reduces its viscosity, thereby increasing the permeability of tissues and vessel walls. Actinohyal, whose mechanism of action is analogous to that of testicular ronidase, is readily soluble in water and physiological saline and 10 times as active as ronidase; the pH optimal for its action (6.5) is higher than that for the latter enzyme.

MATERIALS AND METHODS

The study was conducted on 50 sexually mature Wistar rats (body weight 180-200 g) divided into 5 groups, 10 animals in each. All rats were first subjected to laparotomy under general Hexenal anesthesia (100 mg/kg intramuscularly) to induce the formation of adhesions in the abdominal cavity. Prior to surgery, the rat was fixed on the operating table and the hair coat was shaved off the anterior and lateral abdominal walls. The peritoneal cavity was opened by a median incision and the lateral abdominal wall everted with a special device to expose the parietal peritoneum. A 1×3 cm flap of parietal peritoneum was excised from the lateral abdominal wall and 10 interrupted catgut (3/0) sutures were applied with an atraumatic needle. This procedure was performed bilaterally. The anterior abdominal wounds were then closed with continuous catgut sutures.

Thereafter, starting on day 2 of the postoperative period, the rats were subjected to electrophoretic separation of HR from an actinohyal solution with a galvanic current to prevent the formation of adhesions at the two operated sites. With the rat immobilized on a wooden board, one electrode was bifurcated and positioned on the lateral abdominal walls approximately 0.5 cm from the wound edges at the abdominal midline; the second electrode was placed symmetrically on the rat's back. Both 1.2×1.2 cm liners of the first electrode were moistened either with actinohyal solution (300 IU of HR)) in 30 ml of distilled water to which 2-3 drops of glacial acetic

Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow

0007-4888/95/0012-1205$12.50

TABLE 1. Incidence of Primary and Secondary Postoperative Adhesions in the Five Groups of Rats

Group	Day of examination postoperation:			
	12th		24th	
	No. of sites examined	sites with primary adhesions	No. of sites examined	sites with secondary adhesions
1	20	13 (65)	18	8 (44.4)
2	20	16 (80)	16	10 (62.5)
3	20	15 (75)	16	8 (50)
4	20	17 (85)	18	12 (66.7)
5	20	20 (100)	14	12 (85.7)

Note. Figures in parentheses are percentages. Significant intergroup differences: group 1 *vs.* 5 ($p<0.01$), group 2 *vs.* 5 ($p<0.05$), and group 3 *vs.* 5 ($p<0.02$) on day 12; groups 1 and 3 *vs.* 5 ($p<0.001$) and groups 2 and 4 *vs.* 5 ($p<0.01$) on day 24.

acid had been added, or only with this solvent. The 2.5×2.5 cm liner of the second electrode was moistened with a 0.9% NaCl solution. The HR-containing solution was introduced from the anode in group 1 and from the cathode in group 3, while the solvent was introduced from the anode in group 2 and from the cathode in group 4. Rats of the latter two groups and those of group 5, which received no treatment at all, served as controls. The electrophoresis was carried out with currents in the 7-14 mA range using a Potok-1 apparatus. The rats of groups 1-4 were each subjected to 20-min sessions of electrophoresis once daily for 10 successive days. On day 12 after the operation, laparotomy was performed again under Hexenal anesthesia to evaluate adhesions and reparative processes in the parietal peritoneum of the lateral abdominal walls and to carry out adhesiolysis using microsurgical instruments. Adhesions were counted by a previously described method [1], their distribution, type, and density were recorded, and the data were summed up using a 5-point scoring scale and expressed in terms of density. Nine of the 50 rats did not survive the intervention - one each from groups 1 and 4, two each from groups 2 and 3, and three from group 5.

Thereafter, the survivors were administered a second 10-day course of daily electrophoretic sessions as described above. On day 24 after the first operation all rats were killed by being given a high dose of Hexenal, and the final evaluation of the adhesive process was performed.

The numerical data were subjected to statistical analysis using Student's t test.

RESULTS

In group 5, which did not receive any treatment during the postoperative period, pronounced adhesions were noted on day 12 at both sites in all animals (Table 1). In both actinohyal-treated groups (groups 1 and 3) and in the group that had been receiving the actinohyal solvent from the anode (group 2), the incidence of adhesions was significantly lower than in group 5. The group where the solvent was introduced from the cathode (group 4) also had a lower rate of adhesions than group 5, but the difference proved insignificant. Thus, 15% to 35% of the sites were free of adhesions in groups 1-4 on postoperative day 12; there was no significant difference between these groups.

TABLE 2. Density of Primary and Secondary Postoperative Adhesions ($M\pm m$)

Group	Day of examination postoperation:			
	12th		24th	
	No. of sites examined	mean adhesion density	No. of sites examined	mean adhesion density
1	20	1.25±0.09 (13)	18	0.78±0.14 (8)
2	20	1.70±0.07 (16)	16	1.12±0.15 (10)
3	20	1.35±0.11 (15)	16	1.0±0.18 (8)
4	20	1.80±0.05 (17)	18	1.28±0.12 (12)
5	20	3.35±0.11 (20)	14	2.2±0.28 (12)

Note. Figures in parentheses are the number of sites. Significant intergroup differences: groups 1, 2, 3, and 4 *vs.* 5 ($p<0.001$), and groups 1 and 2 *vs.* 4 on day 12 ($p<0.001$); groups 1, 2, 3, and 4 *vs.* 5 ($p<0.001$), group 1 *vs.* 2 ($p<0.02$), and group 2 *vs.* 3 on day 24.

On postoperative day 12, the density of adhesions in group 5 was assigned 3.35±0.11 points - a score which is 210, 165, 200, and 155% higher than in groups 1, 2, 3, and 4, respectively (Table 2). Such a high score in the untreated group was due to the presence of thick and very dense adhesions. The density of postoperative adhesions in the actinohyal-treated rats was significantly less than in those of the three control groups (2, 4, and 5), regardless of whether the actinohyal solution was introduced from the anode (group 1) or from the cathode (group 3). As can be seen in Table 2, the two groups treated with the actinohyal solvent (groups 2 and 4) also had much lower adhesion densities than group 5.

On day 24 after the first operation, the incidence of adhesions in rats of groups 1, 2, 3, and 4 after the second operation (i.e., after the laparotomy performed on day 12) was 42, 23.2, 35.7, and 19% lower, respectively, than in group 5 (Table 1). On that day, too, groups 1 and 3 had a lower rate of adhesions than the control groups, with 50% and 55% of the examined sites being free of adhesions, although the differences among the first four groups were insignificant ($p>0.05$). The incidence of adhesions also decreased in group 5 (by 14.3%), as did the adhesion density (to 2.2±0.28 points compared with 3.35±0.11 points on day 12), which, however was 182, 96, 120, and 72% higher than in groups 1, 2, 3, and 4, respectively (Table 2). The differences in the density of secondary (recurrent) adhesions between the two actinohyal-treated groups, on the one hand, and the three control groups, on the other, were significant, whereas that between the actinohyal-treated groups themselves was not. As can be seen in Table 2, the use of the actinohyal solvent alone also led to significant decreases in adhesion density (cf. groups 2 and 4 *vs.* group 5).

This study indicates that HR electrophoresis from actinohyal solution can decrease the incidence and density of postoperative adhesions (particularly when the solution is introduced from the anode) is an effective means of preventing their formation and recurrence.

REFERENCES

1. O. A. Mynbaev, *Use of Fibrin Adhesive in Reconstructive/Plastic Surgery on the Uterine Tubes (Synopsis of Dissertation)* [in Russian], Moscow (1992).
2. M. P. Diamond and A. H. DeCherney, *Microsurgery*, **8**, 197-200 (1987).
3. D. Wiseman, in: *Polymeric Site-Specific Pharmacotherapy* (A. J. Domb, Ed.), New York (1994), pp. 370-421.