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An Experimental Study on the Efficacy of Sodium Hyaluronate in Prevention of Postoperative Intraperitoneal Adhesions

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Abstract: The objective of this study was to assess the efficacy of hyaluronic acid in prevention of intra-abdominal adhesions. Adhesion formation is associated with serious postoperative complications, including intestinal obstruction, infertility and chronic pelvic pain. Various medical and operative agents have been used to prevent peritoneal adhesions. Twenty female Albino rats weighing 250-350 g were used in this study. Each rat was anesthetized with 40 mg kg^{-1} of intramuscular ketamine hydrochloride and 10 mg kg^{-1} of xyzaline hydrochloride. A lower midline incision, 3 cm in length, was made. As there were not any adhesions in the abdomen, the ileocecal bowel part was rubbed with sterile hydrophile until a serosal hemorrhage was formed and cecum was replaced in conformity with anatomy. Rats were divided equally into 2 groups, the experimental and control group. The experimental group was given 0.5 mL of intraperitoneal hyaluronic acid and the control group was given 0.5 mL of intraperitoneal physiologic solution. The abdominal incision was closed. At 15 days postoperatively, all the rats were euthanized using anesthesia of ether. Rating of adhesions was performed through a bilateral subcostal incision, evaluating the whole intraperitoneal organs and adhesions macroscopically.

Key words: Intra-abdominal adhesion, hyaluronic acid, rat, chronic pelvic pain, abdomen

INTRODUCTION

Postoperative adhesions are one of the most serious complications following intraabdominal and pelvic surgery (Ellis, 1982; Burns *et al*., 1996; Gomel *et al*., 1996; Stangel *et al*., 1984). Operation, foreign body, infection, abrasive manipulation of tissues, peritoneal ischemia and hemorrhage are the most frequent causes of intraperitoneal adhesion formation (Avsar *et al*., 2001; Hellebrekers *et al*., 2000; Karabulut, 2001; Ryan and Sax, 1995). The incidence ranges between 55 and 100% after abdominal or pelvic surgery (Becker *et al*., 1996; Gunay *et al*., 2005; Menzies and Ellis, 1990; Moreira *et al*., 2000; Risberg, 1997).

Adhesions form as a natural part of the body's healing process. As a part of this process, the body deposits adhesion onto defected and pelvic tissues, by which the activity of the tissue is provided. Adhesion-related complications such as bowel obstruction, infertility and chronic pelvic pain led researchers to investigate the potential of various medical and operative agents in adhesion prevention (Felemovicius *et al*., 2004; Menzies and Ellis, 1990; Urman and Gomal, 1991; Wang *et al*., 2003). Between 54-70% of intestine obstruction and 15-20% of infertility in females are related to intraabdominal adhesion (Dinsmore *et al*., 2000; Gomel *et al*., 1996).

In order to prevent adhesion formation or reduce adhesion, the 1st step is to reduce post traumatic inflammation via various anti-inflammatory agents, antihistaminic, anticoagulant, antioxidant, proteolytic enzymes, plasminogen activators, prostigmin (for the increase of peristalticity of digestive system) and other products such as fluid, paraffin liquid, dextrose, carboxymethylcellulose (for forming physical barrier between serosal surfaces) (Arnold *et al*., 2000; Galili *et al*., 1998; Gul *et al*., 1999; Hemadeh *et al*., 1993; Holtz, 1984; Koc *et al*., 2002).

The use of hyaluronic acid in abdominal and pelvic surgeries has shown a reduction in adhesions

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(Burns *et al*., 1996; Sawada *et al*., 1999; Tang *et al*., 2006; Thornton *et al*., 1998). Hyaluronic Acid (HA) is a straight-chain macromolecular mucopolysaccharide composed of repeat disaccharide units of D-glucuronic acid and N-acetylglucosamine. Mucopolysaccharide complexes bound to proteins *in vivo* to form proteoglycans. This form is hydrophilic and viscous. Inhibition of neovascularization, preservation of tissues and viscous functions are the reasons for its usage in osteoarthritis. These characteristics also present the possibility that it can prevent adhesions (Burns *et al*., 1995; Itokazu and Matsunaga, 1995; Sagliyan *et al*., 2008; Tunay *et al*., 2002).

The aim of this standard empirical adhesion model is to reduce inflammation with the use of HA and consequently, to prevent post traumatic adhesion. In order to prevent morbidity and mortality caused by intraperitoneal adhesions, it is necessary to identify an easy, practical, economic method of adhesion barrier.

MATERIALS AND METHODS

Twenty female Albino rats weighing 250-350 g were used in this study. The animals were acclimated to their environment for 1 week and were provided a diet of standard rat feed and water. The 20 rats were randomized to 4 groups of 5 each in cages.

Each rat was anesthetized with 40 mg kg^{-1} of intramuscular ketamine hydrochloride (Alke-Ketanes 100 mg mL^{-1}) and 10 mg kg^{-1} of xyzaline hydrochloride (Bayer-Rompun 23.22 mg mL^{-1}). The abdomen was shaved and prepared with povidone-iodine and draped in a sterile fashion. A lower midline incision, 3 cm in length, was made. As there were not any adhesions in the abdomen, the ileocecal bowel part was rubbed with sterile hydrophile until a serosal hemorrhage was formed (Fig. 1) and cecum was replaced in conformity with anatomy.

Rats were divided equally into 2 groups, the experimental and control group. The experimental group was given 0.5 mL of intraperitoneal hyaluronic acid (Orthovisc, Anika Therapeutics Inc., USA, Sodium Hyaluronate 15 mg mL^{-1}) and the control group was given 0.5 mL of intraperitoneal physiologic solution. The abdominal incision was closed with usual manner.

At 15 days postoperatively, all the rats were euthanized using anesthesia of ether. Rating of adhesions was performed through a bilateral subcostal incision, evaluating the whole intraperitoneal organs and adhesions macroscopically. The scoring system used for evaluating adhesions in this study was the same as the criteria proposed by Mazuji *et al*. (1964) (Table 1).

Table 1: Macroscopic grading for evaluation of adhesions

Grade	Results
0	No adhesions
1	Thin and local-the adhesion separated from tissue with gentle traction (Fig. 2)
2	Restricted adhesion (Fig. 3)
3	Thick and on a larger scale (Fig. 4)
4	Thick and large adhesion on the front and/or back parts of abdominal wall (Fig. 5)

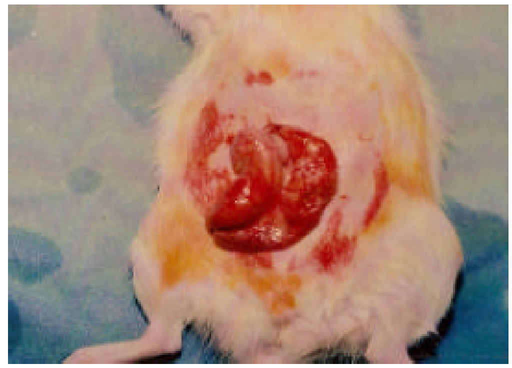

Fig. 1: Serosal hemorrhage

Fig. 2: Thin and local adhesion

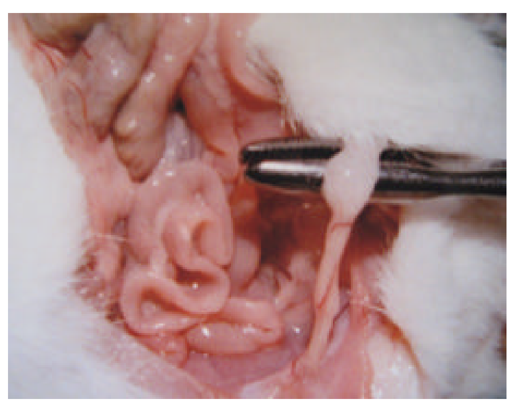

Fig. 3: Restricted adhesion

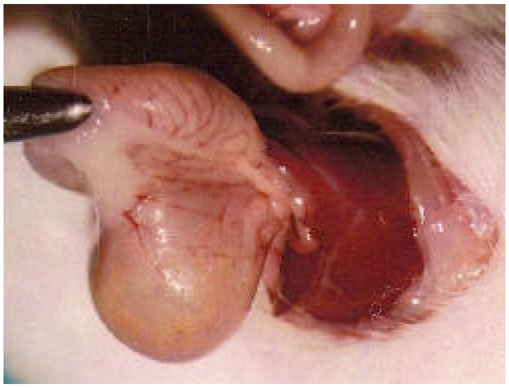

Fig. 4: Thick and on a larger scale

Fig. 5: Thick and large adhesion on the front and/or back parts of abdominal wall

Evaluation of outcomes was made using statistical methods of frequency and percentage. In both evaluation types, differences were analyzed using a χ^2-test.

RESULTS

No complications were observed during the 15 days postoperative period. Macroscopic evaluation of postoperative intraabdominal adhesions and comparison of both groups is shown in Table 2.

With the macroscopic evaluation of formation of postoperative adhesions, the control group was allocated a score of 2 in 1 rat (10%), a score of 3 in 3 rats (30%) and a score of 4 in 6 rats (60%).

The animals within the experimental group (HA) were given a score of 0 in 3 rats (30%), a score of 1 in 5 rats (50%) and a score of 2 in 2 rats (20%).

Comparison of groups in terms of macroscopic adhesion scores showed significant statistical differences between groups ($p<0.05$).

Table 2: Macroscopic evaluation of postoperative intraabdominal adhesions

	Grade (%)					
Groups	0	1	2	3	4	p-value*
Control	-	-	1 (10)	3 (30)	6 (60)	0.030
Experimental (HA)	3 (30)	5 (50)	2 (20)	-	-	0.024

DISCUSSION

Postoperative peritoneal adhesions may develop following any injuries to the peritoneum and they can result in intestinal obstruction, infertility and chronic pelvic pain (Felemovicius *et al*., 2004; Menzies and Ellis, 1990; Urman and Gomal, 1991; Wang *et al*., 2003). Although, many methods have been used for the prevention of adhesion formation, it is still a significant problem. A number of new approaches are being developed to reduce the incidence and severity of postoperative intraabdominal adhesions (Becker *et al*., 1996; Ellis, 1982; Karabulut, 2001; Koc *et al*., 2002; Moreira *et al*., 2000; Risberg, 1997).

The objective of this study was to assess the efficacy of hyaluronic acid in the prevention of intraabdominal adhesions.

In order to develop new methods in prevention of adhesions, the formation of adhesion should be researched in depth (Dinsmore *et al*., 2000; Gunay *et al*., 2005; Kilic, 2005; Koc *et al*., 2003). The common denominator for adhesion formation is the inflammatory response. Peritoneal inflammation leads to inflammation which contains thromboplastin, permality factors, chemotactic agents. Various agents were used for prevention of postoperative agents but no entirely satisfactory solution is available. Corticosteroid, nonsteroid anti-inflammatory agents, plasminogen activator and calcium channel blocking agents are used for their reduction of inflammatory response. The efficacy of this method varies in empirical and clinical studies (Galili *et al*., 1998; Gomel *et al*., 1996; Gul *et al*., 1999; Hellebrekers *et al*., 2000).

A 2.5 cm^2 defect was surgically created in the abdominal wall of experimental rats and replaced with: polypropylene mesh, Sepramesh (SM) and SM plus seprafilm. Each group included 20 animals. Adhesions were complete in polypropylene mesh applied rats (Felemovicius *et al*., 2004). Sumer states that during the application of polypropylene mesh, if there is contact between the mesh and visceral organs, the adhesion formation is dense. In his study, he observed macroscopically score 3 and 4 adhesions. When the polypropylene group is compared with polyethyleneglycole and hyaluronan GF-20 groups there are statistically significant differences in macroscopic adhesion scoring ($p<0.05$). There is also a statistically significant difference between the polypropylene group and the group in which a physical barrier was applied ($p<0.05$). In their empirical study on rats, Ozel *et al*. (2001)

showed that use of Na-Hyaluronate derivatives reduced the scale and extent of postoperative peritoneal adhesion.

Derivatives of Hyaluronic Acid (HA) are applied in cases of articulation defects. HA is directly injected to the articulation cavity and lessens the pain in osteoarthritis and prevents cartilage catabolism. Clinical research has observed that the use of HA in treatment of osteoarthritis and osteochondral defects is safe and efficient (Burns *et al*., 1996; Sagliyan *et al*., 2008; Tunay *et al*., 2002).

Barrier adhesion prevention agents are not resorbed in the abdomen for a period and act as a barrier between defected serosal surfaces and parietal peritonea. The ideal product would be active throughout the peritoneum during the 5-7 days required for serosal reepitelization; be nonreactive; be absorbable following peritoneal rehabilitation. No such product is currently available but oxide regenerated cellulose and Gore-Tex are widely used. The barrier method is more promising than other adhesion preventive agents (Gomel *et al*., 1996). Viscous solution of hyaluronic acid was applied intraperitoneally to rats. The solution covered the surfaces and reduced adhesion formation (Burns *et al*., 1995; Detchev *et al*., 2004; Ozel *et al*., 2001; Sawada *et al*., 1999; Tang *et al*., 2006; Urman and Gomal, 1991). The present study produced the same result.

The present study, employed a standard adhesion model and used hyaluronic acid in prevention of intraabdominal adhesions. The HA applied experimental group showed significant differences in prevention of intraabdominal adhesions when compared with the control group. As a result of macroscopic observation of postoperative adhesions, the following outcomes were recorded within the control group: 1 rat (10%) with a score of 2; 3 rats (30%) with a score of 3; 6 (60%) rats with a score of 4. The outcomes of the hyaluronic acid group were as follows: 3 rats (30%) with a score of 0; 5 rats (50%) with a score of 1 and 2 rats (20%) with a score of 2. Significant statistical differences between the experimental group and control group were observed in terms of macroscopic adhesion scores ($p<0.05$).

CONCLUSION

Consequently, the adhesion prevention effect of hyaluronic acid is clarified by our experiment. HA was assessed as secure, practical and adhesion barrier applicable following intraabdominal surgery.

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