Prevention of Tissue Injury and Postsurgical Adhesions by Precoating Tissues with Hyaluronic Acid Solutions\textsuperscript{1,2}

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INTRODUCTION

Postsurgical adhesions result from the natural wound healing response of tissues to damage that occurs during surgery. Despite considerable efforts to understand postsurgical adhesion formation and prevention, adhesions continue to present clinical complications and challenges to the surgeon. A reported 55 to 97\% of women undergoing pelvic surgery form adhesions [1,2] which can compromise subsequent fertility [1]. Postsurgical adhesions, which occur in 67 to 93\% of abdominal surgical patients [3,4], are also the primary cause of small bowel obstruction following abdominal surgery [3,4], which is reported to occur following 5\% of all general abdominal surgical procedures [4,5].

Tissue injury that causes adhesions can result from two broad categories of surgical trauma: (1) intentional tissue injury that occurs, for example, at incision sites or sites of deperitonealization; and (2) unintentional tissue injury due to abrasive manipulations and desiccation. Virtually all methods of limiting postsurgical adhesion formation have focused on providing wound separation or controlling wound healing events after tissue injury has occurred. Another approach to limit adhesions is to reduce the extent and severity of the unintentional tissue damage that occurs during surgery by precoating tissues with a protective lubricating solution. Precoating tissues with protective solution during surgery includes applying the solution prior to tissue manipulation and desiccation and further protecting tissues by periodically applying lubricating solution throughout the surgical procedure.

Tissue precoating with hydrophilic polymeric solutions of polyvinylpyrrolidone and carboxymethylcellulose have been shown to limit peritoneal and pericardial adhesions in experimental models [6–9]. These studies have also demonstrated that tissue precoating with polymeric solutions more effectively reduces adhesions than applying the solutions at the end of surgery, after the tissue damage has occurred. Tissue precoating with dilute solutions of
METHODS

Test Solutions

Sterile and nonpyrogenic solutions of 0.1, 0.25, and 0.4% w/w HA were prepared in a pH 7 iso-osmolar phosphate-buffered saline solution (PBS). Solutions were supplied by Genzyme Corp. (Cambridge, MA).

Study Procedures

All surgical procedures were conducted according to the guidelines specified in the NIH “Guide for the Care and Use of Laboratory Animals” [14]. Five hundred and twenty-five Sprague-Dawley female rats (200–250 g) were used in this series of experiments. Animals were weighed and then anesthetized by an intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The peritoneal cavity was accessed via a 4-cm midline incision. At this point the animals were randomly assigned to one of five treatment groups with 25 animals per group. Animals in Group 1 did not receive a coating solution before cecal abrasion. Animals in Groups 2, 3, 4, and 5 received 2 ml of either PBS, 0.1, 0.25, or 0.4% HA immediately after opening the cecum before abrasion.

A constant force, constant area rotary cecal abrasion device was employed to impart a consistent degree of tissue trauma in nontreated animals [10]. The abrasion device utilized a motor driven rotating spline shaft (Fig. 1) that delivered a controlled 70-g abrasive force to the cecum. A 1.5-cm diameter flat surface of Type VII surgical gauze (Johnson and Johnson) was secured to the end of the rotating shaft by a rubber septum. The rat cecum was placed in a Teflon holder that contained a 1.5-cm diameter hole to accommodate the abrasion surface. The holder defined the area of the cecum to be abraded and minimized movement of the cecum during abrasion. Each cecum was abraded for 60 revolutions at four sites: (1) the distal portion of the anterior surface, (2) the proximal portion of the anterior surface, (3) the distal portion of the posterior surface, and (4) the proximal portion of the posterior surface. Utilization of this abrasion device helped ensure that the person performing the cecal abrasion did not feel the viscosity of test material prior to and during abrasion. One week following surgery animals were sacrificed by CO₂ asphyxiation [15], and the peritoneal cavity was accessed via a left paramedian incision. Animals were evaluated for adhesions in a random blind sequence. Each cecal adhesion was noted and scored according to the following system [9]:

0 No cecal adhesions
1 Filmy adhesion with easily dissectable plane
2 Adhesion with dissectable plane causing mild tissue trauma
3 Fibrous adhesion with difficult tissue dissection
4 Fibrous adhesion with nondissectable tissue planes

Histology Study

In the second portion of this study, we evaluated the effect of tissue precoating with HA solutions on tissue injury caused by abrasion and desiccation. To evaluate the extent of tissue abrasion and injury, we employed the identical cecal abrasion protocol described above with 25 animals per group. Since we wished to observe the inflammatory response induced by tissue abrasion, the animals were sacrificed 2 days following cecal abrasion to allow sufficient time for an inflammatory response to be observed histologically. The ceca were removed and placed overnight in 10% Bouin’s solution for histological examination by light microscopy. Two sections from each animal were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Random/blind grading of the histo-
logical sections was performed separately by a board certified veterinary pathologist (R.B.). The final histological score was the average score of the two cecal sections. The histology grading scale was: 0, normal serosa; +, very mild hypertrophy of mesothelium; 1+, hypertrophy of mesothelium with mild infiltration of mononuclear inflammatory cells beneath the serosa and possibly a mildly thickened serosa; 2+, significant hypertrophy of mesothelium with moderate infiltration of mononuclear inflammatory cells beneath the serosa and a moderately thickened serosa; 3+, extensive hypertrophy of mesothelium with significant infiltration of mononuclear inflammatory cells and a significantly thickened serosa.

The effectiveness of HA solutions in reducing tissue injury caused by desiccation was studied by employing a standardized method to reproducibly desiccate the cecum. A compressed air tank was connected in series with Tygon tubing to an air flow meter (Short-Rate, Model 6-1355-M, Brooks Instruments B.V., Veenendaal, Holland), a desiccant reservoir, and a 0.22-μm nylon filter. The 0.22-μm filter was placed downstream from the desiccant to remove particulates and potential infective agents. From the filter, the air flowed to an 8-cm diameter cone placed 2 cm above the cecum. The cecum was then exposed for 5 min to an air flow rate of 30 liters per minute. Twenty-five rats were randomly assigned to five test groups: (1) no coating/no desiccation, (2) no coating/desiccation, (3) lactated Ringer’s coating/desiccation, (4) 0.1% HA solution coating/desiccation, and (5) 0.4% HA solution coating/desiccation. Animals assigned to Groups 3, 4, and 5 had 4 ml of study solution applied as previously described for the cecal abrasion protocol prior to desiccation. The ceca were harvested 2 days after surgery and processed for histological examination as described above.

Statistical Analysis

We tested the association between HA concentration and the mean incidence of cecal adhesions by employing a linear regression model. The association between HA concentration, the number of animals with no adhesions, and the number of animals with at least one cecal adhesion of grade 2 or higher was tested with Cochran–Mantel–Haenszel statistics. The association between HA concentration and the number of animals with a histology score of 2+ or higher was also tested by employing Cochran–Mantel–Haenszel statistics.

RESULTS

Efficacy Study

There was no statistically significant difference in trends between the three study sites. Data were therefore pooled from the three study sites for trend analysis. The individual study sites data are given in the Appendix (Tables A1–A3). Ten animals among the three study sites did not recover following surgery. Their deaths are attributed to anesthesia overdose.

The percentage of animals with no adhesions (P > 0.05), the percentage of animals with at least one cecal adhesion of grade 2 or higher (P > 0.05), and the mean incidence of cecal adhesions (P > 0.05) were no different in the PBS and no coating groups (Table 1). As the
FIG. 2. (a) Cecum 2 days after abrasion; the cecum was coated with 0.4% HA solution prior to abrasion; the arrowhead indicates an inflammatory cell on the serosal surface. (b) Cecum 2 days after abrasion; the cecum was coated with PBS solution prior to abrasion; note greater infiltration of inflammatory cells and lack of intact serosa compared to a (All H&E, 400×).
HA concentration in the precoating solution increased from 0% (PBS group) to 0.4% HA, the mean incidence of cecal adhesions decreased in a concentration-dependent manner from 1.6 ± 0.11 to 0.7 ± 0.09 (P < 0.001). The percentage of animals with no cecal adhesions increased from 11% in the PBS group to 50% in the 0.4% HA treatment group. The percentage of animals with at least one cecal adhesion of grade 2 or higher decreased from 79% in the PBS treatment group to only 24% in the 0.4% HA treatment group. The percentage of animals with no cecal adhesions and with at least one cecal adhesion of grade 2 or higher was highly dependent upon HA concentration (P < 0.001).

Histology Study

In the abrasion histology experiment the percentage of animals with histology scores 2+ or higher was similar in the no coating and PBS treatment groups (67 and 65%, respectively) (Table 2). The inflammatory response in these groups was significantly higher than for the 0.25 and 0.4% HA treatment groups (28 and 16% for scores 2+ or higher, respectively) (P < 0.01). A mild inflammatory response was generally observed for ceca abraded following precoating with 0.4% HA solution (Fig. 2a). Some hypertrophy of the mesothelium was observed with infiltration of monocytes (arrow). In the PBS treatment group (Fig. 2b), the serosa was generally poorly defined due to extensive damage and had a high density of infiltrating monocytes.

Because of the group size (n = 5) in the desiccation experiment, statistical comparison of groups was not performed. None of the histology scores in the sham control group were greater than ± (Table 3). Therefore, simply handling the cecum did not significantly damage serosa. One hundred percent of the animals had scores ≥2+ in the no coating and lactated Ringer's groups. In the groups receiving HA precoating prior to desiccation, 80% of the animals in the 0.1% HA treatment group had significant tissue damage and only 20% of the animals had serosal damage of 2+ or higher in the 0.4% HA group.

DISCUSSION

Before initiating the three independent efficacy studies, we felt it was important to develop a method to cause experimental adhesions that minimized operator variability and prevented unintentional operator bias. An objective evaluation of postsurgical adhesion prevention, especially with precoating solutions, should employ a nonmanual method of inducing tissue damage because a person applying manual trauma can discern more lubricious solutions from saline solutions and no coating. The constant force, controlled area rotational device used in this study was specifically designed to provide a nonmanual reproducible tissue injury. Our choice of gauze abrasion was based upon observations of human abdominal surgical procedures in which laparotomy pads were used to pack bowel and other organ structures during surgery. We noted macroscopically visible petechial bleeding of the bowel serosa where the laparotomy pads had contacted the bowel, and we
found that a gauze abrasion device with a constant abrasion load and a constant abrasion surface area caused similar tissue injury.

Almost immediately after peritoneal injury, a serosanguinous exudate forms on the wound site which is quickly transformed into a thin fibrinous band [16, 17]. Normally, native tissue plasminogen activator lysed the fibrinous adhesion before it can become a permanent fibrous adhesion by the action of infiltrating fibroblasts [18, 19]. Ischemic, crushed, and abraded peritoneal tissue has reduced plasminogen activator activity, thus allowing persistence of the fibrinous adhesion and increasing the potential for adhesion formation before normal remesothelialization can occur [18, 19]. Ryan et al. further showed in an experimental model that the combination of tissue desiccation and blood caused adhesions [20]. It seems clear that reducing peritoneal wounding, thus allowing persistence of the fibrinous adhesion before it can become a permanent fibrous adhesion by the action of infiltrating fibroblasts [18, 19]. Ryan et al. also showed in an experimental model that remesothelialization occurring within about 8 days [3, 17]. These studies further suggest that adhesions form very early in the wound repair process but do not continue to form once remesothelialization has occurred. Buchanan et al. [18] points out that fibrinous adhesions can form within a few hours after peritoneal injury and that these either resorb by the action of endogenous plasminogen activator activity or, lacking sufficient plasminogen activator activity, the fibrinous bands mature into fibrous adhesions. Ryan et al., employing a rat peritoneal adhesion model, showed that adhesions present at 1 week were persistent even at 6 months [20]. In a human clinical study Diamond et al. found that adhesion occurrence following percutaneous surgery was the same from 7 to 70 days following initial surgery [21].

A number of methods to limit surgical adhesion formation have been studied with some encouraging but often ambiguous results. Viscous polymer solutions such as polyvinylpyrrolidone, carboxymethylcellulose, dextran, and HA have been instilled at the end of surgery [22–25]. These solutions act to limit adhesions by preventing fibrin deposition or by mechanically separating damaged tissues while they heal. With the possible exception of carboxymethylcellulose [24], polymeric solutions used after tissue injury have not been consistently effective in experimental studies.

In contrast to the use of viscous polymer solutions at the end of surgery, the method of limiting adhesions which we have investigated is intended to prevent unintentional tissue injury during surgery by precoating tissues with hydrophilic polymer solutions. Precoating for tissue protection and adhesion prevention includes coating tissues at the beginning of surgery before significant tissue manipulation and desiccation can occur and periodically throughout the procedure to maintain a protective coating on tissue. These solutions are less viscous than the polymeric solutions that have been used at the end of surgery, thus ensuring that good tissue handling is maintained throughout a surgical procedure. A tissue protective coating during surgery helps maintain the natural lubricity of tissues and organs. The precoating thereby attenuates the unintentional, and often unnoticed, tissue damage from desiccation and surgical manipulations. Precoating tissues to prevent adhesions was initially demonstrated in animal studies with solutions of dextran, polyvinylpyrrolidone, and carboxymethylcellulose [6–9]. Precoating with HA solutions similarly reduced adhesion formation in a rat uterine horn adhesion model in which uterine horn adhesions were caused by a standard laser insult [11, 12] and in a rat cecal adhesion model in which adhesions were induced by gauze abrasion [10]. In these studies the HA solutions were more effective at inhibiting adhesion formation when they were applied prior to tissue injury compared to application at the end of surgery after the tissue injury had occurred. We believe the HA does not significantly reduce adhesions when used after tissue injury because it likely has a short residence time in the peritoneal cavity. Thus, it cannot separate damaged tissues long enough during peritoneal tissue repair to significantly reduce adhesion formation.

The precoating concept is directed toward preventing Type 1-1 de novo adhesions as described by Diamond and Nezhat [27]. These are adhesions that form to sites that had no operative procedure and had no preexisting adhesion lysed at a prior surgery. Diamond and Nezhat differentiate de novo adhesions Type 1-1 from de novo adhesions Type 1-2, which form at sites of operative procedures, and from Type 2 adhesions, which are reformed adhesions. The Type 1-2 and Type 2 adhesions are, therefore, at sites of direct and intentional surgical trauma, where a precoating with an HA solution will not significantly affect adhesion formation, but for which a barrier method of adhesion prevention could be useful.

In the efficacy study presented here, buffered saline did not protect the serosa of the cecum compared to no coating. This fact was visibly apparent by the difference in cecal bleeding following precoating with HA or buffered saline solution (Figs. 3a and 3b). As saline solutions have minimal lubricating qualities this result is not surprising, and it supports previous observations that saline does not prevent tissue desiccation [20] or tissue abrasion [10]. In contrast, precoating with HA solutions significantly reduced peritoneal injury and adhesion formation. Further, these effects were directly related to HA concentration. Because HA is a high molecular weight polymer, its solution viscosity
FIG. 3. (a) Rat cecum precoated with 0.4% HA solution and then gauze-abraded. (b) Rat cecum precoated with PBS and then gauze-abraded shows more bleeding than in a. Arrowheads in a and b indicate two areas of dorsal cecum abraded.
and lubricating properties increase dramatically with increasing HA concentration [26]. Therefore, we hypothesize that the viscosity of HA solutions may influence its precoating properties.

The effectiveness of HA coating solutions to prevent adhesions by limiting tissue trauma was strongly supported by the histological data. The HA solutions effectively reduced trauma caused by gauze abrasion and desiccation to a greater degree than saline solutions; this reduction in tissue trauma was again related to HA concentration and therefore viscosity for a fixed HA molecular weight. As in the adhesion study, precoating the cecum with a saline solution did not prevent tissue injury compared to no coating. These histological results support those of Urman and Gomel [11, 12], which showed that reduced tissue injury and adhesion formation occurred to the rat uterine horn when the horns were precoated with HA solutions, compared to PBS solution, prior to a standardized laser injury.

Because of the numerous types and complexity of surgical procedures it is highly unlikely that a single method of adhesion prevention will be universally successful. Indeed, the lack of consistent success in animal and human studies with the many methods tested to date demonstrates the need for more than one approach to the problem. Our research shows that the use of tissue precoating solutions during surgery can prevent Type 1-1 de novo adhesions which normally form due to unintentional tissue injury caused by normal surgical manipulations and desiccation of tissue. Ultimately, a comprehensive adhesion prevention regimen may include tissue precoating with an HA solution to limit unintentional tissue injury and de novo adhesions, followed by the application of a barrier just prior to surgical closure to separate sites of unavoidable tissue injury from opposing tissue structures.

APPENDIX: ADHESIONS DATA FOR THE INDIVIDUAL STUDY SITES

**TABLE A1**

Site 1 (Genzyme Corporation) Adhesions Data for Three Center Study

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% Animals with no adhesions</th>
<th>Mean incidence of adhesions ± (SEM)</th>
<th>% Animals with adhesions ≥ 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No coating</td>
<td>25</td>
<td>24</td>
<td>1.0 ± 0.15</td>
<td>56</td>
</tr>
<tr>
<td>PBS</td>
<td>24</td>
<td>17</td>
<td>1.2 ± 0.16</td>
<td>79</td>
</tr>
<tr>
<td>0.10% HA</td>
<td>25</td>
<td>36</td>
<td>0.7 ± 0.12</td>
<td>40</td>
</tr>
<tr>
<td>0.25% HA</td>
<td>25</td>
<td>60</td>
<td>0.8 ± 0.22</td>
<td>36</td>
</tr>
<tr>
<td>0.40% HA</td>
<td>25</td>
<td>72</td>
<td>0.4 ± 0.12</td>
<td>12</td>
</tr>
</tbody>
</table>

**TABLE A2**

Site 2 (GLP Testing Laboratory) Adhesions Data for Three Center Study

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% Animals with no adhesions</th>
<th>Mean incidence of adhesions ± (SEM)</th>
<th>% Animals with adhesions ≥ 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No coating</td>
<td>25</td>
<td>8</td>
<td>2.4 ± 0.20</td>
<td>92</td>
</tr>
<tr>
<td>PBS</td>
<td>25</td>
<td>0</td>
<td>1.9 ± 0.18</td>
<td>88</td>
</tr>
<tr>
<td>0.10% HA</td>
<td>25</td>
<td>4</td>
<td>1.3 ± 0.12</td>
<td>40</td>
</tr>
<tr>
<td>0.25% HA</td>
<td>25</td>
<td>24</td>
<td>1.1 ± 0.16</td>
<td>24</td>
</tr>
<tr>
<td>0.40% HA</td>
<td>25</td>
<td>44</td>
<td>0.8 ± 0.18</td>
<td>12</td>
</tr>
</tbody>
</table>

**TABLE A3**

Site 3 (University of Florida) Adhesions Data for Three Center Study

<table>
<thead>
<tr>
<th>Group</th>
<th>% Animals with no adhesions</th>
<th>Mean incidence of adhesions ± (SEM)</th>
<th>% Animals with adhesions ≥ 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No coating</td>
<td>5</td>
<td>1.7 ± 0.18</td>
<td>90</td>
</tr>
<tr>
<td>PBS</td>
<td>17</td>
<td>1.5 ± 0.20</td>
<td>70</td>
</tr>
<tr>
<td>0.10% HA</td>
<td>32</td>
<td>1.5 ± 0.24</td>
<td>64</td>
</tr>
<tr>
<td>0.25% HA</td>
<td>29</td>
<td>1.1 ± 0.20</td>
<td>62</td>
</tr>
<tr>
<td>0.40% HA</td>
<td>33</td>
<td>1.0 ± 0.18</td>
<td>50</td>
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REFERENCES


