Trehalose May Prevent Postsurgical Adhesions in a Rabbit Model of Hysterotomy

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ABSTRACT. The adhesion-preventing effect of trehalose in visceral organs was evaluated in a rabbit model. All rabbits underwent a hysterotomy, during which mild abrasion and desiccation was induced on the surfaces of visceral organs. In the control group, the intestines were kept dry during hysterotomy. In the saline and trehalose groups, saline solution and 7% trehalose solution, respectively, were sprayed on the organ surfaces. Adhesion formation on organ surfaces on which abrasion and desiccation were induced declined significantly in the trehalose group compared with the other groups. In contrast, at the hysterotomy site, there was no significant difference in adhesions between groups, suggesting that trehalose prevents adhesions in organs that have developed minor inflammation by abrasion and desiccation.

KEY WORDS: abrasion, desiccation, hysterotomy, postsurgical adhesion, trehalose.

Post-surgical adhesions are a serious complication of abdominal surgeries. Adhesions occur in more than 90% of the patients who have undergone abdominal surgeries [14] and can lead to conditions such as bowel obstruction [1, 15], chronic abdominal and pelvic pain [16], and infertility [24]. In addition, subsequent laparotomies may carry an increased risk of complications due to the adhesion of visceral organs [9, 12]. In veterinary practice, adhesions that develop after surgery on the small intestine cause clinical problems in 15–22% of horses; colic and intestinal obstruction are observed in such cases [3].

Post-surgical adhesions arise from normal wound healing processes in tissues that are injured during surgery [10, 20]. In addition to direct surgical injuries, unintentional tissue injuries that are caused by abrasive manipulation and desiccation of the organ surface can initiate an inflammatory process. This minor inflammation can lead to the deposition of a fibrin-rich matrix, resulting in permanent adhesion to adjacent tissues [10, 12, 21, 23].

The use of different techniques and agents has been advocated to prevent adhesion. These include minimal trauma to abdominal structures by careful and gentle handling of tissues and moistening of tissues by irrigation or application of a wet sponge to prevent unnecessary desiccation [7, 12, 16, 23]. Adjuvant therapies, such as administration of nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antihistamines, and crystalloid isotonic irrigation containing heparin, have also been used to alter adhesion-inducing inflammatory processes. However, none of these therapies has been effective in reducing the formation of adhesions [12, 22].

Another preventive modality is the use of a solution or materials as a barrier to separate the serosal surface that is injured during the early stages of wound repair. Natural and synthetic solid barriers, such as polytetrafluoroethylene meshes, oxidized regenerated cellulose, bioreversible membranes composed of sodium hyaluronid acid (HA), and carboxymethylcellulose (CMC), are placed over traumatized tissues at the end of surgery; these barriers appear to reduce the incidence of adhesion [6]. However, because these graft materials only cover the surgical sites—not the entire peritoneum—it is difficult to limit adhesions that are caused by unintentional tissue injury due to abrasive manipulation and desiccation.

Trehalose (α,α-trehalose) is a natural disaccharide formed by the 1,1 linkage of 2 D-glucose molecules. It is a nonreducing sugar found in a wide range of organisms such as plants, algae, fungi, yeast, bacteria, insects, and other invertebrates. Because of its unique physical properties, trehalose can protect the integrity of cells against a variety of environmental stresses such as desiccation, dehydration, freezing, and oxidation. Studies on the hydration potential of trehalose suggest that it stabilizes lipid bilayers by ordering the water molecules around the membrane or by interacting directly with polar biomolecules as water is removed.

The protective effects of trehalose in living systems have led to its widespread application in the food and cosmetic industries [5, 8, 18, 19]. Recently, its use has been extended to the medical field for organ preservation during transplantation [4] and for dry eye syndrome [13]. In our previous preliminary study, trehalose prevented adhesion of organ surfaces that underwent desiccation in the rabbit [11]. The aim of this experimental study was to assess the effectiveness of trehalose in reducing postsurgical adhesions due to mild abrasion and desiccation of organ surfaces.
in a rabbit model of hysterotomy.

This study was performed per the Guidelines of the Committee for Animal Care, Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Thirty-six 14-week-old female New Zealand White rabbits (weighing 2.4–2.9 kg; Japan SLC, Inc., Shizuoka, Japan) were used. Anesthesia was induced in all animals by a bolus intramuscular injection of a mixture of ketamine (5 mg/kg), medetomidine (0.2 mg/kg), and midazolam (0.2 mg/kg). After tracheal intubation, anesthesia was maintained with isoflurane and oxygen. The rabbits underwent celiotomy through a 15-cm ventral midline incision. A 2-cm longitudinal incision was made on either uterine horn and closed with a Cushing suture pattern with 5–0 monofilament polydioxanone. During hysterotomy, most of the intestines were exteriorized and exposed to air.

In the control group (n=12), a mild abrasion was made on the surface of the right cecum 4 haustra in length and the duodenum (n=6) or on the right cecum and right lateral peritoneum (8 cm in size) (n=6). The abrasion on the intestines was created using an acrylic brush until subserosal hemorrhage and punctate bleeding were observed. In general, 10 brush strokes were sufficient to achieve the required extent of abrasion. The peritoneum was abraded with a #20 scalpel blade. All injuries were induced such that they faced each other.

Trehalose dihydrate (C11H22O11·2H2O, MW: 378.3 g/M), composed primarily of particles over 75 μm, was obtained from Hayashibara (not endotoxin-free) (Okayama, Japan). Trehalose (77.2 g) was dissolved in 1000 ml distilled water for injection (Otsuka, Tokyo, Japan), and 2.4 g sodium chloride was added to generate an isotonic solution. This solution was sterilized using a membrane filter (Iwaki, Tokyo, Japan) with a pore size of 0.22 μm. The percentage of trehalose in solution (7%) was selected based on a previous study [11] and our preliminary data.

During the abrasion procedure, in the control group (n=12), the intestines were kept dry without any treatment during the 60 min surgical procedure. In the saline group (n=12), physiological saline solution was sprayed on the intestines and other abdominal organs immediately before abrasion and every 15 min (5 times in total) after the abrasion commenced until abdominal closure. In the control group, the number of adhesions per rabbit at these sites was 2.83 and 2.83, respectively, and in the saline group, the number of adhesions per rabbit did not significantly differ between groups.

The total volume of saline or trehalose solution was 60 ml. Trehalose in solution (7%) was repeatedly sprayed using a spray gun similar to that in which the saline solution was sprayed. The percentage of fluid retention suggested that the trehalose solution had been absorbed by 7 days after the treatment.

Figure 1A-C shows the gross appearance of a Grade 2 adhesion between the intestines and between the intestine and uterus. Figure 1D shows the histological appearance of an adhesion with proliferation of granulation tissue. Proliferating fibroblasts and partial infiltration of inflammatory cells, such as lymphocytes and macrophages, were observed.

The numbers of adhesions per rabbit are shown in Fig. 2A. The number of adhesions per rabbit in areas that received abrasion did not significantly differ between groups. In the control group, the number of adhesions per rabbit at the cecum and duodenum and at the cecum and peritoneum was 10.5 and 7.83, respectively. In the trehalose group, the number of adhesions per rabbit at these sites was 2.83 and 2.83, respectively, and in the saline group, the number was 7.83 and 6.16, respectively. The number of adhesions at both areas was totaled in each group and regarded as the site of serosal abrasion.

At the surface of visceral organs that did not have an abrasion, the number of adhesions per rabbit was 2.67 ± 0.90 in the control group, 1.67 ± 0.38 in the saline group, and 0.34 ± 0.14 in the trehalose group (Fig. 2A, a). There were significantly fewer adhesions in the trehalose group compared with the other groups (P<0.01). At the surface of organs with abrasions, the number of adhesions per rabbit in the trehalose group was 0.42 ± 0.19, lower than in the control group (P<0.05) (Fig. 2A, b).

In contrast, at the uterine site where surgery was performed, the number of adhesions per rabbit was 1.75 ± 0.35.
The total number of adhesions per rabbit at sites on an organ surface (excluding those at the surgical site) in the trehalose group was significantly lower than in the control and saline groups ($P<0.01$). The number of adhesions per rabbit of all organs, including the uterus (surgical site), was also significantly lower in the trehalose group than in the other 2 groups.

Figure 2B shows the number of Grade 2 adhesions per rabbit at each site. At the surface of visceral organs without abrasions, the number of Grade 2 adhesions per rabbit was $1.50 \pm 0.56$ in the control group, $1.42 \pm 0.31$ in the saline group, and $0.083 \pm 0.083$ in the trehalose group (Fig. 2B, a). There were significantly fewer adhesions in the trehalose group compared with the other groups ($P<0.01$). At the surface of organs with abrasions, the number of Grade 2 adhesions per rabbit in the trehalose group was $0.33 \pm 0.14$, lower than in the control group ($P<0.05$) (Fig. 2B, b). At the uterine site, the number of Grade 2 adhesions per rabbit was $0.67 \pm 0.22$ in the control group, $0.33 \pm 0.22$ in the saline group, and $0.50 \pm 0.23$ in the trehalose group, and there was no significant difference between the groups (Fig. 2B, c).

The total number of Grade 2 adhesions per rabbit at sites on an organ surface (excluding those at the surgical site) in the trehalose group was significantly lower than in the other 2 groups ($P<0.01$). The total number of Grade 2 adhesions per rabbit, including the uterus, was significantly lower in the trehalose group than in the other 2 groups.

The results of this study suggest that trehalose significantly reduces postsurgical adhesion, particularly at sites that experience abrasive manipulation and desiccation. However, trehalose could not prevent adhesion of organs that underwent surgical procedures, although we did not evaluate various doses and methods of applying trehalose.

In this rabbit model, the surfaces of mildly traumatized intestines and peritoneum faced each other—a model of mild injury that has been incurred by surgical manipulation. The other organ surfaces were kept dry—a model for organ desiccation during surgery. Trehalose prevented adhesion at both sites (sites with mild trauma and those that were kept dry) because of its effects on the organ surface.

Trehalose-mediated protection of biological molecules and the cell membrane can be explained by 3 theories: water replacement, glass transformation, and chemical stability.
The water replacement theory proposes that all biological macromolecules are normally stabilized by water, which forms hydrogen bonds around these molecules. Trehalose appears to have greater flexibility in the glycosidic bond between its 2 D-glucose molecules. This property may allow trehalose to conform to the irregular polar groups of macromolecules.

The glass transformation theory suggests that sugars in solution can transform into or maintain a glass state instead of crystallizing. Trehalose is unique in that it forms a non-hygroscopic glass that is stable at high temperatures, even when it is completely desiccated. Trehalose glass is considered to be more stable, because the addition of a small amount of water may lead to the formation of trehalose dehydrate on the outer edges of the glass, which may result in a structure that encases the remaining glass, isolating it from the destructive effects of crystallization.

The chemical stability of trehalose is remarkable compared with that of other sugars. Due to its 1,1 glycosidic linkage, it is essentially a nonreducing sugar that is highly resistant to hydrolysis. In general, this sugar is chemically inert in its interactions with proteins.

It has been suggested that these 3 mechanisms are not mutually exclusive and that each may contribute to the stabilizing effects of trehalose [19]. These 3 mechanisms might mediate the prevention of adhesion on visceral organs that have undergone mild abrasion and desiccation.

Burns et al. [2] reported that damage to serosal tissues during surgery could be reduced by precoating the tissue with an HA solution at the beginning of surgery. They also reported that this precoating limited the formation of postsurgical adhesions and observed that precoating with saline...
solution did not prevent tissue adhesion. They hypothesized that the viscosity of HA solution influences the effects of precoating.

In this study, we also noted that saline was ineffective in reducing adhesions. In addition, the viscosity of 7% trehalose solution is similar to that of water [17]; thus, the biological molecule-preserving ability of trehalose could be related to its minimizing tissue desiccation and limiting tissue trauma by abrasion.

However, the effects of trehalose are limited to organs that have sustained minor injuries; trehalose is ineffective against surgery-induced major inflammation. The precise mechanism by which trehalose prevents adhesion is not yet fully understood; further studies are required both in vitro and in vivo.

In this study, trehalose solution was sprayed onto organ surfaces. This method was simple and easy to perform during surgery. Most materials that have been used to prevent adhesion have been applied to the surgical wound at the end of surgery. Compared with these earlier methods, trehalose solution may be more advantageous in clinical practice.

In conclusion, use of trehalose significantly reduces adhesion caused by abrasive manipulation and desiccation during surgery and is not accompanied by complications. Although the effect of trehalose in preventing adhesion is insufficient, this agent has tremendous potential in reducing adhesions caused by unintentional tissue injury due to abrasive manipulation and desiccation.

REFERENCES