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Choice of Hemostatic Agent Influences Adhesion Formation in a Rat Cecal Adhesion Model

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Introduction. **Hemostatic agents are frequently used during abdominal surgery and some are linked to adhesion formation. We sought to evaluate the impact of several commonly used hemostatic agents on adhesion formation in a rat peritoneal model.**

Methods. **In our study, Wister outbred rats underwent laparotomy and excision of a portion of their peritoneum to initiate adhesion formation process. One of six different hemostatic agents, namely, activated starch microspheres (Arista AH; Medafor Inc., Minneapolis, MN), glutaraldehyde activated collagen (BioGlue; Cryolife Inc., Kennesaw, GA), thrombin coated collagen microspheres (FloSeal; Baxter Inc., Deerfield, IL), thrombin activated fibrin polymer (Tisseel, Baxter), polyethylene glycol polymer (CoSeal, Baxter), or oxidized cellulose (Surgicel; Ethicon Inc., Somerville, NJ), was placed in the area of peritoneal defect. All animals were sacrificed on post-op day 7 and strength and extent of adhesion formation was determined. Histopathological examination of rat caecum was also performed.**

Results. **Arista and CoSeal showed significantly lower adhesion formation than controls ($P < 0.05$). Higher adhesion scores were seen in BioGlue ($P < 0.05$) treated rats. Additionally, histopathologic examination showed that BioGlue caused statistically more inflammation and necrosis than controls ($P < 0.05$). Total adhesion score increased with residual amount of agent present at 7 d.**

Conclusions. **Use of Arista and CoSeal may help in reducing peritoneal adhesions after intra-abdominal surgeries. Furthermore, there appears to be a relationship between the creation of inflammation and necrosis in tissues and the eventual formation of adhesions. This could aid in improving the design of these agents in the future.**

Key Words: **hemostatic agents; rat peritoneal model; adhesion.**

INTRODUCTION

Peritoneal adhesion formation after laparotomy continues to be a cause of significant morbidity for surgical patients [1]. Being an unpredictable and long-term problem, postoperative adhesions impact surgical workload, hospital resources, and results in considerable health care expenditures [2]. A great deal of effort has been made to elucidate the cause and to prevent the development of adhesions, but results have been mixed. Topical hemostatic agents are commonly applied to bleeding sites during intra-abdominal surgery. A number of these agents have been studied for their antiadhesive properties [3–5]. However, some of these agents may themselves promote adhesion formation. Also, a head-to-head trial comparing the impact of hemostatic agents on intraperitoneal adhesions has yet to be performed.

Arista AH, a topical hemostatic agent, has recently been approved for internal use. The goal of our study is to examine the impact of various hemostatic agents including Arista, FloSeal, Tisseel, Surgicel, BioGlue, on strength and extent of intraperitoneal and cecal adhesion formation in the rat model. We also sought to assess the histopathological changes to the peritoneum

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and bowel after placement of each of these agents. The rat model was selected as the experimental species because of its established use in modeling peritoneal adhesion formation and its similarities to human adhesion formation [6].

MATERIALS AND METHODS

Animals and Preoperative Preparation

After Institutional Animal Care and Use Committee approval, 70 Wister outbred rats (300–350 g) were studied. Each arm included 10 rats with 10 more for protocol development. The animals were quarantined for a minimum of 48 h prior to the beginning of the study. All the animals were fed a commercial diet until 12 h prior to surgery and had continuous access to fresh water. Each rat was weighed just before the surgery.

Surgical Procedure

The cecal adhesion model has been used previously [6]. Under complete aseptic precautions, median laparotomy was done in each of the rats by making a 6 cm midline skin incision and 4 cm abdominal incision. A 1 $\times$ 2 cm area of parietal peritoneum was sharply excised, including a superficial layer of underlying muscle, 1 cm lateral to the midline incision. The cecum was elevated and positioned so that at closure, it would contact the abdominal wall defect. It was gently abraded in a standard manner by scraping with gauze so that a homogenous surface of petechial hemorrhages was created over a 1 $\times$ 2 cm area. The hemostatic agents were applied to the site between the cecum and peritoneal wall defect in accordance with the manufacturer's instructions. All animals were sacrificed on day 7 of the study, immediately after which a repeat laparotomy was performed and cecum and the abdominal sidewall were evaluated for adhesion strength (see below). The adherent abdominal wall and cecum were then sent for histopathological review.

Evaluation Parameters

On repeat laparotomy, the area of the cecum and the abdominal side wall having gross adhesions was measured and expressed as a percent of the total de-peritonealized surface area. The gross scoring system used was a modification of a verified scoring system used in an adhesion model for the rabbit uterine horn [7]. This was further translated into grades 0-4, with no adhesion = 0, cecum to bowel adhesion = 1, cecum to sidewall adhesion over less than 25% of the abraded surface area = 2, cecum to sidewall adhesion between 25% and 50% of the abraded surface area = 3, and cecum to sidewall adhesion over 50% of the surface area = 4. Each animal was evaluated for strength of adhesion formation and graded 0–3 based on the following grading scale: 0 = no adhesion, 1 = gentle traction required to break adhesion, 2 = blunt dissection required to break adhesion, 3 = sharp dissection required to break adhesion. The extent of adhesion formation was also graded 0–3 on the following adhesion scale: 0 = no adhesion, 1 = filmy adhesion, 2 = vascularized adhesion, 3 = opaque or cohesive adhesion. These three scores were summed for a total gross adhesion score. Histopathologic evaluation was also performed on all rat cecum – abdominal wall adhesion to evaluate the extent of inflammatory response, acute necrosis, and presence of residual agent. All were graded on a 0–4 scale, with 0 being not present and 4 being present over >75% of the adhesion. If there was no adhesion between the two, the bowel wall and abdominal wall were examined separately. Both the gross observer (SHM) and the veterinary pathologic observer (HJK) were blinded as to the material used at the time of their evaluations.

Safety of test devices was assessed by daily monitoring of the weight, behavioral changes, and any early mortality in the rats. Biodegradation was assessed by microscopic examination of the tissue.

Test Devices

All hemostatic agents were prepared and applied according to the manufacturer's instructions. Arista AH (Medafor Inc., Minneapolis, MN) is a plant-starch derived powder-like agent formed into tightly engineered microporous particles with closely controlled porosity and spherical diameters. When applied to bleeding sites, these particles behave as molecular sieves that rapidly absorb the fluid and small molecular (less than 40,000 Da MW) components of blood thereby concentrating platelets, thrombin, fibrinogen, and other proteins on the outer surfaces of the particles [8, 9]. BioGlue (CryoLife Inc., Kennesaw, GA) is a two-component surgical adhesive composed of purified bovine serum albumin and glutaraldehyde. Both molecules covalently bind (cross-link) to each other and, upon application, to the tissue proteins at the repair site. FloSeal (Baxter Inc., Deerfield, IL) comprises of a bovine-derived gelatin matrix and a bovine derived thrombin component mixed immediately prior to application. Surgicel (Ethicon Inc., Somerville, NJ) is a white knit fabric prepared by the controlled oxidation of regenerated cellulose. Tisseel (Baxter Inc., Deerfield, IL) is made by the mixing of two self-activating components, concentrated fibrinogen with aprotinin, and thrombin with calcium chloride. CoSeal (Baxter Inc., Deerfield, IL) is made by mixing two distinct polyethylene glycol polymers that combine to form a flexible, watertight seal.

Statistical Analysis

Animals were randomized to one of the seven study groups. The study endpoints were adhesion area (mm^2) and pathologic adhesion score (0–4) measured at sacrifice day 7. For continuous endpoints, analysis of variance was used to obtain a pooled estimate of variance. Each treatment group was then compared with the control group using a t-test based on the pooled variance estimate. Group comparisons regarding pathologic adhesion score were done using the nonparametric rank-sum test. All tests were two-sided at α-level 0.05.

RESULTS

A total of eight animals died during the course of this study. One was sacrificed due to excess application of one of the agents, which we felt would have influenced the scoring of the adhesions. Three others were sacrificed due to wound breakdown caused by the rats. The final four were due to anesthesia complications. These were in various groups (three control, two BioGlue, two CoSeal, one Surgicel), and the sacrificed rats were replaced such that all groups had 10 rats analyzed. Although hemostasis was not a specific endpoint for the study, it should be noted that all agents were able to stop the mucosal hemorrhage created by the cecal and muscle abrasions. No rat died of postoperative hemorrhage.

At second look laparotomy, adhesions were examined. The model system showed 100% adhesion formation in the control rats with a mean adhesion total score of 6.0. The other groups had varying adhesion scores, as can be seen in Table 1. Arista had a lower mean adhesion score than control (3.9 *versus* 6.0, $P < 0.05$). CoSeal use resulted in a lower mean adhesion score as well (3.6 *versus* 6.0, $P < 0.05$). FloSeal, Tisseel, and Surgicel

TABLE 1

Gross Adhesions Scores (Mean ± SD) for Each Group of Rats

	Area score	Strength score	Extent score	Total score
Arista	1.5 ± 1.2	1.0 ± 0.8	1.0 ± 0.8	3.5 ± 2.7*
CoSeal	1.1 ± 1.1	1.5 ± 1.4	1.1 ± 1.1	3.6 ± 3.4*
Surgicel	1.8 ± 1.7	1.0 ± 1.0	1.0 ± 1.0	3.8 ± 3.6
FloSeal	1.2 ± 0.9	1.7 ± 0.9	1.7 ± 0.9	4.6 ± 2.6
Control	2.5 ± 1.1	1.8 ± 0.6	2.1 ± 0.6	6.4 ± 2.1
Tisseel	2.5 ± 1.6	2.6 ± 1.0	2.3 ± 1.0	7.4 ± 3.4
BioGlue	3.1 ± 1.4	3.0 ± 0.0	2.5 ± 0.5	8.6 ± 1.4†

*$P < 0.05$ total score statistically lower than control.
†$P < 0.05$ total score statistically higher than control.

adhesion scores were not statistically different from controls. On the other hand, adhesion scores were higher in rats receiving BioGlue (8.6 *versus* 6.0, $P < 0.01$).

None of the four pathologic adhesion scores was found to significantly correlate with gross adhesion score (results not given), i.e., no histologic measure of reaction to the hemostatic agent could be used to predict the intensity of adhesion. Acute inflammation (Fig. 1) was increased compared with controls in the rats treated with BioGlue and CoSeal. Acute necrosis (Fig. 1) was increased compared with controls only in the rats treated with BioGlue. Varying degrees of residual agent were found at day 7 (Fig. 1). Of note, only Arista was completely resorbed by the rats at day 7 in all test animals.

DISCUSSION

In this comparative laboratory trial, Arista and CoSeal limited adhesion formation the most. The use of Thrombin, Tisseel, and Surgicel did not differ from controls and BioGlue promoted adhesions formation when compared to controls. There has been a considerable concern regarding postoperative intra-abdominal adhesions and the problems during a second laparotomy. Several agents, including topical hemostats, are being tested for their role in preventing adhesions. However, some hemostatic agents may themselves cause foreign body reaction and inflammation and increase adhesion formation when applied intraperitoneally. In the present study, we compared several hemostatic agents for their propensity to cause adhesions. Although it would seem appropriate that increasing foreign body reaction and inflammation would increase the propensity to form adhesions, it should be noted than none of the pathologic measures of reaction to the hemostatic agent correlated with gross adhesion score. It is interesting to note that the two hemostatic agents that require a polymeric reaction (other than thrombin/fibrin) *in situ* to cure showed the strongest inflammation (CoSeal and BioGlue). Tisseel and FloSeal also require polymeric reactions, but they are based on the thrombin/fibrin system. This could explain the lack of inflammatory response seen with their use. However, it again should be noted than inflammation score did not correlate with gross adhesion score.

Comparing these results to the literature is a difficult task. One agent, Arista, tested here has no other literature regarding adhesions, but many of the others have had mixed results in different models. In this model system, Arista and CoSeal limited gross adhesions the most, with Arista having the additional benefit of showing limited inflammation at the surgical site. Use of

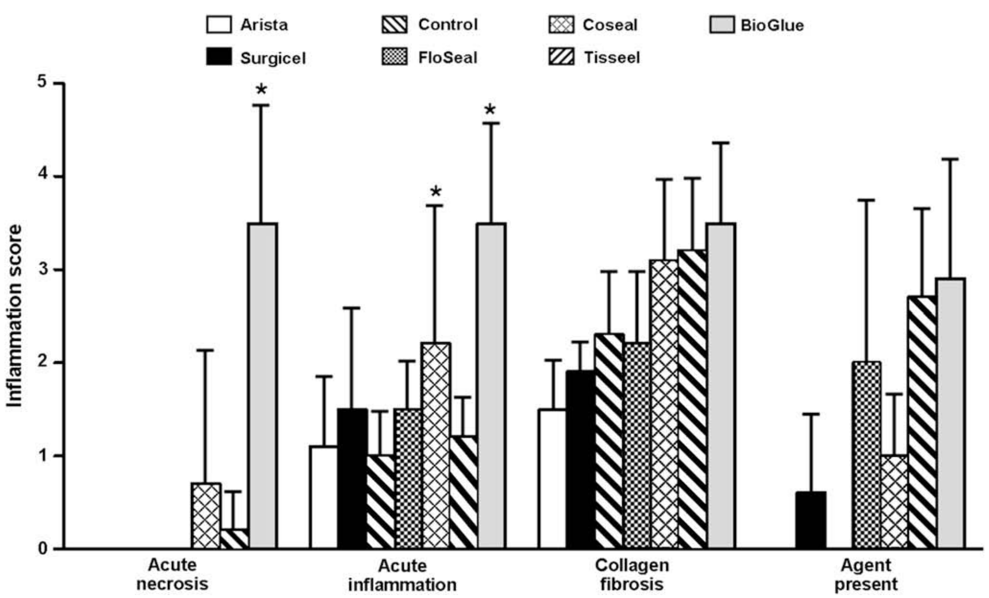

FIG. 1. Pathologic measure of acute necrosis, acute inflammation, collagen fibrosis, and agent present at 7 d after the first procedure. BioGlue shows a higher necrosis and inflammation score than control ($P < 0.05$). A CoSeal shows a higher inflammation score than control ($P < 0.05$).

CoSeal has been shown to reduce pericardial adhesions in rabbit models [10], as well as intraperitoneal adhesions to the abraded uterine horn of the pig [11] .

Several animal studies have shown reduced abdominal adhesion formation with the use of Surgicel [4, 12, 13] and FloSeal similar to our study. Tisseel, a fibrin sealant has also shown reduced, adhesion formation as compared to controls in various studies [5, 14]. Other studied have shown no difference with fibrin sealants [15]. We instead found no difference in adhesion scores with use of Surgicel, FloSeal, and Tisseel compared with controls. It should be noted that this study was originally powered to find a 1-point difference in mean adhesion score with a variance of 1 in each group. The variance for several of the groups were higher than expected, and further animals could show that use of Surgicel does indeed show lower adhesion scores.

BioGlue, which is used primarily as a tissue adhesive, was definitely associated with increased adhesion scores in our study. Review of the literature in the use of this adhesive shows no prior work with tissue adhesions. However, the cardiac literature has noted tissue toxicity from the leakage of glutaraldehyde as the product is resorbed [16] . In dural closures, Klimo et *al*. recommended discontinuing its use due to wound complications [17]. However, Nadler *et al.* recommended BioGlue for use in partial nephrectomy over others due to its improved hemostasis [18]. Other recommendations are for hemorrhoidal repairs [19] and for aortic repairs [16]. In addition to higher adhesions scores, acute tissue necrosis was seen with BioGlue use. It would seem appropriate to recommend BioGlue only in tissue that can withstand a rim of necrosis without patient complication.

Admittedly, this study looks at a single point in time for determining the adhesion promotion or inhibition of these products. Seven days was chosen as this appears to be a standard timeframe for evaluating adhesions in these models. However, this has its limitations. For example, the Tisseel product in this study also contains a proprietary blend of growth factors in order to promote wound healing. Certainly, this could promote adhesions in the early phase of wound healing, but as these growth factors are resorbed by the body, the effect could change over time. The ultimate effect on limiting postoperative adhesions could be different than seen here. Additionally, these new growth factors could also explain why the Tisseel preparation here showed no difference than controls when it had been shown to limit adhesions in other studies.

CONCLUSION

Arista and CoSeal can potentially limit adhesion formation when used for hemostasis during intra-abdominal surgeries. Arista also shows no residual agent and no difference in acute inflammation when compared to controls. Additionally, BioGlue appears to promote adhesions and acute tissue necrosis. We suggest that Arista and CoSeal can be used to promote hemostasis without adhesions after intra-abdominal procedures. Additional laboratory and human trials are needed.

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REFERENCES

1. diZerega GS. Contemporary adhesion prevention. Fertil Steril 1994;61:219.
2. Holmdahl L, Risberg B, Beck, et al. Adhesions: Pathogenesis and prevention-panel discussion and summary. Eur J Surg Suppl 1997;577:56.
3. Galan N, Leader A, Malkinson T, et al. Adhesion prophylaxis in rabbits with Surgicel and two absorbable microsurgical sutures. J Reprod Med 1983;28:662.
4. Larsson B, Nisell H, Granberg I. Surgicel–an absorbable hemostatic material–in prevention of peritoneal adhesions in rats. Acta Chir Scand 1978;144:375.
5. Lindenberg S, Steentoft P, Sorensen SS, et al. Studies on prevention of intra-abdominal adhesion formation by fibrin sealant. An experimental study in rats. Acta Chir Scand 1985;151:525.
6. Harris ES, Morgan RF, Rodeheaver GT. Analysis of the kinetics of peritoneal adhesion formation in the rat and evaluation of potential antiadhesive agents. Surgery 1995;117:663.
7. Boyers SP, Diamond MP, DeCherney AH. Reduction of postoperative pelvic adhesions in the rabbit with Gore-Tex surgical membrane. Fertil Steril 1988;49:1066.
8. Ereth M, Dong Y, Nuttall G, et al. Efficacy of hemostatic starch microspheres in bleeding time incision model. Proceedings of the American Society of Anesthesiologists Meeting, Orlando, FL, 2002:A537
9. Murat FJ, Ereth MH, Dong Y, et al. Evaluation of microporous polysaccharide hemospheres as a novel hemostatic agent in open partial nephrectomy: Favorable experimental results in the porcine model. J Urol 2004;172:1119.
10. Marc Hendrikx M, Mees U, Hill AC, et al. Evaluation of a novel synthetic sealant for inhibition of cardiac adhesions and clinical experience in cardiac surgery procedures. Heart Surg Forum 2001;4:204.
11. Ferland R, Mulani D, Campbell PK. Evaluation of a sprayable polyethylene glycol adhesion barrier in a porcine efficacy model. Hum Reprod 2001;16:2718.
12. Nishimura K, Bieniarz A, Nakamura RM, et al. Evaluation of oxidized regenerated cellulose for prevention of postoperative intraperitoneal adhesions. Jpn J Surg 1983;13:159.
13. McGaw T, Elkins TE, DeLancey JO, et al. Assessment of intraperitoneal adhesion formation in a rat model: Can a procoagulant substance prevent adhesions? Obstet Gynecol 1988;71:774.
14. Lindenberg S, Lauritsen JG. Prevention of peritoneal adhesion formation by fibrin sealant. An experimental study in rats. Ann Chir Gynaecol 1984;73:11.
15. Shinohara K, Kobayashi E, Yoshida T, et al. Effect of fibrin glue on small and large bowel anastomoses in the rat. Eur Surg Res 1998;30:8.

16. Furst W, Banerjee A. Release of glutaraldehyde from an albumin-glutaraldehyde tissue adhesive causes significant in vitro and in vivo toxicity. Ann Thorac Surg 2005;79:1522.
17. Klimo P, Jr., Khalil A, Slotkin JR, et al. Wound complications associated with the use of bovine serum albumin-glutaraldehyde surgical adhesive in pediatric patients. Neurosurgery 2007; 60:305.
18. Nadler RB, Loeb S, Rubenstein RA, et al. Use of BioGlue in laparoscopic partial nephrectomy. Urology 2006;68:416.
19. Anghelacopoulos SE, Tagarakis GI, Pilpilidis I, et al. Albumin-glutaraldehyde bioadhesive ("BioGlue") for prevention of postoperative complications after stapled hemorrhoidopexy: A randomized controlled trial. Wien Klin Wochenschr 2006; 118:469.