The Effect of Tranexamic Acid in Fibrin Sealant on Adhesion Formation in the Rat

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Abstract: The objective of the research was to determine the effect of the type, dose, and volume of anti-fibrinolytic agents (tranexamic acid, aprotinin) added to fibrin formulations, on adhesion development. Adhesions were induced in 228 male rats by creating apposing parietal and visceral peritoneal defects. Animals were randomized to receive no treatment or a fibrin formulation containing aprotinin or tranexamic acid. Seven days later the incidence of adhesions, and the force and energy required to detach them, were determined. Adhesions developed in 13/13 rats in the control and aprotinin groups. Treatment with fibrin (100 mg/ml tranexamic acid) resulted in adhesions in 4/14 rats (as strips, $p \leq 0.0005$), 4/10 rats (as spray, $p \leq 0.0036$), and 12/15 rats (by drip). The reduction of adhesions was dependent on the concentration of tranexamic acid with strip and spray application. Using commercial formulations, tranexamic-acid–containing fibrin (10/15, $p = 0.042$), but not aprotinin-containing fibrin (13/15), reduced the incidence of side-wall adhesions from 15/15 in controls. Fibrin containing either tranexamic or aprotinin reduced the incidence and severity of adhesions. This effect was greater when tranexamic acid was used and was dependent on the mode of administration, the volume, and to a degree, the concentration of tranexamic acid. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 68B: 222–230, 2004

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INTRODUCTION

Adhesion formation is an adverse consequence of surgery and results from the self-repair of tissue following trauma. Tissues that are normally separate become stuck together by fibrous scar tissue called adhesions. Adhesions can lead to serious complications including small bowel obstruction, female infertility, chronic debilitating pain, and difficulty with future operations.[1] Although a number of important advances have been made recently, the problem of postoperative adhesions remains without an ideal solution.[2]

The few products available around the world, although effective to some degree, have certain limitations. INTERCEED® (Ethicon, Inc., Somerville, NJ) [3] does not function in the presence of bleeding[4] and requires the attainment of meticulous hemostasis. SEPRAFILM® (Genzyme Corp. Cambridge, MA)[5,6] is somewhat brittle, is difficult to handle, and cannot be applied easily through a laparoscope. Both INTERGEL® (Lifecore Biomedical, Chaska, MN)[7] (at the time of writing, this product has been withdrawn from the market, pending an assessment of possible side effects) and ADEPT® (ML Laboratories Ltd., Leicester, UK)[8] are easy to apply but do not completely prevent adhesions. The search continues for agents that are easy to apply, function in the presence of bleeding, may be used on a variety of tissues, may be used to deliver drugs to a surgical site, completely prevent adhesions, do not reduce wound healing, do not potentiate infection, and do not evoke adhesions or fibrosis.

Because it theoretically possesses many of these properties, fibrin has been considered for use in the prevention of adhesions. Fibrin is the end product of the clotting cascade formed by the action of thrombin on its precursor protein, fibrinogen. Thrombin cleaves fibrinopeptides A and B to

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form fibrin monomers that associate spontaneously as a weak gel. A three-dimensional clot is formed after the transamidase, Factor XIII, cross links glutamine and lysine residues.[9]

The use of fibrin to prevent adhesions may at first appear counterintuitive. In the common view of the pathogenesis of adhesions, fibrin deposition leads to the maturation of fibrous adhesions from fibrinous ones. Thus the deliberate placement of fibrin at a surgical site might enhance adhesion formation.[10] This is unsupported by several studies using fibrin-derived materials in animals [11–22] and humans. [23–26]

One reason fibrin may be used to reduce rather than enhance adhesions is that once polymerization is complete, an adhesion barrier may form that cannot stick to other surfaces. The hemostatic and sealing properties of fibrin sealants, as well as their widespread availability, make them attractive candidates for use in adhesion prevention.

A number of factors may affect the performance of the fibrin preparation, including the source of fibrin, the method of processing, its concentration, the presence of fibrinolytic inhibitors, and the concentration of thrombin and calcium ions. These in turn may affect parameters such as polymerization kinetics and tissue adhesion.[27]

Thus, in order to interpret any studies involving fibrin preparations, the source of the material must be known. Animal or human studies have used autologous fibrin,[20] cryoprecipitate,[12,16] or commercial fibrin sealants such as Beriplast™ [19,22,26] or Tisseel® (or its equivalents, TissuCol™, Hemaseel™), which contain a fibrinolysis inhibitor (aprotinin).[11,13–15,17,18]

The effectiveness of the fibrin preparation may be partly related to its persistence on tissue, which in turn is related to its ability to resist degradation by plasmin. Some commercial products contain aprotinin, a protein derived from bovine lung. Given systemically, aprotinin has been reported to reduce adhesion formation in rats[28] and humans.[29] However, due to the safety concerns about the use of bovine-derived products, it is preferable that other fibrinolytic inhibitors be used.

One alternative is tranexamic acid [trans-4-(aminomethyl) cyclohexanecarboxylic acid], a synthetic cyclic competitive inhibitor of plasminogen activation that has been used for many years in hemophiliac patients, undergoing tooth extractions, to reduce hemorrhage.

Quixil® is a fibrin sealant preparation (Omrix biopharmaceuticals, SA, Rhode-St-Genèse, Belgium) recently introduced in Europe, and in the U.S. as CROSSEAL™ (American Red Cross, Washington, DC). It contains human fibrinogen with tranexamic acid and human thrombin.

Given the clinical use[23–26] of fibrin preparations for adhesion prevention, the objective therefore was to determine the effect on adhesion development of the type, dose, and volume of antifibrinolytic agents (tranexamic acid, aprotinin) added to fibrin formulations.

METHODS

In the first part of the study, the influence of volume of sealant, dose of anti-fibrinolytic, and method of delivery was examined. This part was conducted in Nes Ziona, Israel. In the second part, the efficacy in reduction of postsurgical adhesions of a fibrin sealant containing an optimized concentration of tranexamic acid was compared with a commercially available fibrin sealant containing aprotinin. This part was conducted in Dallas, TX.

All protocols were approved by the respective Animal Ethics or Institutional Animal Care and Use Committees and were performed in accordance with the NIH guidelines as described in the Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996.

Animal Model

The model is essentially as reported previously[30,31] in which abdominal adhesions are induced by creating specific abdominal wounds. These wounds are allowed to make contact naturally without the use of sutures or other foreign bodies. This model produces reliable and consistent adhesions between the traumatized surfaces. The adhesion analysis provides objective measurements of the force required to separate the adhering surfaces.

Male Sprague-Dawley rats (approximately 250 g) were obtained either from Harlan Biotech, Rehovot, Israel or from Harlan Sprague-Dawley, Houston, TX.

Preparation of the Animals

Rats were weighed before surgery and 7 days after the operation. In Part 1 animals were anesthetized with a 0.4-ml IM injection of a mixture of 85/15 ketamine HCl (100 mg/ml, Vitamed, Israel) and Xylazine HCl 20 mg/ml (Port Dodge Pty. Ltd., Australia). In Part 2 anesthesia was induced by placing the rats in an induction chamber into which isoflurane 5% in oxygen was introduced. Anesthesia was maintained by placing a mask on the rats on the operating table.

Depilation of the surgical site was accomplished with an electric animal clipper. After the area was cleaned, it was painted with an aqueous iodophor solution of 1% available iodine.

The abdomen was entered via a 6-cm midline incision. After the procedure, the abdominal wall was closed with continuous 4-0 polypropylene (Part 1) or 4-0 Vicryl (Part 2) and the skin with 4-0 nylon (Part 1) or steel wound clips (Part 2).

Adhesion-Inducing Procedure

With the muscle wall exposed, a 5-cm incision in the muscle was made along the linea alba through the peritoneal cavity. A defect in the right abdominal wall was created by removing a 2 × 1-cm patch of parietal peritoneum. The medial edge of this defect was located 1 cm lateral from the midline incision and parallel to it. The cecum was elevated and positioned so that upon closure, the cecum would contact the abdominal-wall defect. The cecum was abraded in a standard manner by scraping with a scalpel so that a homogeneous surface of petechial hemorrhages was formed over a 1 × 2-cm area. The

TABLE I. Formulation Differences between TissuCol, Hemaseel and Quixil

	TissuCol/Hemaseel	Quixil
Clottable Protein	75–115 mg/ml (fibrinogen)	40–60 mg/ml (mainly fibrinogen and fibronectin)
Total protein	100–130 mg/ml	65–85 mg/ml
Thrombin	500 IU/ml	900–1100 IU/ml
$CaCl_2$	4.44 mg/ml (40 μmoles)	5.6–6.2 mg/ml
Aprotinin	3000 KIU/ml	0
Tranexamic acid	0	85–105 mg/ml

cecum and abdominal-wall defect were dried by exposure to the air for 10 min. The other areas of the abdominal wall and the cecum were protected from drying by placing moist gauze over them during this period. Both areas were exposed for 10 min after the application of the investigational product. The cecum was approximated to the side-wall defect for 1 min prior to closure. After creation of the defects, the surgeon was made aware of the group assignment of the animal.

Investigational Products

Quixil® (Omrix Biopharmaceuticals, SA, Rhode-St-Genèse, Belgium) is a double viral-inactivated fibrin sealant containing 40–60 mg/ml of clottable protein, plus thrombin (1000 IU/ml). The commercial formulation contains 100 mg/ml of tranexamic acid.

In Part 1 of this study, Quixil was also prepared with the following concentrations of tranexamic acid: 100 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0 mg/ml. In Part 2, only the commercial preparation of Quixil with 100-mg tranexamic acid/ml was used.

Two other commercially available preparations were used in this study. In Part 1 TissuCol® VH Kit (2 ml) (Baxter-Immuno, Vienna, Austria) was used. In Part 2 an identical preparation manufactured by Baxter but sold and distributed by another company (Hemaseel™ APR Haemacure Corp., Sarasota, FL) was used. Both preparations contain aprotinin (Table I).

Quixil Preclotted Strip

Quixil (0.3 ml), formulated with various concentrations of tranexamic acid, was sprayed onto a 3 × 2-cm Teflon frame to produce a preclotted strip of sealant 1 mm thick. The strip was then kept in a sealed Teflon cast to prevent evaporation of water. The precast strips were stored at 2–8 °C and used within 1 week.

Application of Investigational Products

In the first part of the study, the groups of animals received 0.3 ml of the various Quixil formulations applied to the side-wall defect by dripping, or 0.3 or 0.8 ml applied by spraying, with the Quixil application device for both modes of application. TissuCol was applied by spraying according to the same procedure used for Quixil®, but with a TissuCol spraying device. The preclotted strip of Quixil was applied directly to the side-wall defect.

In Part 2 both Quixil and Hemaseel were reconstituted according to the manufacturer's directions and delivered using their respective and proprietary double-barreled syringe systems, with an air pump driven by compressed air regulated at 2.0–2.2 bars of pressure.

Group Assignments

Controls consisted of animals that received no treatment prior to closure. Group assignments for Part 1 of this study are shown in Table III.

In the second part of the study, animals were randomized either to receive no further treatment, or to be treated with 0.3 ml Quixil or 0.3 ml Hemaseel applied to the side-wall defect.

Evaluation of Adhesions

Seven days after surgery, animals were euthanized with the use of pentobarbitone (Part 1) or carbon dioxide intoxication (Part 2). The skin and muscle layers of the abdomen were incised lateral and distal to the location of the original defect. The resulting U-shaped flap was slowly lifted to reveal the adhesion, if present. Care was taken not to disturb the adhesions between the cecum and the side wall. After carefully noting, grading, and separating any extraneous adhesions, silk suture was used to loop around the terminal end of the cecum.

The rat was placed on a small board. The caudal edge of the U-shaped flap was secured with a clamp so that the peritoneal wall was at an angle of 35–45° to the horizontal. This permitted traction of the adhesions.

The other end of the suture was passed horizontally through a pulley and then vertically to a hook mounted on the underside of a load cell which was mounted on a crosshead. The cross head was moved at a constant rate, which caused the division of the adhesions. The force required to remove the cecum from the side wall was recorded electronically and was plotted against the displacement of the gauge. From this, the energy required to divide the adhesions was determined by calculating the area under the force-displacement curve. The parameters for the acquisition of this data in the two parts of the study are shown in Table II.

In several cases, adhesions were so fragile that they broke during the setup for tensiometric testing. These adhesions were given a grading of 1, and nominal values of 0.1 N and 1 mJ were assigned to these cases for statistical purposes.

Tenacity of Adhesions

Adhesions to the various abdominal organs were evaluated by the method of Harris et al.[30]

TABLE II. Parameters used in Data Acquisition

	Part 1 (Israel)	Part 2 (Texas)
Load Cell	Chatillon model DFI 2 (Chatillon, Greensboro NC) (1-kg range)	SMTI-2.2 load cell (Interface, Inc., Scottsdale, AZ) (10-N Range)
Servohydraulic system	Chatillon model TCD-200	INSTRON (Model 1321)
Crosshead speed	12.33 mm/min	12.5 mm/min
Frequency of readings taken	2 Hz	5 Hz
Data-acquisition system	Custom software—IDS	INSTRON 2490 intelligent interface with 12-bit analog-to-digital converter for computer data acquisition Instron FLAPS computer control and acquisition software.
Data recorded by	Paradox 4.0	Microsoft Excel 7.0
Amplifier	Chatillon DFI 2	Model SGA Amplifier/Conditioner (Interface, Inc.)

Grade 0 No adhesions
Grade 1 Filmy adhesions, easily removed
Grade 2 Moderate, difficult to separate
Grade 3 Highly inseparable; requires sharp dissection

Assessment of Residual Fibrin (Part 1)

Upon examination of the animal at necropsy and after inspection of the surgical site, the remnant of the Quixil preclotted strip was detached from the animal wall defect, washed intensively with saline, and then mixed with a solution of 0.5-M NaOH and 7-M urea to dissolve the fibrin. After overnight incubation, the protein (fibrin) concentrations were evaluated by differential spectrophotometeric readings at 280 versus 320 nm (this wavelength accounts for light scattering) against a known calibrated fibrin standard.

STATISTICAL PROCEDURES

Randomization

Animals were randomized to treatment groups, with their allocation only being revealed to the surgeon at the point where application of the test material was required. Evaluations were conducted in a blinded manner.

Statistical Analysis

Peak force and total energy (area under the curve) were calculated together for each adhesion force-displacement curve. Summary statistics, mean and standard error of the mean (SEM), were calculated for each group.

Data Analysis—Part 1

All treatment groups were compared, with respect to the occurrence of adhesions, with the use of a chi-square test, whereas pairwise comparisons between each treatment group and the control group, were performed with the use of Fisher's exact test. Because 14 treatment groups were compared with the control, a Bonferroni adjustment for multiple testing was used, which required a p value ≤ 0.0036 (0.05/14) for statistical significance. For the incidence of adhesions, other predefined comparisons of interest were also performed; however, no adjustment for multiple testing was employed. Both force and energy (area under the curve) were analyzed with the use of a one-way analysis of variance. An adjustment for multiple testing was employed with the use of Dunnett's test to compare each of the treatment groups against the control group. For force and area under the curve, other predefined comparisons of interest were also performed; however, no adjustment for multiple testing was employed. All calculations were performed with the use of SAS 8.1 software (Cary, NC).

Occurrence of Adhesions—On Site, Off Site, and Incidence of Adhesions

Three measures of adhesion occurrence were defined:

On-site adhesions	The number (%) of rats with adhesions to the sidewall defect
Off-site adhesions	The number (%) of rats with adhesions to sites other than the sidewall defect
Incidence of all adhesions	The number (%) of rats with adhesions at any site

These parameters were compared between the treatment groups with the use of χ^2 and Fisher's exact tests.

Data Analysis—Part 2

Control and Hemaseel groups were compared with the Quixil group with the use of Dunnett's t test[32] for multiple comparisons of the peak force and energy required to detach the adhesions.

Incidence of Adhesions

The numbers of rats in each group with adhesions to the side wall were compared with the use of Fisher's exact test.

TABLE III. Part 1: Number and % of Animals with On-Site, Off-Site, and Any Adhesions, Peak Force and Energy Required to Break Cecal–Side-Wall Adhesions

Tx	Tranexamic acid (mg/ml)	N[a]	On Site[b] %	Off Site[b] %	Incidence (All)[b] %	Peak Force[c] Newton	Peak Force[c] SEM	Energy (AUC)[c] mJ	Energy (AUC)[c] SEM	N/ Total[d]
1	Control	13	100	85	100	1.35	0.13	31.68	5.00	10/13
				Strip Quixil (0.3 ml)						
2	100	14	7**	29*	29**	0.53		5.40		1/14
3	2.5	14	14**	36*	43**	0.00		0.00		0/14
4	1.25	14	21**	50	93	0.91		10.38		1/14
				Spray, Quixil (0.3 ml)						
5	100	10	30**	10**	40**	0.08		0.41		1/10
6	2.5	10	50*	20**	60*	0.38*	0.06	2.13**	0.50	4/10
7	1.25	20	65*	40*	85	0.48**	0.07	4.95**	1.07	12/20
				Spray, TissuCol (0.3 ml) (aprotinin 3000 IU)						
8	0	12	100	42*	100	1.03	0.15	14.49*	2.29	12/12
				Spray, Quixil (0.8 ml)						
9	2.5	10	20**	20**	40**	0.55	0.18	4.27*	2.38	2/10
10	1.25	10	10**	40*	40**	1.28		14.82		1/10
				Drip, Quixil (0.3 ml)						
11	100	15	53*	53	80	0.81	0.20	8.07*	2.71	4/15
12	5.0	10	60*	30*	70	0.37**	0.13	5.04**	2.33	5/10
13	2.5	10	80	50	90	1.18	0.23	23.36	7.96	6/10
14	1.25	10	50*	40*	90	0.45*	0.07	4.84**	1.23	4/10
15	0	10	60*	50	100	0.78	0.23	13.99*	4.68	6/10

[a] Number of animals in group.
[b] Percent of animals in group with adhesions at site of injury (On Site), away from site of injury (off site), or at any site (All)
[c] Peak force and energy required to break adhesions between cecum and side wall (± standard error of the mean).
[d] Number of animals/total with adhesions between cecum and side wall.
* $p \leq 0.05$.
** $p \leq 0.0036$.

Tenacity of Adhesions

The tenacity of adhesions for each animal in Part 2 were arranged in rank order and mean rank positions were calculated for each group.[33] With the use of Dunnett's t test[32] for multiple comparisons, control and Hemaseel groups were compared with the Quixil group. p values ≤ 0.05 were regarded as statistically significant, except where noted.

RESULTS OF PHASE 1

Animal Welfare and Disposition

A total of 183 rats were operated upon during a 3-month period, and each week 15–20 rats were entered into the study and the designated formulations were applied. One of the control rats died on Day 5 after surgery. Death was caused by an injection of an anesthetic. All other animals recovered uneventfully after surgery.

Occurrence of Adhesions—On-Site, Off-Site, and Incidence of Adhesions (Table III)

An overall comparison of the 15 treatment groups with respect to on-site lesions, off-site lesions, and incidence of adhesions, with the use of the χ^2 test found treatment group differences with respect to the on-site and all lesions groups ($p < 0.001$), but not off-site adhesions ($p = 0.0885$).

For each parameter of adhesion occurrence evaluated, pairwise comparisons between each treatment group and controls were made with the use of a two-sided Fisher's exact test (Table III), with the Bonferroni adjustment.

Reductions in on- and off-site and all adhesions were noted with various Quixil formulations, the most effective being the strip formulation (Group 2) containing 100 mg/ml tranexamic acid. Strip formulations containing lower doses at either 2.5 (Group 3) or 1.5 mg/ml (Group 4) were less effective, as were the spray formulations.

The drip formulations of Quixil were less effective than the other modes of application. The group treated with the formulation containing aprotinin showed only a marginal reduction in the off-site adhesions, but no reduction in the overall incidence of adhesions.

To compare the two commercial formulations, the data from three groups in which Quixil was used at a volume of 0.3 ml (i.e., strip, No. 2; spray, No. 5, and drip, No. 11) were pooled and compared with the results from the TissuCol group. Quixil ($N = 39$) and TissuCol ($N = 12$) yielded 31% and 100% ($p < 0.0001$) for on-site adhesions, 33% and 42%

for off-site adhesions, and 51% and 100% ($p = 0.0017$) for all adhesions, respectively.

Dose-response relationships for tranexamic acid and adhesion formation were examined with the use of the Mantel–Haenszel χ^2 test. A more pronounced dose-response observation was made for the groups treated with 0.3 ml of Quixil spray, possibly because of the lower effectiveness of this mode of application in preventing on- and off-site adhesions. Although the Mantel–Haenszel p value approached significance (0.073) for both the on-site and off-site analyses, only with the all-adhesions analysis did it reach significance ($p = 0.012$). At the 0.8-ml volume with only two tranexamic acid dose levels, no dose-response relationship could be discerned, although the increased volume was superior at the 1.25 mg/ml dose of tranexamic acid ($p = 0.03$).

Peak Force and Energy Required To Break Cecal–Side-Wall Adhesions (Table III)

Cecal–side-wall adhesions formed in 10/13 control animals with peak forces for detachment (1.35 ± 0.13 N) of a similar order of magnitude to those reported previously.[30,31] An analysis of variance found significant differences between the groups for both parameters ($p < 0.001$).

Several Quixil formulations, in addition to reducing the overall incidence of adhesions, also reduced the peak force and/or energy required to detach adhesions in a statistically significant manner, notably, Group 7 (spray Quixil, 1.25 mg/ml tranexamic acid, 0.3 ml volume). Although the incidence of adhesions was not reduced in the TissuCol group, the energy required to detach the adhesion was significantly reduced.

To compare the two commercial products, data from the three groups in which Quixil was used at a volume of 0.3 ml (i.e., strip, No. 2; spray, No. 5; and drip, No. 11) were pooled and compared with the data from the TissuCol group. Quixil ($N = 6$) and TissuCol ($N = 12$) required peak forces of 0.642 ± 0.177 N and 1.032 ± 0.145 N ($p = 0.1260$) and energies of 6.35 ± 2.13 mJ and 14.48 ± 2.29 mJ ($p = 0.038$), respectively, to detach the adhesions.

The relationships between the dose of tranexamic acid and the strength of the adhesions were examined. For some comparisons, dose-response trends appeared present, but because of the small group sizes, statistical significance was not achieved.

The relationship between tranexamic concentration and effect for the groups sprayed with 0.3 ml Quixil approached significance ($p = 0.077$) for the energy measurements only.

Among the groups in which Quixil was applied by dripping, there was no linear dose-response relationship. The group treated with 2.5 mg/ml tranexamic acid gave a response (1.18 ± 0.23 N, 23.36 ± 7.96 mJ) which approached that of the control group. Pairwise comparisons of this dose group and the adjacent groups showed differences for both force (5 mg/ml, 0.0076; 1.25 mg/ml, 0.0209) and energy (5 mg/ml, 0.0182; 1.25 mg/ml, 0.0243) parameters.

TABLE IV. Part 1: Effect of Tranexamic Acid Concentration on Recovery of Clottable Protein in a Strip after 7 Days of Attachment to the Abdominal-wall Defect

Mode of Administration of Quixil	Fibrin before Application	Fibrin after 7 days	Recovery (%)
Strip with 100 mg/ml tranexamic acid	44.04	29.60	67
Strip with 2.5 mg/ml tranexamic acid	46.58	28.39	61
Strip with 1.25 mg/ml tranexamic acid	50.40	22.85	45

Residual Fibrin Protein

Attempts (Part 1) to collect and measure the amount of sealant left on the wall defect yielded variable amounts from all treatment groups, except the preclotted strips. The volume recovered from formulations containing tranexamic acid correlated well with the tranexamic The higher the concentration of tranexamic acid, the larger the volume of sealant remaining 7 days after surgery.

Where strips were used, almost full-shape strips were recovered, independent of the tranexamic acid concentration used (see Table IV). The amount of fibrin recovered in the strip correlated well with the tranexamic acid concentration.

RESULTS OF PHASE 2

Animal Welfare and Disposition

Forty-five animals were entered into the study. Four animals failed to recover from anesthesia due to a calibration error in the vaporizer and their treatments were reassigned to spare animals. In one animal a cecotomy was made inadvertently. This animal was excluded from the study and its treatment assigned to a spare animal.

All other animals recovered uneventfully. With the exception of one animal (Hemaseel group), all animals maintained or increased body weight. There appeared to be no differences between the groups.

Adhesion Development

Adhesions formed in all control animals. Detachment of these adhesions required peak forces greater than, but of a similar order of magnitude, to those reported previously[30,31] (2.43 ± 0.27 N). The energy required for adhesiolysis in controls was 59 ± 9.6 mJ.

Effect of Test Formulations on Adhesions (Table V)

One third (5/15) of the animals treated with Quixil did not form adhesions to the side wall compared with only 2/15 in the Hemaseel and 0/15 in the control groups. In animals treated with Hemaseel both the peak force and energy required to detach the adhesions were less than in the control

TABLE V. Part 2: Effect of Quixil and Hemaseel on the Formation of Adhesions in a Rat Cecal-Side-Wall Model

Group	Peak Force (Newtons[a])	p[b]	Energy mJ[c]	p[b]	Tenacity[d]	Adhesion-free[e]	N
1. Control	2.425 (0.27)		59.0 (9.6)		0/1/14	0 (0)	15
2. Quixil							
All animals	0.459 (0.16)	**, ††	5.0 (1.9)	**, †	5/3/7**	5 (33.3)‡	15
Animals with adhesions[6]	0.688 (0.21)	**, †	7.5 (2.5)	**, ‡‡			10
3. Hemaseel							
All animals	1.5 (0.24)		27.9 (5.4)		2/1/12	2 (13.3)	15
Animals with adhesions[f]	1.731 (0.21)		32.2 (5.3)				13

[a]Mean peak force ± SEM.
[b]p values for comparisons indicated.
[c]Mean energy required to break adhesions (mJ ± SEM).
[d]The number of animals with no adhesions/filmy adhesions/cohesive adhesions.
[e]The number (%) of animals with no adhesions to the side wall.
[f]Only those animals with adhesions were considered for this analysis.

**$p < 0.01$ (Dunnett's t test) versus control.
†$p < 0.05$ (Dunnett's t test) versus hemaseel.
††$p < 0.01$ (Dunnett's t test) versus Hemaseel.
‡$p = 0.042$ (Fisher's exact t test) versus control.
‡‡$p = 0.00059$ (Student's t test) versus Hemaseel.

group, but these reductions only approached statistical significance. However, in the animals treated with Quixil statistically significant reductions were found in the peak force and energy required to detach the adhesions.

Material Handling

Preparation of Quixil was accomplished very quickly after thawing. However TissuCol and Hemaseel were supplied as lyophilized powders and reconstitution required a period of warming and agitation of the solutions. The application devices for the three products were easy to use.

Gross Tissue Reactions

No evidence of gross tissue reaction, toxicity or irritation was noted in animals treated with the investigational products.

CONCLUSIONS

In two laboratories, fibrin sealant containing tranexamic acid was shown to reduce the overall incidence of adhesions. When adhesions did form, they were less tenacious than those that occurred in the control animals.

The most effective mode of application of fibrin sealant for prevention of adhesions was as a preclotted strip containing tranexamic acid, although spray application was also effective. Application by dripping was the least effective method. An inverse correlation between the dose of tranexamic acid and the incidence or strength of adhesions could be discerned. Increasing the volume of sealant sprayed onto the tissue also appeared to enhance its effectiveness.

Formulations containing tranexamic acid were also more effective than those containing aprotinin, an effect seen in both parts of the study. These data conflict somewhat with those of Rodeheaver's group, who, using the same model, found a reduction in the incidence of adhesions in one study[30] but not in another.[31] In both studies, the peak force requirement was reduced by about 50% by the aprotinin-containing formulation. In the present study there was also a reduction but of a smaller magnitude. Another difference between the present study and that of Rodeheaver was that sealant was applied only to the side wall (present study) or to both cecal and side-wall surfaces (Rodeheaver).

There are several other differences between the aprotinin- and tranexamic acid- containing formulations that may have accounted for these results (Table I). Although the amount of clottable protein in Quixil is less than that in Hemaseel, the higher concentration of thrombin used may result in a faster clotting time. In studying differences between commercial fibrin sealant preparations, Kjaergard et al.[27] suggested that rapid adhesion of the fibrin to tissue ensures that it functions on contact and remains where placed, reducing the opportunity for displacement by blood or movement of tissue.

There are several pharmacologic differences between aprotinin and tranexamic acid that may have accounted for the differences observed in the present study. Aprotinin is a nonspecific inhibitor of serine proteases and thus inhibits not only plasmin(ogen) but also other enzymes that might be part of the healing process, a problem not encountered with tranexamic acid, a specific plasmin(ogen) inhibitor.[34] Furthermore, because plasmin is involved in later stages of wound contraction and remodeling, such as the regulation of matrix metalloproteinases, the persistence of aprotinin, by virtue of its molecular size, may have a deleterious effect on wound healing. Because of its small molecular size, tranexamic acid diffuses away quickly,[35] maintaining molecular as well as temporal specificity.

In patients undergoing coronary artery bypass with extracorporeal circulation, aprotinin[36] but not amino-carboxy acids (e.g. tranexamic acid), reduced the increase in the levels of IL-10 and IL-6. How this relates to the development of adhesions must rely, *inter alia*, on a balance between the actions of these two cytokines, because IL-10 is regarded as generally anti-inflammatory and reduces adhesions,[37,38] and IL-6 is regarded as pro-inflammatory and increases adhesions[39] or is associated with pelvic adhesions.[40] Last, aprotinin may initiate local allergic reactions,[41–43] which could potentiate fibrosis and may also contribute to its reduced effectiveness compared with tranexamic acid.

Interestingly, the application of a fibrin sealant containing either aprotinin or tranexamic acid had some effect on off-site adhesions, suggesting that there is a more generalized consequence of applying the formulation locally. Fibrin sealant without any inhibitor effected a modest reduction in off-site adhesions, although this was only used in the drip group.

It is unclear why anti-fibrinolytic agents such as aprotinin or tranexamic acid should have a positive effect on the reduction of adhesions, but these off-site effects support the notion that fibrin sealants might enhance wound healing and ameliorate the inflammatory process for the remainder of the peritoneum and viscera,[16] possibly by accelerating or stimulating fibrinolysis via a local feedback loop. Others have noted that under most experimental conditions, both aprotinin and tranexamic acid *in vitro* may paradoxically accelerate plasminogen activation.[34]

Accordingly, the use of fibrin-based anti adhesion agents may provide an example of a challenge to the current paradigm for preventing adhesions in which an absorbable barrier placed between two adjacent and adhesive surfaces resorbs at the point of demesothelialization when it is no longer needed. The new paradigm, into which fibrin-based materials may well fit, involves optimization of the wound-healing process[2] and the use of barriers that would provide temporary protection, a scaffold for neoserosal regeneration, and possibly a reservoir for agents that have localized effects on wound healing.

In conclusion, both tranexamic-acid–containing, and aprotinin-containing fibrin sealants reduced the incidence and severity of adhesions in this model, although the effect was greater with the tranexamic-acid–containing formulation. The effect was also confirmed in a second laboratory. Given the current use of these types of products clinically, further studies are warranted to study this phenomenon.

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