JOURNAL OF SURGICAL RESEARCH 59, 759–763 (1995)

Local Release of Fibrinolytic Agents for Adhesion Prevention[1,2]

JENNIFER L. HILL-WEST, M.S.,* RANDALL C. DUNN, M.D.,†[3] AND JEFFREY A. HUBBELL, PH.D.*,‡[4]

*Departments of *Biomedical and ‡Chemical Engineering, University of Texas, Austin, Texas 78712; and the †Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas 77030*

Submitted for publication June 17, 1994

Tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), and streptokinase were evaluated for their ability to reduce postsurgical adhesion formation in a rat uterine horn devascularization and serosal injury model in a blinded, randomized study. Small doses of tPA, uPA, or streptokinase were delivered over approximately a 4-day period either from a biodegradable hydrogel matrix or as four daily intraperitoneal injections. The hydrogel was formed upon the uterine horns by photopolymerization of an aqueous precursor solution containing dissolved drug. A control group that received no treatment had an average extent of adhesion formation of 72 ± 15% (mean ± SEM, percentage of the length of the uterine horns involved in adhesions). Application of this formulation of the hydrogel alone reduced the extent of adhesion formation to 22 ± 10% by functioning as a mechanical barrier. When tPA was released from the hydrogel, adhesion formation was reduced to 4 ± 3%, while when tPA was given by intraperitoneal injection, adhesion formation was only reduced to 49 ± 8%. Local delivery of urokinase reduced adhesion formation to 6 ± 6%, but intraperitoneal injection of urokinase did not reduce adhesion formation. Streptokinase did not reduce adhesion formation when administered by intraperitoneal injection and increased adhesion formation to 45 ± 9% when locally released relative to the hydrogel alone. These results suggest that both tPA and uPA may be used to prevent adhesion formation when delivered locally.

INTRODUCTION

Postoperative adhesions develop when the peritoneum or serosa is disrupted by manipulation, and mast cells are activated, releasing histamine and vasoactive kinins. These substances increase capillary permeability, resulting in the formation of a serosanguinous exudate. If this exudate is not resorbed, it may form a fibrinous bridge between adjacent tissues which may subsequently become ingrown with collagen-secreting fibroblasts, resulting in permanent adhesions. In the abdomen and pelvis, ischemic mesothelium has been shown to produce decreased amounts of plasminogen activator [1–3]. Thus, the ability of the tissue to degrade fibrin may be compromised by surgically induced ischemia.

A number of studies have examined the effect of administering exogenous plasminogen activators following surgery to compensate for reduced native plasminogen activator activity. Several plasminogen activators have been investigated. Tissue plasminogen activator (tPA) is a two-chain glycoprotein that is a highly effective activator of surface-bound plasminogen. Postsurgical adhesion formation has been successfully reduced by the administration of recombinant tPA in abdominal [4–6] and pericardial [7] animal models and in humans [8]. Urokinase plasminogen activator (uPA) is a two-chain glycoprotein that can activate both surface-bound plasminogen and liquid-phase plasminogen. However, uPA is a far less effective plasminogen activator than tPA [9]. The results of animal studies using uPA in adhesion prevention have been mixed. A study in rabbits with intraperitoneal administration of uPA found that uPA reduced adhesion formation when blood was also added to the injured site [10]. Other studies, such as one in rats comparing intravenous, intraperitoneal, and intragastric administration [11], have found uPA to be ineffective in the prevention of postoperative adhesions. Streptokinase is a bacterial protein which has no enzymatic activity of its own, but which forms an equimolar complex with plasminogen. This complex has plasminogen activator activity. Streptokinase was originally found to reduce adhesion formation in a rabbit model in 1950 [12]. Subsequent studies have had negative results, however [13, 14]. Nonetheless, streptokinase–streptodornase was administered to a group of children following abdominal

[1] Presented in part at the Society for Gynecological Investigation, Chicago, Illinois, March 23–26, 1994.

[2] This research was supported by a grant from Focal, Inc., Cambridge, MA.

[3] Current address: Obstetrical and Gynecological Associates, Houston, TX.

[4] To whom correspondence should be addressed at: California Institute of Technology, Division of Chemistry and Chemical Engineering 210-41, Pasadena, CA 91125.

0022-4804/95 $12.00

laparotomy and a significant reduction in the incidence of adhesion formation was observed [15].

In the present study, tPA, uPA, and streptokinase were evaluated side-by-side in a randomized and blinded rat uterine horn model of devascularization and serosal injury. All three were evaluated when locally released from a biodegradable hydrogel formed directly upon the uterine horns and when given by daily intraperitoneal injection. Both methods of administration provided plasminogen activators for approximately a 4-day period, and the total doses administered were the same in each case.

METHODS

The hydrogel precursor polymer was synthesized and purified as described elsewhere [16]. It consisted of an 8000 kDa polyethylene glycol chain extended with an average of five lactic acid residues per end and further capped at each end with a reactive acrylate unit. This material was provided by Focal, Inc., Cambridge, MA. The precursor was stored as a dry powder and was dissolved in sterile physiological Hepes-buffered saline (10 m*M*) at a concentration of 15% w/v. The long-wavelength ultraviolet photoinitiator 2,2-dimethoxy,2-phenylacetophenone (Aldrich, Milwaukee, WI) was dissolved in *N*-vinyl pyrrolidone (Aldrich) at a concentration of 600 mg/ml, and 1.5 μl of this solution was added to each 1 ml of the aqueous precursor solution to achieve a final concentration of 900 ppm initiator. A 3 mg/ml solution of tPA (Genentech, South San Francisco, CA), a 1.8 mg/ml solution of uPA (Abbott Laboratories, Westborough, PA), and a 1.2 mg/ml solution of streptokinase (Astra Pharmaceutical Products, North Chicago, IL) were prepared in aliquots of the precursor solution or in saline. The dosages of each drug were equivalent fractions of the clinical dose used to treat myocardial infarction. The solutions were sterilized by passage through a 0.2-μm filter with a 0.8-μm prefilter.

A first set of experiments evaluated the efficacy of the fibrinolytic drugs when given daily as intraperitoneal injections. In this case, the duration of treatment was 4 days, but the drug was diffused throughout the intraperitoneal cavity. Female Sprague–Dawley rats (250–300 g) were anesthetized with pentobarbital (50 mg/kg) given intraperitoneally, and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature of the horns was systematically cauterized using bipolar electrocautery; the most distal and most proximal vessels were not cauterized. Following this, the antimesenteric surface of each horn was cauterized for 1 sec at each of two spots 1 mm in diameter, each separated by 2 cm and centered along the length of each horn [17]. After the injury was made, the animals were randomly assigned to four groups: saline, tPA, uPA, and streptokinase. Each group consisted of seven animals. The uterine horns were replaced in the peritoneal cavity, and the musculoperitoneal layer was closed with continuous 4-O Vicryl sutures (Ethicon, Inc., Somerville, NJ). The cutaneous layer was closed with 9-mm staples. The first injection (0.375 cc) was given at the conclusion of the surgical procedure. Additional injections (0.375 cc) were given daily, for a total of four. On the 7th postoperative day, the rats were sacrificed by CO_2 asphyxiation, and the extent and severity of adhesion formation were evaluated in a blinded fashion. Extent was reported as the fraction of the horn length involved in adhesions. Severity was reported on a qualitative scale. A grade of 0 was given when no adhesions were present, 1 when filmy adhesions were present, and 2 when dense, vascularized adhesions were present.

A second set of experiments evaluated local delivery of the fibrinolytic drugs from a hydrogel formed directly upon the uterine horns after injury. After the electrocautery injury was made as described above, the rats were randomly assigned to treatment groups: control (injury with no treatment), hydrogel without drug, hydrogel with tPA, hydrogel with uPA, and hydrogel with streptokinase. Each group consisted of seven animals. The appropriate solution (1.5 ml) was applied to the uterine horns, and the hydrogel was formed by exposure to long wavelength ultraviolet light for 20 sec at an irradiance of approximately 70 mW/cm^2 (Black Ray, UVP, San Gabriel, CA). On the 7th postoperative day, the rats were sacrificed, and the extent and severity of adhesion formation were evaluated as described above.

Statistical analyses were by the Kruskal-Wallis test for the extent of adhesion formation and by the χ^2 test for the severity of adhesion formation.

RESULTS

In the set of experiments evaluating fibrinolytic agents given by four daily intraperitoneal injections, tPA was found to be somewhat effective, reducing adhesion formation by 36% relative to controls. The severity of the adhesions that did form was not changed by the administration of tPA. Neither uPA nor streptokinase reduced the extent or severity of adhesion formation when administered by daily intraperitoneal injection. One animal in the streptokinase group died several hours after surgery due to anesthesia complications. The results of this set of experiments are summarized in Table 1.

In the experiments evaluating fibrinolytic agents released from a localized hydrogel matrix, the hydrogel alone reduced adhesion formation by 69% relative to the untreated control, consistent with previous observations [17]. Incorporation of tPA further reduced adhesion formation, by 95% relative to the untreated control or by 82% relative to the hydrogel-treated control. Surprisingly, uPA was as effective as tPA when locally released from the hydrogel. Treatment with uPA re-

TABLE 1

Prevention of Adhesions by Daily Intraperitoneal Injection of Fibrinolytic Agents

Group (n)[a]	Extent (Mean % ± SEM)	Significance of extent relative to control[b]	Severity (0–2)	Significance of severity relative to control[c]
Control (7)	77 ± 6		1, 1, 1, 2, 2, 2, 2	
Streptokinase (6)	83 ± 5	$P > 0.7$	1, 2, 2, 2, 2, 2	$P > 0.5$
uPA (7)	78 ± 7	$P > 0.7$	1, 1, 2, 2, 2, 2, 2	$P > 0.5$
tPA (7)	49 ± 8	$P < 0.05$	1, 1, 1, 2, 2, 2, 2	$P = 1.0$

[a] n, number of animals in group.
[b] By the Kruskal–Wallis test.
[c] By the χ^2 test.

duced adhesion formation by 92% relative to the untreated control or by 73% relative to the hydrogel-treated control. No dense adhesions were noted in any animals treated with tPA or uPA delivered from the hydrogel. Treatment with locally released streptokinase resulted in the formation of more extensive and severe adhesions than in the hydrogel-treated control. These results are summarized in Table 2.

DISCUSSION

A number of techniques have been previously investigated for the prevention of postoperative adhesions. These have included the use of polymeric barrier materials and numerous pharmacological approaches including fibrinolytic agents. Local delivery of fibrinolytic agents may provide a greater reduction in adhesion formation than one could expect to achieve with systemic administration of similar doses. Local delivery allows one to use lower total doses to create a locally high concentration at the site of application and may avoid possible systemic side effects. Local delivery may also protect sensitive drugs from destruction. Local delivery may be accomplished using pumps or polymer matrices. Pumps offer very precise control over delivery of the drug but may not sufficiently localize drug solutions to the target organs within the peritoneal cavity. Drug-containing polymeric materials generally release drug molecules by diffusion through pores in the polymer or as the polymer matrix erodes. Some polymers, such as the hydrogel used in this study, may be applied to tissues as liquids and then converted *in situ* to a gel that is adherent to underlying tissue. These types of systems allow the drug to be localized with high precision by open or minimally invasive surgery.

Local delivery has been utilized for the administration of anti-adhesion agents in a number of studies. Streptokinase, tPA, and Fb-Fb-CF, a tPA analog, were evaluated in a pericardial adhesion model in rabbits when applied to tissue as an aqueous solution or when locally released from an oxidized regenerated cellulose fabric [7]. In this study, tPA could only be administered

TABLE 2

Prevention of Adhesions by Local Release of Fibrinolytic Agents

Group (n)[a]	Extent (Mean % ± SEM)	Significance of extent relative to hydrogel alone[b]	Severity (0–2)	Significance of severity relative to hydrogel alone[c]
Untreated (7)	72 ± 5	$P < 0.01$	1, 1, 2, 2, 2, 2, 2	$P < 0.01$
Hydrogel (7)	22 ± 3		1, 1, 1, 1, 1, 1, 1	
Hydrogel + streptokinase (7)	45 ± 9	$P < 0.05$	1, 1, 2, 2, 2, 2, 2	$P < 0.01$
Hydrogel + uPA (7)	6 ± 3	$P < 0.01$	0, 0, 1, 1, 1, 1, 1	$P < 0.15$
Hydrogel + tPA (7)	4 ± 1	$P < 0.01$	0, 1, 1, 1, 1, 1, 1	$P > 0.25$

[a] n, number of animals in group.
[b] By the Kruskal–Wallis test.
[c] By the χ^2 test.

from the fabric, as bleeding complications were too severe to continue the study when the tPA was administered as a single bolus. Streptokinase and Fb-Fb-CF were both more effective when applied in the fabric than when given systemically [7]. A number of studies have administered tPA abdominally via minipumps [18–20]. Several studies have investigated the use of tPA administered in a localized hyaluronic acid gel [4, 6, 21]. A study investigating the length of administration of tPA following uterine horn cautery in rabbits found that optimal results were achieved when tPA was given continuously for a 4-day period [19]. All of the local delivery forms discussed above lengthen the duration that therapeutic concentrations of fibrinolytic drugs are present in the region of manipulation by preventing destruction of the drugs and by maintaining slow release rates.

The hydrogel material used in this study as a local delivery vehicle is a water soluble, biodegradable polyethylene glycol derivative which is applied to tissues as a liquid and converted to a hydrogel *in situ* by brief exposure to long-wavelength ultraviolet light. This photopolymerization process creates a hydrogel coating, approximately 200 μm thick, that conforms to the tissue upon which it was formed but which is not adherent to other tissues [17]. The hydrogel has been previously described by our laboratory as a barrier material for postsurgical adhesion prevention in several animal models [17, 22, 23]. The hydrogel degrades via nonenzymatic hydrolysis over approximately a 4-day period and remains adherent to the underlying tissue throughout the degradation process [23].

The hydrogel precursor is comprised of a central chain of polyethylene glycol, with flanking regions of oligomers of lactic acid on both ends, with end caps of acrylate groups at each terminus. The polyethylene glycol region provides biocompatibility and water solubility, the lactic acid regions provide biodegradability, and the acrylate termini provide photopolymerizability. When an aqueous solution of the precursor is exposed to long-wavelength ultraviolet light in the presence of the photoinitiator, photopolymerization occurs to form a three-dimensional hydrogel network. The network conceptually resembles a three-dimensional net, the nodes in the net being the reacted acrylate groups and the links being the polyethylene glycol chains. The lactic acid regions reside between the links and the nodes. Macromolecular drugs, e.g., tPA, uPA, and streptokinase in the present study, may be entrapped within the network by incorporation in the precursor solution. When the lactic acid oligomers degrade by nonenzymatic hydrolysis, the pore sizes in the gel increase and the entrapped drug is thereby slowly released.

In previous experimentation, the hydrogel barrier has been observed to degrade over a period of approximately 4 days: a gel barrier of an initial thickness of 176 μm degraded to 32 μm within 4 days. On the 5th day, the gel did not form a continuous barrier, but was present only in patches, with an average thickness of 4.9 μm [23]. The composition used in this study was not the optimum formulation (10% precursor solution) previously found for adhesion prevention [17], but was altered somewhat (15% precursor solution). This altered formulation was selected both to provide for more sustained drug release and to provide for lesser efficacy with the hydrogel alone, so as to investigate possible enhancements due to a released fibrinolytic enzyme.

The results of this study show that both tPA and uPA are highly effective when delivered from a localized hydrogel matrix, nearly eliminating adhesion formation in this model. While tPA reduced adhesion formation when given by daily intraperitoneal injection, it was far more effective when the same amount of drug was released from the hydrogel matrix. Moreover, uPA was effective only when administered locally from the hydrogel. When administered from the hydrogel, uPA and tPA had statistically indiscernible efficacy. The hydrogel material presumably enhanced the efficacy of fibrinolytic agents by localizing them at the site of trauma and protecting them from rapid clearance or inactivation that may occur in the peritoneal fluid. In addition, the hydrogel material employed in this study released fibrinolytic agents continuously over approximately a 4-day period, which has been shown by Dunn and Mohler to be the optimum duration of treatment for adhesion reduction by continuous catheter delivery of fibrinolytic agents [19].

In our model, streptokinase did not reduce adhesion formation regardless of the mode of administration. Moreover, streptokinase released from the localized hydrogel matrix significantly decreased the efficacy of the hydrogel as a barrier, by 51%. This may be attributable to the immunogenic nature of streptokinase. Streptokinase is a bacterial protein derived from pathogenic strains of the *Streptococcus* family. Most mammals, including rats [24], are naturally immunized against streptokinase as a result of streptococcal infections. Sufficiently high titers of anti-streptokinase antibodies to completely neutralize normal doses given to treat myocardial infarction are present in many humans (25). Streptokinase is known to cause anaphylactic shock mediated by IgE antibodies, toxic immune complex disease due to IgG antibodies, and lymphocyte-mediated inflammatory reactions [26]. When streptokinase was locally released from a hydrogel matrix over a 4-day period, adhesion formation was greater than in the control with the hydrogel alone. This increase in adhesion formation was likely due to an immune response to streptokinase, causing inflammation. While streptokinase has reduced adhesion formation in some studies in rabbits [12], it has not been found to be effective in rats [14]. Moreover, the local, sustained

release of an immunogen may elicit a more vigorous immune reaction than a single or multiple exposure.

Wound-healing complications and bleeding complications are of concern when fibrinolytic agents are administered postsurgically. It should be noted that no such complications were observed in any animals that received the fibrinolytic agents administered from the hydrogel matrix. Four of the animals that received tPA by daily intraperitoneal injection had small hematomas at the closure site, none of which were severe or problematic. Although the group sizes were small, it appears that administration via the hydrogel matrix may serve to decrease side effects in addition to enhancing efficacy. Further experimentation with larger group sizes would be necessary to substantiate this.

Previous studies evaluating the efficacy of this hydrogel in adhesion reduction have found that at a precursor concentration of 10%, application of the hydrogel reduced adhesion formation by 87% in the same animal model used in the current study [17]. While these results were very encouraging, we sought to even further enhance the efficacy of the nonpharmacological barrier by using the resorbable barrier to release a bioactive compound. The results of the present study suggest that the combination of a highly biocompatible hydrogel barrier with either uPA or tPA may further reduce the formation of postsurgical adhesions.

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