Paclitaxel-Loaded Crosslinked Hyaluronic Acid Films for the Prevention of Postsurgical Adhesions

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Received December 14, 2001; accepted January 4, 2002

Purpose. Post surgical adhesion formation results in significant morbidity for surgical patients. The purpose of this study was to investigate the use of paclitaxel (PTX) as an inhibitor of adhesion formation in rats and to design and characterize a controlled release film formulation of the drug for application to exposed surgical sites.

Methods. The rat cecal side wall abrasion model was used to investigate the anti-adhesion properties of PTX. The drug was administered by either intraperitoneal injection (i.p.), as the cremophor formulation (Taxol®) or by application of carbodiimide crosslinked hyaluronic acid (HA) films containing PTX. The HA films were also characterized by measurements of elasticity, degree of swelling in water and drug release rates.

Results. Taxol® administered by i.p. injection at 4 mg/kg on a daily basis for between 3 and 5 days resulted in a significant reduction in adhesion formation. All animals in the control group (n = 10) had some form of adhesion following abrasion whereas the percent of animals without adhesions significantly increased and the mean incidence of adhesion formation decreased in the three Taxol® treated groups. The application of 5% PTX loaded HA films had a similar significant effect in increasing both the % of animals without adhesions and in reducing the mean incidence of adhesions.

Conclusions. Paclitaxel is an effective inhibitor of adhesion formation in rats. HA crosslinked with 2 mM water soluble carbodiimide and containing 10% glycerol and 5% PTX are flexible, mucoadhesive, biocompatible controlled release films suitable for application to surgical sites for the prevention of adhesion formation.

KEY WORDS: paclitaxel; surgical adhesions; hyaluronic acid films.

INTRODUCTION

Postsurgical adhesions are abnormal tissue attachments that result from cuts or abrasions to tissues during surgery. These adhesions develop as part of the normal wound healing response of the tissues to the trauma and occur in over two thirds of all abdominal surgical patients (1,2). The consequences of these adhesions are varied and depend upon the surgical site involved. Problems may include pain, infertility, obstruction of the intestines, and even an increased risk of death after cardiac surgery (3–5).

The process of adhesion formation initially involves the establishment of a fibrin framework and normal tissue repair. The normal repair process allows for fibrinolysis alongside mesothelial repair. However, in surgical adhesion formation the fibrin matrix matures as fibroblasts proliferate into the network and angiogenesis occurs resulting in the establishment of an organized adhesion within 3 to 5 days (6,7).

Interventional attempts to prevent the formation of postsurgical adhesions have included the use of hydroflotation techniques or barrier devices. Hydroflotation involves the instillation of large volumes of polymer solutions such as dextran (8) or carboxymethyl cellulose (9) into the surgical space in an attempt to keep the organs apart. However, this technique has produced only marginally beneficial effects in animals or humans (10). Synthetic barrier membranes made from oxidized regenerated cellulose (Interceed ™) or polytetrafluroethylene (Gore-tex surgical membrane) have demonstrated some limited inhibition of adhesion formation in humans. More recently, a fully resorbable membrane made from a modified hyaluronic acid/carboxymethylcellulose (HA/CMC) combination (Seprafilm ™) was shown to significantly reduce, but not eliminate, postsurgical adhesion formation in both animals and humans (11–13). The success of these HA/CMC membranes was thought to derive from their ability to provide tissue separation during the peritoneal wound repair process when adhesions form. The membranes were observed to form a clear viscous coating on the injured tissue for 3–5 days after application, a time period that is compatible with the time course of postsurgical adhesion formation (14).

Attempts to prevent postsurgical adhesion formation by pharmacological means have been largely unsuccessful. The intraperitoneal administration of anti-inflammatory agents such as dexamethasone or corticosteroids produced only marginal inhibition of adhesion formation (4,15). Antiproliferative agents, such as retinoids or antiangiogenic agents such as those targeting vascular endothelial growth factor receptors might offer therapeutic potential. However, the pathophysiology of postsurgical adhesion formation indicates that a compound that inhibits both cell proliferation and angiogenesis might be an effective anti-adhesion agent. Paclitaxel is a potent anti-angiogenic agent (16) that also inhibits the proliferation of cells such as fibroblasts at very low concentrations (17). However, paclitaxel is also known to inhibit wound healing (18), which may potentially compromise the clinical use of this drug in the prevention of postsurgical adhesions.

The objective of this study was to design a biocompatible, mucoadhesive film containing the drug paclitaxel that could be applied to the abraded tissues to release the drug over a 2–3 day period. This time period was considered to be long enough to allow inhibition of the early processes of postsurgical adhesion formation but short enough to allow clearance of the drug from the tissue site and subsequent normal wound repair. In this study we report the *in vitro* characterization and *in vivo* efficacy of paclitaxel loaded crosslinked hyaluronic acid (HA) films designed for the prevention of postsurgical adhesion formation.

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ABBREVIATIONS: PTX, Paclitaxel; HA, hyaluronic acid; HA/CMC, hyaluronic acid/carboxymethylcellulose; EDAC, Ethyl-3-(dimethylamino) carbodiimide.

MATERIALS AND METHODS

Materials

Medical grade sodium hyaluronate (HA) was obtained from Lifecore Scientific (Chaska, NJ, USA). Solvents were HPLC grade (Fisher Scientific). Paclitaxel (PTX) was obtained from Hauser (Hauser Chemical Company, Boulder, CO, USA). Sticky-back Teflon sheeting was obtained from Norton Performance Plastic Corporation (Wayne, NJ, USA). Ethyl-3-(dimethylamino) carbodiimide (EDAC) was obtained from Sigma (St. Louis, MO, USA). Taxol ® (5 mg/ml injection) was obtained from Bristol Myers Squibb (Princeton, NJ, USA) and was diluted 1:4 in saline prior to injection to give a final concentration of 1.2 mg/ml

Preparation of HA Films

All films were made by pipetting 4 g of the 1% HA solution (± glycerol) into 2.5 cm diameter plastic petri dishes and drying for 24 h at 60°C. Paclitaxel loaded films were made by adding a known volume of a 2% PTX solution in ethanol to the viscous HA solution. Similarly, crosslinked films were prepared by the addition of a known volume of a 400 mM solution of EDAC in water, to give final concentrations of 0.8 to 8 mM. HA solutions were well mixed before casting. For animal studies, larger films were made by pouring 16 g of the 1% HA dispersion onto 4.5 × 4.5 cm^2 Teflon sheets, followed by drying at 60°C for 24 h. These films were terminally sterilized using gamma irradiation from a cobalt-60 source and exposed to 2.5 Mrad (over 8 h) of radiation

Elasticity Determinations

Rectangular films measuring 1 cm × 2.5 cm were placed in a device that clamped the films at the 1 cm ends. The thickness of the film was measured using a digital micrometer (Mitutoyo, Japan) and the length of the film between the clamps was measured using calipers. This clamp device was fixed at one end and suspended in the optical path of a microscope (clamped and oriented in the horizontal direction) with a calibrated eyepiece micrometer. The microscope was then focused on a mark on the film. Increasing weights were then applied to the lower end of the film and the extension of the film was measured using the eyepiece micrometer. Films always returned to their original length when weights were removed.

Stress was determined as the force applied per unit area [9.81 × weight applied (kg)/width × thickness (m^2)] N/m^2

Strain was determined as the change in film length (extension)/original length (m).

Swelling Studies

Film swelling was determined by measuring the weight gain of films placed in water for different times. Pieces of film weighing approximately 2 mg were placed on 2.5 cm diameter filters (Millipore membrane filter 0.45 μm pore size) and the combination was accurately weighed. The filter and film were then placed on a vacuum filter head. Excess water was applied to the film. At given time points, the vacuum was applied for 10 s to remove excess water, the filter/film combination was then placed on a tissue to remove water from the base of the filter and the combination was reweighed. The weight of water absorbed by the filter alone was determined as above using separate filters.

Drug Release Studies

Five mg films were placed in small cages (stainless steel sieve mesh with 60 μm openings) and placed at the bottom of 50 ml screw cap test tubes. The tubes were held in a vertical position in an orbital shaker (New Brunswick Scientific, Edison, NJ, USA) at 100 rpm. Ten ml of phosphate buffered saline, pH 7.4, (PBS) were gently pipetted into the tube to completely cover the cage. Five ml of n-octanol was then added, the tube was capped and shaken at 100 rpm at 37°C. The concentration of PTX in the n-octanol phase was determined by UV/VIS absorbance at 232 nm. The sample of octanol used for each detemination was returned to the original sample tube. The solubility of PTX in n-octanol was determined to be greater than 5 mg/ml or more than 5000-fold that in PBS (approx. 1 μg/ml).

Animal Studies

Two separate studies were conducted each using 40 rats in 4 groups of 10. In both studies surgical trauma was induced as follows: Forty mature Sprague Dawley rats, each weighing 225–350 g were obtained from Charles River Laboratories, Wilmington, MA. Only animals which appeared grossly normal (showing a clean unruffled coat, bright clear eyes and an active posture) were used in the study. Animals were randomly assigned to one of four groups, weighed and anesthetized with a single injection of ketamine hydrochloride (6 mg/kg), administered in the large muscle of the thigh. The abdomen was shaved and cleaned with alcohol. A 4 cm incision was made in the skin beginning approximately 2 cm caudal to the linea alba while the muscle was tended with forceps. The cecum was abraded four times on the ventral and dorsal surfaces with a mechanical abrading device, which permits operator independent, controlled abrasion over a defined area.

Taxol® Treatment Study

The treatment schedule for this study is outlined in Table I. The animals in the control group received no treatment whereas the animals in Groups 1–3 received paclitaxel administered as the Cremophor-EL and ethanol formulation of the

Table I. Efficacy of Intraperitoneal Injections of the Cremophor-EL: Ethanol Formulation of Paclitaxel (Taxol®) in the Rat Cecal Abrasion Model of Surgical Adhesion Formation

Group	Treatment (days)	Mean incidence[a] +/–SEM	% Animals with no adhesions
Control (n = 10) (no treatment)	NA	1.6 +/– 0.3	0
Group 1 (n = 10)[b]	–1, 0, 1, 2, 3	1.1 +/– 0.4	40
Group 2 (n = 9)	0, 1, 2, 3	0.4 +/– 0.3	78
Group 3 (n = 8)	0, 1, 2	0.5 +/– 0.3	63

[a] Mean incidence is the total number of adhesions observed in the group of animals divided by the number of animals in the group.
[b] Groups 1–3, Taxol® at 4 mg/kg.

drug (Taxol®). The drug was administered directly into the open peritoneal cavity at a dose of 4 mg/kg following abrasion and the incisions were closed with 3.0 Dexon suture. Paclitaxel was also administered postoperatively in daily doses of 4 mg/kg by intraperitoneal injection of Taxol®. Animals in the control group received no treatment, animals in Groups 1 and 2 received Taxol® at the time of the operation and once a day for three postoperative days whereas animals in Group 3 received Taxol® on the day of the operation and for two postoperative days. Animals in Group 1 had also received Taxol® one day before surgery. Seven days postoperatively, the animals were euthanised and evaluated for the presence of grade 1 (or higher) postoperative adhesions. The evaluator was unaware of group assignment. Adhesions to the cecum were evaluated and scored according to a predefined scoring system: 0 = no adhesions, 1 = filmy adhesion with easily identifiable plane, 2 = mild adhesion with freely dissectable plane, 3 = moderate adhesion with difficult dissection of plane, 4 = dense adhesion with non-dissectable plane.

HA-PTX Film Study

The assignment of groups is given in Table II. Following abrasion of the cecum, animals in Group 1 received no treatment. Animals in Groups 2–4 received HA films (4.5 × 4.5 cm) all containing 10% glycerol and either no drug, 1% PTX (contained 1.6 mg of drug) or 5% PTX (contained 8 mg of drug) respectively. The films were wrapped around the cecum. The incisions were then closed with 3.0 Dexon suture. Seven days postoperatively, the animals were euthanised and evaluated for the presence of grade 2 (or higher) postoperative adhesions.

Statistical analysis was performed using a Chi-Square analysis between each pair of groups. In all cases a p value < 0.05 was considered statistically significant.

All studies were performed in accordance with the NIH guidelines as described in the Principles of laboratory animal care (NIH publication # 85-23, revised 1985) and were approved by the Genzyme Institutional Animal Care and Use Committee.

RESULTS

It was observed that films prepared using HA alone (no glycerol or EDAC crosslinking) were brittle and dissolved within minutes in water. However, the addition of glycerol to the HA solution produced films that were elastic and did not tear on handling. These films also dissolved in water within minutes. The addition of both EDAC and glycerol to the HA solution produced films that were elastic and swelled, but did not dissolve in water.

The effect of adding increasing amounts of glycerol to the HA films on the stress:strain properties of the films is shown in Fig. 1. Films prepared using HA alone were brittle and possessed almost no elastic properties as shown by the steep gradient of the stress:strain curve. The addition of 10% glycerol produced films that were elastic and showed a reduced gradient in the stress:strain curve in Fig. 1. This increase in elasticity was dependent on glycerol concentration as shown in Fig. 1. However, the increase in elastic behavior induced by the addition of the lowest glycerol concentration (10%) was so dramatic that films containing this concentration of glycerol were felt to have excellent handling properties and to be suitable for application to potential surgical adhesion sites.

When HA films containing 10% glycerol were placed in water they swelled and then fully dissolved within 30 m. When the same films were placed on the filter units they were found to swell very rapidly as shown in Fig. 2. However, the weight decreased after 30 s on the filter unit. This was due to the rapid dissolution of the films in water. The effect of adding increasing concentrations of EDAC to HA films containing 10% glycerol from 0.8 mM to 8 mM was to cause a concentration dependent decrease in the swelling of the films. By 15 m the films containing 0.8 mM EDAC had swollen by more than 5000% whereas films containing 8 mM EDAC had swollen by less than 2000%. The films containing 0.8 mM EDAC began to dissolve after 15 m as shown by the apparent decrease in swelling of these films in Fig. 2.

Generally, the rate of paclitaxel release from all films was rapid. Films containing between 1% and 10% PTX, with or without glycerol and crosslinked or non-crosslinked, released almost all of the loaded drug within three days. Crosslinking HA films with EDAC slowed the rate of drug release over the first three days of the drug release study. Representative data for 10% PTX loaded films are shown in Fig. 3. The addition of glycerol at 10% (w/w) had no effect on the release profile of PTX from any films (data not shown). The addition of 0.8 mM EDAC had very little effect on the release rate of PTX from HA films compared to non-crosslinked films. However, drug release was slowed in films crosslinked with 2 mM EDAC, which released approximately 50% of the loaded drug in 10 h compared to more than 70% released from either non-crosslinked or 0.8 mM crosslinked films. These films continued to release drug in a controlled manner over 48 h (Fig. 3). The use of 8 mM EDAC crosslinking caused a significant lag phase in the drug release from HA films which began at 10

Table II. Efficacy of 2 mM EDAC Crosslinked HA Films Containing Paclitaxel in the Rat Cecal Abrasion Model of Adhesion Formation

Group	Mean incidence[a] +/– SEM	% Animals with adhesions >grade 2	% Animals with no adhesions
Group 1 (n = 10) (surgical control)	0.8 +/– 0.2	60	30
Group 2 (n = 10) (control HA film)	2.5 +/– 0.5	80	20
Group 3 (n = 10) (1% paclitaxel in HA film)	0.7 +/– 0.3	30	50
Group 4 (n = 9) (5% paclitaxel in HA film)	0.2 +/– 0.1	11	78

[a] Mean incidence is the total number of adhesions observed in the group of animals divided by the number of animals in the group.

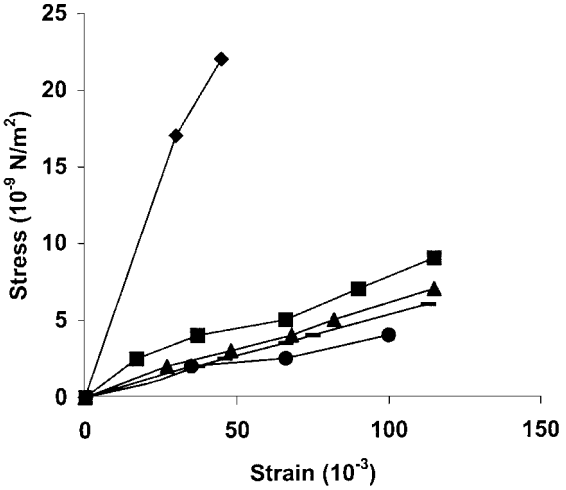

Fig. 1. Effect of the addition of glycerol on the stress:strain relationship for hyaluronic acid (HA) films containing glycerol at ♦, No Glycerol, ■, 10% (w/w) Glycerol; ▲, 20% Glycerol; –, 30% Glycerol; ●, 40% Glycerol.

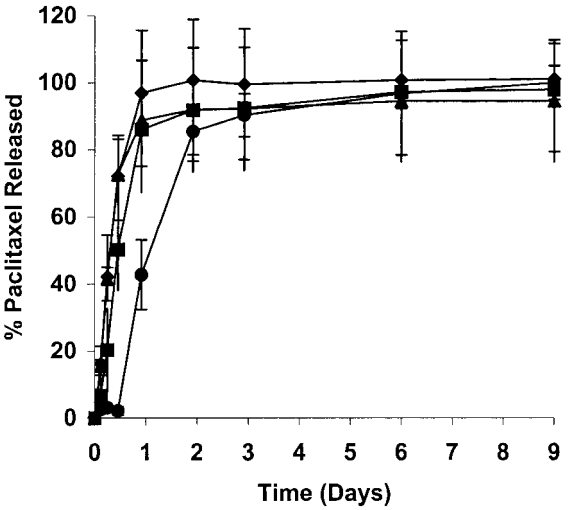

Fig. 3. Effect of EDAC crosslinking of HA films on the release rate of paclitaxel from the films. All films contained 10% glycerol, 10% paclitaxel and ♦, No EDAC; ▲, 0.8 mM EDAC; ■, 2 mM EDAC; ●, 8 mM EDAC.

h and continued for approximately 5 days as shown in Fig. 3. This delay in drug release from 8 mM EDAC crosslinked films was found to be dependent on the loading of PTX in the films as shown in Fig. 4. At a drug loading of 1%, there was no lag phase and the films released all encapsulated drug within 2 days. At a 10% drug loading there was a half day lag phase in drug release followed by a controlled release over 5 days. At a 5% drug loading, the release profile had 3 apparent phases, slow release for 24 h, rapid release over the following day and then very slow release over the next several days.

Due to the delayed PTX release from HA films at higher drug loadings, 2 mM EDAC crosslinking was used for all further studies. The cumulative release of PTX from 2 mM EDAC crosslinked films containing different drug loadings can be seen in Fig. 5. All these films had released all loaded drug within 2–3 days.

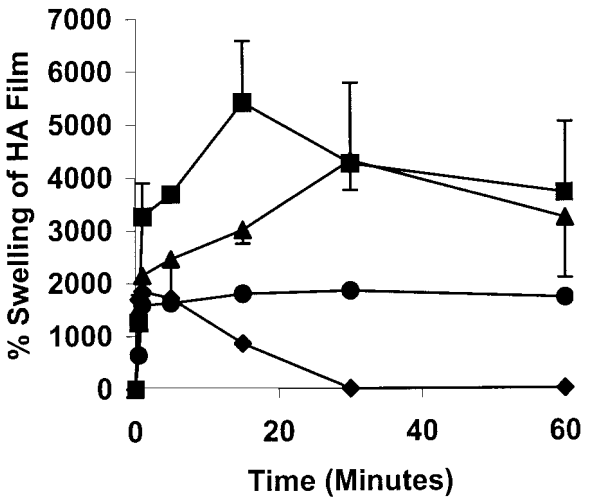

Fig. 2. Effect of EDAC crosslinking of HA films on % swelling of films as measured by water uptake. All films contained 10% glycerol and ♦, No EDAC; ■, 0.8 mM EDAC; ▲, 2 mM EDAC; ●, 8 mM EDAC.

Animal Studies

The surgeons performing the abrasion technique used a mechanical abrading device that is intended to give reproducible degrees of abrasion on the cecum. Within each experiment, the same surgeon performed all the abrasions to allow for uniformity of technique. No comparisons of the level of adhesion formation within similar animal groups in different animal experiments were made. The use of a single surgeon for a complete experiment and the use of larger animal groups (n = 10) within each experiment facilitated the collection of statistically relevant data.

Study 1: Intraperitoneal Taxol®

This study, using the Cremophor-EL formulation for PTX, was focused on an investigation of the pharmacological activity of PTX against adhesion formation and the effect of different treatment schedules on such activity. The Cremophor formulation has not been proposed as a system for de-

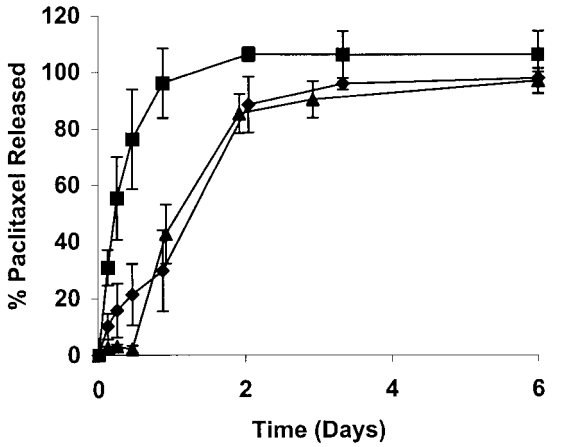

Fig. 4. Effect of paclitaxel loading in HA films on the release of paclitaxel. All films contained 10% glycerol, 8 mM EDAC and ■, 1% Paclitaxel; ♦, 5% Paclitaxel; ▲, 10% Paclitaxel.

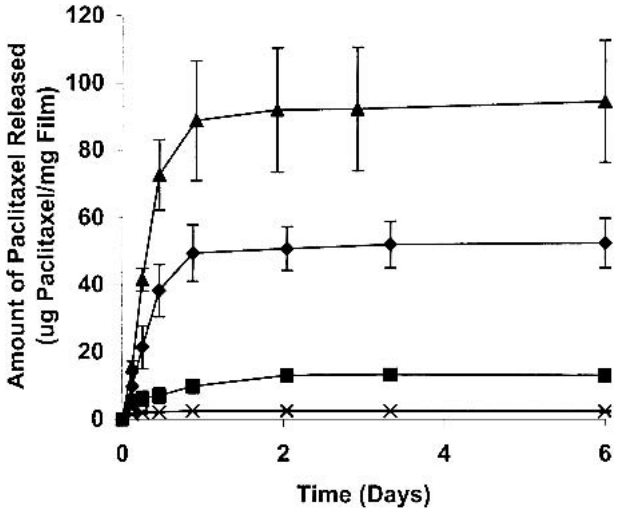

Fig. 5. Effect of paclitaxel loading on the amount of paclitaxel released from HA films. All films contained 10% glycerol, 2 mM EDAC and ×, 0.2% Paclitaxel; ■, 1% Paclitaxel; ♦, 5% Paclitaxel; ▲, 10% Paclitaxel.

livering the drug. Therefore, only the mean incidence of adhesion formation and the percent of animals with no adhesion were determined in this experiment. All animals in the control group had some degree of postsurgical adhesion formation following cecal abrasion. Animals receiving different schedules of intraperitoneal Taxol® treatment (Groups 1 to 3) all had a reduced mean incidence in adhesion formation and the percent (%) of animals with no adhesions increased as shown in Table I. The increases in the % of animals with no adhesions were all statistically significant from control. The percent of animals with no adhesions were: Group 1; 40% ($P = 0.025$), Group 2; 78% ($P = 0.0004$) and Group 3; 63% ($P = 0.0033$). There was no statistical difference between the increases within these groups. Animals in the control group had a mean incidence of adhesions of 1.6 (± 0.3). Animals in Groups 1–3, receiving Taxol®, all had a reduced mean incidence of adhesion formation. Compared to controls, these reductions were significant for Groups 2 and 3 ($P = 0.0045$ and $P = 0.019$, respectively) but not for Group 1 ($P = 0.2$). There was no statistical difference between the reductions in the mean incidence of adhesions between the three groups (Table I).

Study 2: Crosslinked HA-PTX Film Study

Sixty percent of animals in the surgical control group (Group 1-no treatment) had grade 2 (or higher) adhesions following cecal abrasion and only 30% of animals were adhesion free as shown in Table II. For control HA films (Group 2) the mean incidence of adhesion formation increased significantly in this group compared to surgical controls (Group 1) ($P = 0.0144$). Films loaded with 1% PTX (Group 3) were effective (compared to the control film-Group 2) in decreasing the mean incidence of adhesion formation ($P = 0.0132$) and in decreasing the % of animals with adhesions of grade 2 or higher ($P = 0.0246$). These films also increased the % of animals with no adhesions, although this increase was not statistically significant ($P = 0.1596$) (Table II). Five percent PTX loaded films were effective in reducing surgical adhesion formation in animals following cecal abrasion (Group 4). These films resulted in an increase in the % of animals with no adhesions and decreased the mean incidence of adhesion formation and the number of animals with grade 2 or higher adhesions as compared to both untreated animals (Group 1) or control membrane treated animals (Group 2). All these reductions in adhesion formation (Group 4 vs Group 1 or Group 2) were statistically significant.

While the 5% PTX loaded films demonstrated a significant reduction in postoperative adhesion formation, all of the animals in this group had excess fluid in the abdominal cavity at the time of necroscopy (3–5 ml on average). Normally an immeasurable amount of fluid is present coating the abdominal viscera. This finding was not observed in animals from other groups.

DISCUSSION

PTX is an antiproliferative, antiangiogenic agent that is approved for use against a number of cancers including ovarian and lung cancer (19). Since surgical adhesion formation also involves both proliferative and angiogenic processes, PTX was chosen as a potential pharmacological inhibitor for use against this disease in this study.

In cancer treatment, PTX is given systemically in a 50:50 Cremophor EL®:ethanol formulation (Taxol®). Using the rat cecal model of surgical adhesion formation, Taxol® was shown to have potent inhibitory effects when given daily at 4 mg/kg (Table I). These data demonstrated the efficacy of PTX in preventing postsurgical adhesion formation in rats The reason for the reduced efficacy in the pre-treated group, Group 1, (as compared to Groups 2 and 3) is not known. However, collectively, these data indicated that a 2–3 day PTX treatment schedule might be effective for a controlled release system of the drug.

The next phase of this study was to load PTX into a polymeric film formulation to provide localized, controlled release of PTX at the site of the surgical trauma. This would avoid both the needs for repeated intraperitoneal injections of the Cremophor PTX formulation and the associated toxicities, which have been reported for the Cremophor EL vehicle (20,21).

Bioresorbable membranes based on HA have been shown to possess suitable biocompatibility and mucoadhesive properties (11). The addition of 10% glycerol (w/w) allowed for the preparation of films with good flexibility and elasticity. However, when such films were placed in water, they completely dissolved within 30 m (see swelling data in Fig. 2.) making these films unsuitable as controlled release vehicles for PTX.

A number of methods have been previously reported to inhibit or prevent the dissolution of HA including derivatization to the ester form (22–24), hydrazide crosslinking (25,26) diepoxy crosslinking (27) or complexation with derivatized CMC (11,12). All these methods produce HA films or gels with significantly longer dissolution or degradation lifetimes. We employed a carbodiimide crosslinking method previously described by Tomihata *et al.* (28). The addition of the water soluble ethyl-3-(dimethylamino) carbodiimde (EDAC) to HA films was found to have a concentration dependent effect on the swelling and dissolution rate of HA films (Fig. 2). The rate of dissolution of films in water was measured gravimetri-

cally (data not included). In these studies, films crosslinked with 2 mM or 8 mM EDAC were observed to fully dissolve in one and two days respectively. The inclusion of PTX in films slowed the dissolution of the films. After 2 days in water, 2 mM EDAC crosslinked films containing 1%, 5% and 10% PTX had dissolved by 100%, 87% (±6%) and 74% (± 6%) respectively. Similarly, 8 mM EDAC crosslinked films containing 1%, 5%, and 10% PTX had dissolved by 100%, 56% (± 8%) and 67% (± 5%) respectively after this time.

It is interesting to note that HA is one of the components of a barrier system currently used to prevent adhesion formation. This product is called Seprafilm™ and is made of modified HA/carboxymethyl cellulose. These films have been found to be fully biocompatible and reasonably effective in reducing adhesion formation. The PTX-loaded HA films used in this study were not intended to constitute physical barriers to adhesion formation. HA was selected as a vehicle for the pharmacological intervention of adhesion formation, not as a physical barrier.

The release rate of PTX from films was reduced at degrees of crosslinking of 2 mM or greater. At 10% PTX loading, there was a significant lag phase of 10–12 h followed by controlled release. PTX was present as a solid dispersion within all HA/glycerol films. When films were observed using polarized optical microscopy PTX crystals could be observed in films containing 5% and 10% drug. Although most of these crystals were homogenously dispersed throughout the films, there was also evidence of crystal aggregation in films. This was particularly noticable in 10% PTX loaded films where aggregates as large as 100 to 200 μm were observed. Since there was little evidence of PTX crystals in 1% loaded films it was likely that at higher loadings of the drug the films became saturated with dissolved PTX resulting in extensive drug precipitation. In view of this, it is likely that the mechanism of drug release from the films involved uptake of water, swelling of the HA/glycerol matrix and dissolution of dispersed PTX. The PTX could be released from the film by a combination of diffusion and dissolution and erosion of the HA/glycerol matrix. Increasing the degree of crosslinking reduced the extent of swelling of the film and probably decreased the diffusion rate of drug through the matrix. More extensive crosslinking and the presence of PTX also increased the dissolution lifetime of the films thus reducing the rate of drug release from the films. The reason for the lag phase in the release profile at the higher PTX loadings of 5% and 10% and high crosslinking (8 mM) (Figs. 3 and 4) is poorly understood. However, it could be related to a reduced dissolution rate of drug due to either aggregation of PTX crystals or decreased wetting and water uptake into a highly hydrophobic PTX-loaded matrix.

It was previously hypothesized that to prevent surgical adhesion formation, a 2–3 day delivery of PTX to the site might be appropriate. This might allow for the inhibition of angiogenesis and cell proliferation without prolonged inhibition of normal wound healing. The 2 mM EDAC crosslinked films containing 10% glycerol released almost all loaded PTX over a two day period and were considered to be a suitable formulation for evaluation in the surgical adhesion model in rats. These films were used in all further studies. The amount of drug released over a two day period depended on the initial loading (Fig. 5).

Both 1% and 5% PTX loaded films were found to inhibit adhesion formation. Only 22% of animals treated with the 5% PTX loaded films showed any sign of adhesion formation (Table II). This treatment is equally as effective as barrier methods based on HA/CMC (such as SeprafilmTM), that remain in place for a week and reduce adhesion formation to between 20–24% (11,12), There was clearly no barrier effect exerted by the use of control 2 mM EDAC crosslinked films (PTX absent) since adhesion formation in the rats treated with nondrug loaded films was higher than in untreated animals. Since the crosslinked HA films used in this study are highly mucoadhesive it is possible that these films may have caused neighboring tissues to remain in close proximity until the films dissolved. Such an effect might actually enhance adhesion formation as observed in the control group. In animals treated with control films there was no evidence of a foreign body reaction to the films which might have caused an adhesion-like binding of tissues. There was no evidence of white blood cell infiltration, inflammation or capsule formation in any animals treated with these films. The biocompatibility of HA is well established and the films broke up and dissolved in a matter of days so that foreign body reactions would be most unlikely in these studies. There was no evidence of toxicity (such as listlessness or weight loss) or inhibition of wound healing observed in animals treated with PTX loaded films in this study. One animal in the 5% PTX treated group had an abscessed cecal perforation but this was considered to likely have resulted from a nick at the time of surgery. This animal was not included in the analysis in this study. There was evidence of excess abdominal fluid at the higher dose of PTX (5% PTX loaded films).

The data obtained from this study demonstrate the efficacy of PTX in preventing surgical adhesions. Furthermore, a controlled release film composed of HA/glycerol /PTX was shown to be a suitable formulation for application to surgical sites. However, none of the drug schedules used in this study (i.p. injection of Taxol® or PTX loaded films) fully eliminated adhesion formation in all animals. Future work in our laboratory is being directed towards optimizing the formulation of the films and maximally suppressing postsurgical adhesion formation.

ACKNOWLEDGMENTS

This work was supported by a grant from Angiotech Pharmaceuticals. We would like to thank Horton Middletoe in the GPR laboratory for his technical expertise in preparing the viscous hyaluronic acid solutions and for checking the mucoadhesive properties of the films used in this study.

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