Slow-Releasing Paclitaxel in Polytetrafluoroethylene/Polylactide-co-glycolide Laminate Delays Adjustment after Strabismus Surgery in Rabbit Model

Ho-Kyung Choung,1,2 Su-Eon Jin,2,5 Min Joung Lee,4 Chong-Kook Kim,5 and Jeong-Min Hwang5

PURPOSE. To determine the usefulness of slow-releasing paclitaxel in polytetrafluoroethylene/polylactide-co-glycolide (PTFE/PLGA) laminate for delayed adjustable strabismus surgery.

METHODS. A prospective, masked-observer, controlled study was performed in 25 rabbits. Fifty rabbit eyes were divided randomly into three groups. After recession of the superior rectus muscle (SRM), a PTFE/PLGA laminate containing paclitaxel, PTFE alone, or physiologic saline was applied beneath and over the SRM in the PTFE/PLGA-paclitaxel group (group paclitaxel), the PTFE group (group PTFE), and the control group, respectively. Delayed adjustment was performed by a masked observer once on each SRM at 3 or 5 weeks after surgery. Adjustment, adjustment lengths, forces required, and adhesion degrees were evaluated.

RESULTS. In the control group, adjustment was impossible in any eye at 3 or 5 weeks after surgery. In group PTFE, adjustment was possible in 5 of 8 eyes at 3 weeks after surgery and in 5 of 10 eyes at 5 weeks after surgery. In group paclitaxel, adjustment was possible in 6 of 9 eyes and in 7 of 7 eyes at 3 and 5 weeks after surgery. On comparing adjustability, a significant difference was observed between group paclitaxel and the control group (P = 0.016 and P = 0.001, respectively). A significant difference was observed between group paclitaxel and the control group (P = 0.003) in terms of adhesion between SRMs and sclera 5 weeks after surgery.

CONCLUSIONS. Slow-releasing paclitaxel in PTFE/PLGA was found to reduce adhesion and allowed delayed adjustment in most eyes for up to 5 weeks after surgery. (Invest Ophthalmol Vis Sci. 2008;49:5340–5345) DOI:10.1167/iovs.08-1694

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Adjustable suture strabismus surgery has become an effective method of adjusting binocular alignment during the immediate postoperative period.1,2 The ability to modify undercorrection or overcorrection and to lower the reoperation rate is the main advantage of adjustable suture strabismus surgery. However, binocular alignment may drift over time even after the eyes are placed in a suitable position by adjustable strabismus surgery.3 Therefore, delayed adjustment avoiding the effects of anesthesia and muscle injury after initial surgery may produce better postoperative results.4,5 However, the postoperative healing process causes adhesions that inhibit such delayed adjustment.

Efforts have been made to facilitate delayed adjustment by implanting physical barriers such as silicone,6 viscoelastic material,7 oxidized regenerated cellulose compound (Interceed TC7; Johnson & Johnson Medical, Arlington, Texas),8 polyglactin 910 mesh,9 polytetrafluoroethylene (PTFE; W.L. Gore & Associates, Flagstaff, AZ),9 or bioresorbable sheet (SurgiWrap; MacroPore Biosurgery, Inc, San Diego, CA),10 by using antiproliferative agents such as mitomycin C,11 or by using combinations of these various physical barriers and antiproliferative agents.12–14 Adjustment can be delayed with the use of silicone for up to 11 days in humans12 and for up to 8 weeks in rabbits.6 However, silicone can cause discomfort because of its thickness and rigidity and may trigger infection, extrusion, or granuloma formation. PTFE barriers have also been shown to allow delayed adjustment for up to 4 weeks and to have some advantages over other physical barriers.9,13 All barriers induced foreign body reactions and required frequent instillations of anti-inflammatory agents.

Poly (lactide-co-glycolide; PLGA) copolymers have been used for site-specific (local, including the eyes) and systemic long-term administration of controlled-release drugs.15 As one of the biodegradable polymers approved by the United States Food and Drug Administration for specific clinical use in humans, PLGA is the most commonly studied biodegradable polymer degraded by simple hydrolysis of the ester bonds into lactic and glycolic acid and removed from the body through normal metabolic pathways.16,17 Thus, this offers the possibility of a drug delivery system that allows the slow release of an anti-inflammatory or an antiadhesive drug at a sustained therapeutic concentration over several days or weeks. It has recently been reported that PTFE/PLGA containing dexamethasone or tramilast allow delayed adjustment up to 5 weeks in rabbit eyes.18 However, steroid agents may have adverse effects, such as elevation of intraocular pressure and formation of cataract, that can be clinically significant considering that the drug is released over several weeks.

Paclitaxel (Taxol; Sigma, St. Louis, MO) is a chemotherapy drug used for the treatment of breast, ovarian, lung, bladder, prostate, melanoma, esophageal, and other solid tumor cancers. It belongs to a class of chemotherapy drugs called plant alkaloids, which specifically attack cancer cells during various phases of division. Paclitaxel ultimately interferes with the growth of cancer cells and slows their growth and spread in
the body. At high concentrations, paclitaxel is cytotoxic and promotes apoptosis. At low concentrations, cells are inhibited predominantly in the G0/G1 and G1/G2 premitotic phases.19 As a consequence, the antiproliferative and antiinflammatory effects of paclitaxel are devoid of necrosis or apoptosis induction. In particular, local adaptation of low concentrations of paclitaxel reduces cell proliferation.20–25

We considered designing a system capable of continually releasing paclitaxel to prevent adhesions after strabismus surgery. In this experimental study in a rabbit model, we evaluated the effectiveness of controlled-released paclitaxel and PTFE for the prevention of postoperative adhesions that would allow a delay in adjustment after strabismus surgery without frequent instillation of a topical steroid.

MATERIALS AND METHODS

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital.

Twenty-five New Zealand White rabbits, each weighing 2 to 3 kg, underwent 5-mm recession of both superior rectus muscles (SRMs) with the use of double-armed, nonabsorbable 5-0 polyester (5-0 Ethibond; Ethicon, Somerville, NJ) sutures and were subsequently adjusted at 3 and 5 weeks after surgery. PTFE/PLGA containing sustained-release paclitaxel was used as a physical barrier in group paclitaxel, whereas PTFE not containing paclitaxel was used in group PTFE. In the control group, no physical barrier was used. Fifty SRMs were divided among three experimental groups as follows: control group (10 muscles; 5 for examination at 3 weeks and 5 at 5 weeks), group PTFE (20 muscles; 10 for examination at 3 weeks and 10 at 5 weeks), and group paclitaxel (20 muscles; 10 for examination at 3 weeks and 10 at 5 weeks). Two eyes of each rabbit were randomly assigned to three groups to minimize interrabbit variation.

Procedures

General anesthesia was induced by the administration of 30 to 45 mg/kg ketamine hydrochloride and 5 to 10 mg/kg xylazine hydrochloride intramuscularly, and topical anesthesia was achieved using propa- racaine hydrochloride. Muscle recession procedures with and without a physical barrier were performed as previously described.19

Polyvinylpyrrolidone-iodine was applied to the eyelids for preoperative surgical antisepsis. Limbal peritomy was performed from 10 to 11 o’clock. The eyeball was retracted using the contralateral globe, and the conjunctiva incision site was opened with the tip of a curved needle holder or scissors. The superior rectus muscle was dissected before conjunctival incision was opened with the tip of a curved needle holder or scissors. The superior rectus muscle was dissected before conjunctival incision was opened. The superior rectus muscle was dissected before conjunctival incision was opened.

Delayed Adjustment

In a masked random fashion, the SRMs were adjusted under anesthesia at 3 weeks or 5 weeks after surgery in all three groups. The delayed adjustment procedure was performed as follows. The previous conjunctival incision site was opened with the tip of a curved needle holder or scissors. The superior rectus muscle was dissected before adjustment, and the muscle was exposed to allow the experimenter much free movement as possible. After the SRM was fully exposed, a PTFE or PTFE/PLGA membrane was removed before adjustment. A dial tension gauge (DT-50; Tectol, Okaya, Japan) was used to precisely control the amount of force applied. The noose suture attached to the SRM was hooked using the bar of a dial tension gauge. The SRM was then moved anteriorly as much as possible. This adjustable distance was measured using a Castroviejo caliper, and the force required was measured using the gauge.

Preparation of Polymeric Laminate Fabrication

Poly (DL-lactide-co-glycolide) 75:25 (PLGA; average molecular weight, 90,000–126,000 Da; Sigma, St. Louis, MO) and PTFE laminate coated with drug-containing PLGA were manufactured with a solvent-casting method. Paclitaxel (94 mg) and PLGA (627 mg) were dissolved in 10 mL dichloromethane (Fisher Scientific, Suwanee, GA) with magnetic stirring for 20 minutes at room temperature. Paclitaxel-containing polymer solution was cast onto glass plates and PTFE laminate–coated glass plates. Cast polymer laminates were dried in the refrigerator for 24 hours at 40°C. PTFE and PTFE laminates coated with PLGA were peeled from the glass plates and cut into a defined size (1 cm × 2 cm). Drug concentrations in the laminates were approximately 1 mg/cm².

Determination of Paclitaxel Loaded in Polymer Laminate

Polymer laminates were dissolved in 2 mL acetonitrile (Fisher Scientific) and were vortexed for 1 minute. Polymer solutions obtained were then centrifuged at 10,000g for 60 minutes at 4°C to remove all alien materials. Acetonitrile (4.5 mL) was added to 0.5 mL of the supernatant and vortexed for 30 seconds. One hundred microliters of this solution was diluted to 900 μL with mobile phase. 100-μL aliquot was directly injected into a high-performance liquid chromatograph (HPLC), and the amounts of paclitaxel loadings were measured as described.

The HPLC system used was a Waters system (Alliance 2690; Waters, Milford, MA) with UV detector (2487; Waters) and a C18 reverse-phase column (5 μm, 250 mm × 1.5 mm; Capcell Pak MG; Nihon Shimazu, Kyoto, Japan) with column oven at 40°C. Paclitaxel was detected at 227 nm. Data were analyzed using specialized software (Masslynx; Micromass, Manchester, UK). The mobile phase consisted of 55% (vol/vol) acetonitrile and 45% (vol/vol) 2 mM phosphoric acid. The volume injected was 10 μL, and the flow rate used was 0.1 mL/min. Under these conditions, the linear calibration curve of paclitaxel was obtained in the concentration range 12.5 to 400 ng/mL (r² > 0.99).

In Vitro Release of Paclitaxel

Paclitaxel release from the polymer laminate was studied in phosphate-buffered saline (PBS). A polymer laminate sample (1 × 2 cm) was placed in 23 mL PBS (pH 7.4) containing 0.1% sodium azide as a preservative under sink conditions and was maintained in a circulating water bath at 37°C. At defined time intervals (0, 0.04, 0.25, 0.5, 2, 3, 4, 5, 7, 13, 20, 23, 30, 32, and 37 days), 200-μL aliquots of the medium were collected after magnetic stirring for 30 seconds and were replaced with an equal volume of fresh PBS filtered through a 0.45-μm membrane filter, and 10 μL solution was directly injected into the HPLC for paclitaxel quantification, as described.

Evaluation of Adhesions

At the time of adjustment, adhesions among muscle, PTFE or PTFE/PLGA membrane, sclera, and conjunctiva were evaluated and recorded. Adhesions were classified as SRM/C (superior rectus muscle/conjunctiva) or as SRM/S (superior rectus muscle/sclera) when located
above or below an SRM, respectively. Adhesion severities were scored from 0 to 4 according to criteria described in a previous report, where 0 = no adhesion, 1 = filmy adhesion easily separated with blunt dissection, 2 = mild to moderate adhesion with freely dissectible plane, 3 = moderate to dense adhesion with difficult dissection, and 4 = nondissectible plane. Animals were killed after delayed surgery adjustment by the intravenous administration of 10 mL sodium pentothal.

**Postmortem Histologic Examination**

Histopathologic studies were performed to evaluate inflammation and fibrosis among sclera, SRM, and conjunctiva. Involved tissues in each of three groups were examined macroscopically and microscopically using hematoxylin and eosin (H&E) staining and Masson trichrome staining after kill.

**Statistical Analyses**

Statistical analyses were performed using Fisher exact test to identify differences among three groups with respect to adjustability. Advancement distances and forces needed to advance SRMs were analyzed using the Mann-Whitney U test. For statistical analysis, adhesion severities were divided into low (score 0, 1, 2) or high (scores 3, 4) grade and were compared using Fisher exact test. Statistical significance was accepted for \( P \leq 0.05 \). When statistical significance was identified among three groups, post hoc pairwise comparisons adjusted by Bonferroni method were performed.

**RESULTS**

During the follow-up period, before adjustment, two eyes were lost because of the death of a single rabbit. The two eyes were incidentally included in group PTFE. Four eyes in group paclitaxel were excluded because of infection.

**In Vitro Release of Paclitaxel**

The release profile of paclitaxel from PLGA-coated PTFE film in vitro for 37 days occurred in a triphasic manner, with an initial lag release followed by a low-release phase and a high-release phase (Fig. 1). An initial lag release of paclitaxel was attributed to the poor solubility of paclitaxel. The second and third release phases of paclitaxel were probably explained by a combination of drug diffusion through the PLGA laminate and the hydrolytic degradation of PLGA. Paclitaxel (772.71 ± 227.07 ng/mL) was released for 37 days, and the value was the small quantity compared with the initial amount of paclitaxel. As previously mentioned, a low concentration of paclitaxel (<10 μM) enhanced the reduction of cell proliferation rather than the induction of cell necrosis or apoptosis. Thus, we predicted that our paclitaxel-PLGA laminate could not promote necrosis or apoptosis induction but could prevent cell proliferation during the postoperative period.

**Adjustability**

In the control group, adjustment was impossible in any eye at 3 and 5 weeks after surgery. In group PTFE, adjustment was possible in 5 of 8 eyes at 3 weeks after surgery and in 5 of 10 eyes at 5 weeks after surgery. In group paclitaxel, adjustment was possible in 6 of 9 eyes at 3 weeks and in 7 of 7 eyes at 5 weeks after surgery (Table 1). On comparing adjustability, a significant difference was found among three groups \( (P = 0.0014) \) at 5 weeks after surgery but not at 3 weeks after surgery \( (P = 0.593) \). Subsequent pairwise comparisons showed that the difference between group paclitaxel and the control group was significant at 3 and 5 weeks, respectively \( (P = 0.016 \text{ and } P = 0.001; \text{Bonferroni corrected}) \). Subsequent pairwise comparisons showed that the difference between group PTFE and the control group was significant \( (P = 0.024 \text{; Bonferroni corrected}) \) at 3 weeks but not at 5 weeks \( (P = 0.053) \) after surgery. No significant difference was observed between groups PTFE and paclitaxel at 5 weeks \( (P = 0.858) \), but there was a significant difference between the two groups \( (P = 0.026) \) at 5 weeks after surgery.

**Adjustment Amount and Force Required**

In group PTFE, average adjustment (advancement) amount and force were 2.10 mm and 54 g at 3 weeks after surgery and 2.35 mm and 50.8 g at 5 weeks after surgery. In group paclitaxel, the average adjustment amount and force were 3.17 mm and 47.33 g at 3 weeks and 2.64 mm and 54.29 g at 5 weeks after surgery.

**Table 1.** Adjustable Eye Numbers and Adjustment Tractional Forces and Distances

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (wk)</th>
<th>Adjustment Possible (No. eyes)</th>
<th>Adjustment Not Possible (No. eyes)</th>
<th>Force (g)</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PTFE</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>54.00 ± 15.03</td>
<td>2.10 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>50.80 ± 21.94</td>
<td>2.35 ± 0.70</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>47.33 ± 9.18</td>
<td>3.17 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>54.29 ± 15.55</td>
<td>2.64 ± 0.94</td>
</tr>
</tbody>
</table>

Force and length values are mean ± SD. None are shown for control because it was impossible to move the muscle given the adhesion. PTFE, polytetrafluoroethylene; paclitaxel, polytetrafluoroethylene/poly(lactide-co-glycolide) + paclitaxel.
surgery (Table 1). On comparing adjustment amount, a considerable difference was found between groups PTFE and group paclitaxel at 3 weeks after surgery but did not reach significance \((P = 0.052)\). For adjustment forces, no difference was found between groups PTFE and paclitaxel at 3 or 5 weeks after surgery \((P = 0.329\) and \(P = 0.876\), respectively).

Degree of Adhesion between SRM and Conjunctiva

No significant difference was observed among the three groups with respect to the degree of adhesion between SRMs and conjunctivae at 3 or 5 weeks after surgery \((P = 0.117\) and \(P = 0.185\), respectively). Pairwise comparisons revealed no significant differences between group PTFE and the control group, group paclitaxel and the control group, or groups PTFE and paclitaxel at 3 and 5 weeks after surgery (Fig. 2).

Degree of Adhesion between SRM and Sclera

A significant difference was observed among three groups at 5 weeks after surgery \((P = 0.088)\), but no significant difference was observed in terms of degree of adhesion between SRMs and sclerae at 3 weeks after surgery \((P = 0.482)\). Pairwise comparisons revealed significant differences between group paclitaxel and the control group \((P = 0.033;\) Bonferroni corrected) and between groups PTFE and paclitaxel \((P = 0.033;\) Bonferroni corrected) at 5 weeks after surgery, but no significant difference was observed between group PTFE and the control group \((P = 0.354)\), group paclitaxel and the control group \((P = 0.147)\), or groups PTFE and paclitaxel \((P = 0.963)\) at 3 weeks after surgery (Fig. 2).

Histologic Examination

Histologic examination using H&E staining showed some degree of inflammation with fibrosis around SRMs in the control group and in group PTFE at 3 weeks after surgery, and these were reduced at 5 weeks after surgery. Mild inflammation and fibrosis were observed around SRMs in group paclitaxel (Fig. 3). Masson trichrome staining also revealed that fibrosis was least prominent in group paclitaxel than in group PTFE or the control group. No specific toxic reaction was observed in group PTFE or group paclitaxel.

DISCUSSION

PTFE has been used as a graft material in several types of organ surgery, including ocular surgery, for many years and has many favorable characteristics for delaying adjustment.18,24–28 Between an expanded porous 1 mm-thick sheet and a 0.1 mm-thick nonporous surgical membrane, nonporous 0.1 mm PTFE is soft and flexible and can be applied to complex shapes without difficulty. Moreover, because of its thinness, it is well tolerated by patients and generally does not cause discomfort. Given these advantages, we considered nonporous 0.1 mm...
PTFE would be the most suitable nonabsorbable material choice for delaying adjustment.

Criteria for the ideal drug-release device for our purpose—sustained and uniform release, easy implantation, handling, and sterilization—are discussed in our previous paper.18,29

In the present study, a 0.1 mm–thick PTFE film coated with PLGA was used. PTFE coated with PLGA is easier to handle than PLGA film, which is difficult to manipulate because it tends to roll up and stick to itself.14

Our previous study suggested that PTFE alone might allow adjustment to be delayed for up to 4 weeks after surgery in 40% of rabbit eyes2 and that the combined use of PTFE and 5-fluorouracil, or the addition of viscoelastic solution (Viscoat; Alcon, Fort Worth, TX), could allow adjustment to be delayed for up to 4 weeks after surgery in 80% of rabbit eyes.13 Recently, PTFE/PLGA laminate containing tranilast also allowed adjustment to be delayed for up to 5 weeks after surgery in 80% of rabbit eyes.30

In this study, we evaluated adjustability at 3 weeks and 5 weeks after surgery based on a release profile of paclitaxel, which showed sustained release up to 5 weeks. Only group paclitaxel was significantly more adjustable than the control group at 3 and 5 weeks after surgery. In addition, group paclitaxel was significantly more adjustable than group PTFE at 5 weeks after surgery. Moreover only group paclitaxel showed significantly less adhesion between SRMs and sclera than the control group or group PTFE at 5 weeks after surgery. The present study produced favorable results without the need for frequent topical steroid instillation. These results demonstrate that PTFE/PLGA, which allowed sustained paclitaxel release, effectively reduced adhesion development and improved adjustability after strabismus surgery in our rabbit model.

Paclitaxel is a diterpenoid extracted from the bark of a rare, slowly growing Pacific yew or Western yew tree (Taxus brevifolia) first discovered in the early 1960s as part of a National Cancer Institute screening study to identify natural compounds with antineoplastic activity.31 The action mechanism of paclitaxel has been intensively investigated, and results have suggested that paclitaxel binds to β-tubulin, thereby inhibiting microtubule depolymerization in a dose-dependent fashion.32 Because microtubules are ubiquitous in the cytoplasm and nuclei of most cells, this mechanism affects numerous cell types and processes, including cellular division and motility, secretory processes, and signal transduction pathways. Paclitaxel aids polymerization of tubulin dimers to form microtubules and thus stabilizes the microtubules. The microtubules formed because of paclitaxel action are stable and thus dysfunction, leading to cell death.33–35 At high concentrations, paclitaxel is cytotoxic, exerts cell cycle arrest in the G2/M phase, and promotes apoptosis. Conversely, cells are inhibited predominantly in the G2/M phase and G1/G2 premiotic phases at low concentrations.19 As a consequence, the antiproliferative and antimigratory effects of paclitaxel are devoid of necrosis or apoptosis induction. In particular, paclitaxel locally adapted at low concentration reduces cell proliferation.20–23

The release profile of paclitaxel from PLGA-coated PTFE film showed sustained release up to 5 weeks at a very low concentration. Thus our paclitaxel-PTFE/PLGA laminate could not promote necrosis or apoptosis induction but could promote cell proliferation in the postoperative period.

Paclitaxel has also been evaluated in some ocular diseases and has especially demonstrated antiproliferative effects in rabbits. It is reported that paclitaxel is useful in the treatment of experimental proliferative vitreoretinopathy by reducing the incidence of tractional retinal detachments36,37 The use of paclitaxel powder at the conclusion of filtration surgery improved the outcome of the surgery as measured by the magnitude of intraocular pressure lowering and the duration of surgical success.38 No toxic drug effect was observed.

Delaying adjustment allows the surgeon a better idea of the stabilization of a patient’s motility. Hwang (Hwang JM, unpublished data, abstract presented at 24th Annual Meeting of the American Association for Pediatric Ophthalmology and Strabismus, March 1998) showed that in humans, delayed adjustable strabismus surgery is possible with the use of a thin polytetrafluoroethylene plate, and Shokida et al.39 found that delayed adjustment surgery using a silicone sheet produced better results in patients with exotropia who underwent reoperation than in patients who underwent immediate adjustment. Results of the present study suggest that paclitaxel-releasing PTFE/PLGA may be useful in delaying adjustment up to 5 weeks.

In conclusion, we describe a new drug and a new drug delivery system based on the extraocular sustained release of paclitaxel from a PTFE/PLGA film. This system provided significantly better adjustability in delayed suture strabismus surgery in rabbit eyes for up to 5 weeks after surgery. Moreover, this study demonstrates for the first time the effects of paclitaxel on delayed suture strabismus surgery.

References


