Polylactic Acid for Visualizing the Vitreous Body during Vitrectomy

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PURPOSE. To investigate the possibility of using polylactic acid (PLA) as a surgical adjuvant for visualizing the vitreous body during vitrectomy.

METHODS. After a core vitrectomy, 1 mL of PLA suspension was injected into the rabbit vitreous in two groups: group A, 2.5% PLA (n = 5), and group B, 1% PLA (n = 9). Vehicle injection instead of PLA was used as a control (group C, n = 5). The clinical signs and electroretinogram (ERG) were evaluated for 28 days, and histologic findings were evaluated on day 28. Next, intraocular pressure (IOP) after intracameral injection of a PLA suspension was evaluated in the rabbits (n = 6). Last, the visualization of the vitreous body by PLA suspension was evaluated during vitrectomy in monkey eyes (n = 4).

RESULTS. The white granules of PLA disappeared from the vitreous cavity in 10 eyes within 3 weeks; however, a small amount of PLA remained in four eyes for 4 weeks. Mild inflammation of the anterior chamber was observed in one eye in group B and 1 eye in group C. No cataract or retinal hemorrhage was found in any eyes. The amplitude of ERG on each time point did not differ between the groups. IOP remained within normal range except for the initial spike. Retinal structure was well preserved histologically. During vitrectomy in monkey eyes, the vitreous body was well visualized, and the posterior vitreous separation was performed easily and safely.

CONCLUSIONS. PLA can be a new surgical adjuvant to visualize the vitreous body during vitrectomy. (Invest Ophthalmol Vis Sci. 2007;48:3277–3282) DOI:10.1167/iovs.06-1020

...pars plana vitrectomy (PPV) has been established as the treatment of various vitreoretinal diseases, such as proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR). The major goal of PPV is to remove the vitreous or fibrovascular membrane from the retina, and the failure of this procedure sometimes results in severe complications.1 Despite the development of various surgical techniques and instruments, removing the vitreous is an uncertain procedure because the vitreous is transparent.

Recently, Peyman et al.2 and we3 reported that intraocular injection of triamcinolone acetonide (TA) during vitrectomy allows visualization of the transparent vitreous, which helps greatly in the complete separation of the posterior vitreous from the retina. This method is effective for diabetic macular edema, proliferative vitreoretinopathy, uveitis, and other disorders, and the procedure is comparatively safe.4–7 Regardless of that, there is still concern about the intravitreous use of a steroid agent. Moshfeghi et al.8 reported that endophthalmitis after intravitreous TA is not uncommon. Considering the high standards of patients today, TA may not be suitable as a surgical adjuvant for visualizing the vitreous body.

Polylactic acid (PLA) is a biodegradable substance without any biological effect. In 2004, the U.S. Food and Drug Administration approved a poly-L-lactic acid (PLLA)-based injectable medical device for restoration and/or correction of the signs of facial fat loss in people with human immunodeficiency virus.9 As a result, the properties of the PLA microparticles have attracted considerable interest in the medical community. At the same time, PLA has been used in orthopedic surgery and its safety has been reported.10 PLA granules are originally white and adhere to the gel-like structure so easily that they assist in visualizing the transparent vitreous as do TA granules but with less-harmful consequences.

In the present study herein, we examined the effect of intravitreous PLA on the ocular tissues of rabbit and monkey eyes and investigated the possibility of PLA as a surgical adjuvant for visualizing the vitreous body.

MATERIALS AND METHODS

All the animal experiments were approved by each institutional review board of Kagoshima University, Kyushu University, and Senju Pharmaceutical Co., Ltd., in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals

For the safety study, Japanese White rabbits (male; age, 9–10 weeks; weight, ~2 kg) were purchased from Kitayama Labes Co., Ltd. (Nagano, Japan). Cynomolgus monkeys (Macaca fascicularis; male; age, 4 years; weight, ~3.5 kg) were purchased from Hamri Co., Ltd. (Ibaraki, Japan). For the measurement of intraocular pressure (IOP), Dutch belted rabbits (male; age, 15 weeks, weight ~2 kg) were purchased from Bio-Tech Co., Ltd. (Saga, Japan).

Preparation of PLA Granules

PLA was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The PLA granules were prepared by a freeze-dried method. Briefly, a solution of PLA in acetone and EtOH was poured into polyvinylpyrrolidone (PVP) and mannitol solution in water with stirring, and the mixture was freeze dried to obtain the PLA granules. PVP
and mannitol were purchased from BASF Japan Ltd. (Tokyo, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan), respectively.

The PLA sample was prepared for observation of the size and surface structure by scanning electron microscope (S-2150; Hitachi, Tokyo, Japan). Briefly, the PLA granules were freeze dried and critical-point drying was performed (HCP-2; Hitachi), and sputtered (E-101; Hitachi) with gold of 20-nm thickness. The sample was studied by scanning electron microscope and a micrograph was obtained.

Intravitreous Injection for Safety Study

For the animal study, PLA granules were mixed with vehicle solution (containing 0.13 mM NaCl, 21.5 mM KCl, 7 mM Na2HPO4·12H2O and AcOH; pH 7.0) and 2.5% or 1.0% PLA suspension was formed. Vehicle solution was used as the control.

The right eyes of 19 rabbits were used in the following study. The rabbits were anesthetized with intramuscular injection of ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (10 mg/kg). The pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride eye drops (Santen Pharmaceutical Co., Ltd., Osaka, Japan). The sclera was exposed and a 1-mm sclerotomy was made with a disposable ophthalmic knife 2 to 3 mm from the limbus by using an operating microscope (Carl Zeiss Meditec, Inc., Oberkochen, Germany).

After core vitrectomy, the suspension of 2.5% PLA (n = 5), 1% PLA (n = 9), or vehicle (n = 5) was injected into the vitreous (1 mL to each eye). Then, the sclerotomy and overlying conjunctiva were closed with medical glue for surgery and topical 0.3% gatifloxacin hydrate (Senju Pharmaceutical Co., Ltd.) was applied to the eyes of each animal, to minimize the risk of infection after surgery. Then, clinical signs were observed with a slit lamp biomicroscope (Kowa Co., Ltd., Tokyo, Japan), and fundus observation was performed by using a binocular indirect ophthalmoscope (Neitz Instruments Co., Ltd., Tokyo, Japan) over 28 days. In addition, electroretinogram (ERG) was examined in each eye before surgery and on days 7, 14, and 28, according to our previously described method.11 Ganzfeld ERG was recorded with evoked response recorder (Neuropack II; Nihon Kohden, Tokyo, Japan). The amplitudes of the a- and b-waves are expressed as the percentage of the amplitude in each eye before surgery.

A histologic study was performed in each eye by a standard light microscopic method. Briefly, after the animals were euthanatized by intravenous injection of 5% sodium pentobarbital, the eyes were enucleated and fixed with 1% glutaraldehyde and 4% formaldehyde in 0.1 M of phosphate buffer (pH 7.0–7.5) for 24 hours and embedded in paraffin. Paraffin-embedded sections were cut at ~3 μm and stained with hematoxylin and eosin. The randomly selected sections were examined by the observer in a masked fashion.

Intraocular Pressure

Since PLA granules can increase IOP by plugging the trabecular meshwork, the PLA suspension was injected into the anterior chamber and IOP was monitored to simulate an extreme clinical case.

Indomethacin (50 mg/kg body weight; Wako Pure Chemical Industries, Ltd.) was intraperitoneally administered 1 hour before the surgery, to reduce postoperative inflammation of the anterior chamber. For the surgery, the ocular surface was anesthetized with topical instillation of 0.2% oxybuprocaine hydrochloride eye drops (Senju Pharmaceutical Co., Ltd.). After the aqueous humor was withdrawn at the equivalent volume of PLA suspension by paracentesis, 2.0% PLA suspension was injected into the anterior chamber (1.3 mg/eye; n = 5). Then, IOP was measured by applanation tonometry over 28 days. As the control, no treated eyes (n = 5) were set. The eyes that received 2.0% PLA were enucleated and examined histologically by the method described in the previous section.

Intraoperative Use of PLA for Monkey Eyes

The usefulness of PLA as a surgical adjuvant was evaluated during vitrectomy in monkey eyes (n = 4). All surgical procedures were performed in animals under general anesthesia. General anesthesia was induced with 15 mg/kg intramuscular ketamine and 1.5 mg/kg intramuscular xylazine, along with a single subcutaneous injection of atropine sulfate (0.05 mg/kg). The animals were intubated and breathed 100% oxygen. Heart and pulse rates and arterial oxyhemoglobin saturation were monitored continuously. Body temperature was maintained at 37°C with a warm-water heating pad. The pupils were fully dilated with 1.0% tropicamide and 2.5% phenylephrine.

The conjunctiva, lids, and lashes were disinfected with 10% povidone iodine (Isodine; Meiji Seika, Tokyo, Japan). Each eye was then

**FIGURE 1.** Scanning microscopic image of PLA granules showing a spheroid to crystal-like shape with a gritty surface.

**FIGURE 2.** Photographs of rabbit eyes 7 days after intravitreous injection of a PLA suspension. There was no serious inflammation in the anterior chamber (A). Some PLA granules were found beneath the lens, but no cataract was observed (B, arrow).
completely draped, and an eyelid speculum was inserted. After excision of the conjunctiva, three sclerotomies were formed 1.5 mm from the limbus, and a standard three-port pars plana vitrectomy was performed. After the core vitrectomy, intravitreous injection of 2.5% PLA suspension was performed on the area of interest. The vitreous body with PLA granules was then removed with a vitrectomy probe. The intraoperative observation by surgical microscope (Carl Zeiss Meditec, Inc.) was recorded on video tape and evaluated by two examiners after the operation.

After removing the vitreous body with PLA granules as much as possible, the wound was closed with 6-0 Vicryl. The conjunctiva was also closed. Antibiotic ointment was applied at the end of surgery and antibiotic eye drops were applied three times a day for 1 week. Clinical observation was performed for 1 month. After that, the animals were euthanatized, and the eyes were enucleated and placed directly into 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M of phosphate buffer. Half of the eye was used for light microscopic examination, and the other was for transmission electron-microscopic examination. The tissue for electron microscopy was postfixed in osmium, dehydrated, embedded in plastic, thin sectioned, stained with uranyl acetate-lead citrate, and examined and photographed with an electron microscope.

**Statistical Analysis**

Data are expressed as the mean ± SEM. Statistical significance between the control and PLA-injected groups was determined with the two-tailed t-test. \( P < 0.05 \) was considered significant.

**RESULTS**

**PLA Granules**

After PLA was mixed with the vehicle solution, the PLA granules dispersed within a few minutes. By scanning electron microscopy, each PLA granule had a spheroid- to crystal-like shape with a gritty surface (Fig. 1). The average diameter of each granule was approximately 20 \( \mu \)m, ranging from 5 to 70 \( \mu \)m, as measured by a laser diffraction particle size analyzer (SALD-2100; Shimadzu Co., Kyoto, Japan).

**Intravitreous Injection for Safety Study**

**Clinical Findings.** Conjunctival injection and/or edema was observed in all eyes after surgery and disappeared gradually within 2 weeks. Mild to minimal inflammation was observed transiently in some eyes: one eye in the control group, one eye in the 1.0% PLA group, and no eyes in the 2.5% PLA group. No serious inflammation was noted in the other eyes (Fig. 2A). Although PLA was observed on the posterior retinal surface in one eye (Fig. 2B, arrows), no cataract was found in any eyes. In the vitreous cavity, a white, cloudy structure was visible in all the eyes that received PLA injection. It gradually and completely disappeared in 10 of 14 eyes within 3 weeks: 7 eyes in the 1.0% group and 3 eyes in the 2.5% PLA group (Fig. 3); however, a small amount of PLA remained in two eyes of the 2.5% PLA group.

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each group even after 28 days. No retinal hemorrhage or exudate was found in any eyes throughout the period.

**Electroretinogram.** The percentage of a- or b-wave amplitude in each eye in relation to that before treatment is depicted in Figure 4. ERG a-wave amplitudes declined after 7 days and began to increase gradually thereafter (Fig. 4; top row). There was no significant difference between the groups at any time point. The average amplitude of the b-wave (Fig. 4, bottom row) increased over time similarly in the three groups (2.5% PLA, 1% PLA or control), but there was no statistically significant difference between any of the groups at each time point.

**Histologic Study.** The retinal structure was well preserved in every specimen and neither inflammatory infiltrate nor degenerative change was observed in any of three groups (Fig. 5).

**Intraocular Pressure**

There was no significant difference in baseline IOP between the groups. After injection of PLA intracamerally, the half volume of the anterior chamber was filled with white PLA granules. IOP rose transiently as high as 40 mm Hg 1 hour after the injection and then lowered gradually (Fig. 6A). Subsequently, IOP was 7 to 15 mm Hg for several days and gradually returned to the original level. After 14 days, IOP did not differ in each group significantly (Fig. 6B). Through the observation period, intracameral inflammation was slight or nonexistent, and the cornea was sound and clear. A histologic study showed no pathologic changes such as inflammation or degeneration in the eyes with intracameral injection of PLA (Figs. 6C, 6D).

**Intraoperative Use of PLA in Monkey Eyes**

Immediately after injection, PLA granules adhered to the surface of the posterior vitreous, which caused the vitreous body to be visualized clearly as a white membrane (Fig. 7). Without PLA, the membrane was not identifiable. Surgical posterior

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**FIGURE 5.** Light micrograph of rabbit eyes on day 28. The retinal structure was well preserved and neither inflammatory infiltrate nor degenerative change was observed in any of three groups. Hematoxylin and eosin. Original magnification, ×50.

**FIGURE 6.** Results in rabbit eyes with injection of PLA. After intracameral injection of PLA, IOP increased to a maximum after 1 hour and then decreased gradually (A). Subsequently, IOP was ~10 mm Hg for several days and returned to the original level. After 14 days, IOP did not differ significantly in each group (B). Histologic study showed neither inflammation nor degeneration in any ocular tissue, such as the cornea (C), trabecular meshwork, iris, or ciliary body (D). Hematoxylin and eosin. Original magnification: (C) ×25, (D) ×10.
Vitreous detachment was performed easily and safely. The vitreous body with PLA was removed with surgical forceps. Floating granules were removed by suction with a vitrectomy probe, and only few granules were left at the end of the vitrectomy. The granules disappeared completely within 7 days in all four eyes. No abnormal inflammation of the anterior segment or cataract was observed. The examination of ocular fundus did not show any pathologic change such as retinal hemorrhage, detachment, or exudate for 4 weeks. Light microscopic study showed that the retinal structure was well preserved, and no inflammatory cell infiltration was found (Fig. 8). Electron microscopic study showed that the intracellular structure of retinal cells was well preserved. The vitreoretinal border and internal limiting membrane were also intact (Fig. 8).

**Discussion**

In this study, the rabbit eyes received intraocular injections of PLA suspension and clinical, functional, and histologic examinations were performed for 4 weeks thereafter. Furthermore, the visualization of the vitreous body during vitrectomy was evaluated in monkey eyes.

Without question, the most greatest concern in using a new substance in the clinical field is safety. Clinical examinations showed no serious adverse events. Minimal to mild inflammation was noticed in some eyes, but it resolved soon spontaneously. PLA granules were found for several days but disappeared over time, and no inflammation was found even in the surrounding tissues. In histologic study, there was not any significant change in the examined eyes. In the functional study by ERG, the amplitude of the a-wave decreased after 7 days and gradually returned to baseline over time, whereas the average amplitude of the b-wave increased slightly over time. We could not determine why these changes occurred; however, the similar trend of these ERG changes in PLA and control groups suggests that it did not have anything to do with the use of the PLA suspension, but was caused by other factors (e.g., the surgical method itself).

PLA granules injected into the anterior chamber affected the IOP, but not when injected into the vitreous, in our preliminary study (data not shown). Thus, an unusually large amount of PLA granules was injected into the anterior chamber instead of the vitreous cavity, to simulate extreme clinical cases. As a result, the IOP decreased mildly after a spike of 1 hour and gradually returned to the original level with no additional treatment. The initial spike was probably due to the effect of the granules’ sticking to the trabecular meshwork. The exact cause of the decrease of IOP was unclear, but it is important to mention that the IOP remained within the normal range after the initial spike. Therefore, the effect of intravitreous or intracameral injection of PLA on IOP is thought to be minimal. What is more important, unlike silicone oil tampon-
ade or drug injection into the vitreous, most of the PLA granules used as a surgical adjuvant will be removed at the end of the surgery, although in the present study the PLA granules were not removed, which simulated unusually extreme cases. Considering these results, it is safe to say that intravitreous or intracameral PLA granules apparently were not toxic to the rabbit eyes.

PLA has been used clinically for various purposes. For example, it has been used widely in more than 30 countries throughout the world to treat a variety of facial volume and contour deficiencies, with no toxic effect.12 PLA has also been used for substance for controlled release of hormone antagonist.13 Furthermore, in ocular treatment, PLA has been used as a carrier of drugs for the treatment of various vitreoretinal diseases in animals, and in those studies PLA has been shown to be biologically silent.14,15 These results are compatible with those in the present study.

To evaluate clinical usefulness, PLA granules were tested during vitrectomy in monkey eyes. The results showed that PLA granules aided in visualizing the posterior vitreous clearly and vividly, which greatly facilitated the surgical procedure. The usefulness of TA granules as a PPV surgical adjuvant has been reported, with its major role being to enable visualization of the vitreous body. At this moment, it is difficult to conclude whether PLA granules are superior to TA, but the present problems with IOP and ocular infection caused by intraocular TA seem to indicate that PLA granules would be a good alternative.

References