Toxicity of a Biodegradable Microneedle Implant Loaded with Methotrexate as a Sustained Release Device in Normal Rabbit Eye: A Pilot Study

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Abstract

Purpose: Primary intraocular lymphoma is a term that refers to nonmetastatic malignant lymphoid neoplasia that arises primarily within the eye. Primary vitreo-retinal lymphoma (PVRL), a subtype of primary intraocular lymphoma that comprises at least 85% of cases, provides a therapeutic challenge because of its diverse clinical presentations and variable clinical course. One of the currently available treatment options for PVRL is intravitreal injection of methotrexate. To achieve and maintain sufficient therapeutic levels of methotrexate in the eye to eradicate PVRL, the patient must undergo multiple intravitreal injections with attendant potential toxic peaks and sub-therapeutic troughs of intraocular drug concentrations. In this pilot study, we investigated the intravitreal concentration of methotrexate over time, and toxicity associated with slow sustained release of the drug from a biodegradable device containing methotrexate implanted in a deep scleral pocket of the eyes of normal rabbits.

Methodology: Biodegradable microneedle implants (~8 mg) loaded with 10%wt of methotrexate were fabricated using solvent cast method. All the implants were inserted surgically in a deep lamellar scleral pocket created in each eye of 3 albino New Zealand rabbits. The left eye received a placebo implant, and the right eye received an implant loaded with methotrexate. Postoperatively, the animals were monitored regularly for complications related to the surgery, implant, or drug. The animals were sacrificed 4 weeks after the surgical implantation, and the eyes were enucleated. The eyes were studied histopathologically to look for evidence of inflammation related to the implants and toxicity related to the implant or drug.

Results: The biodegradable microneedle methotrexate implants were inserted successfully into deep lamellar scleral pockets of the rabbits without any intraoperative or postoperative complications. Histopathologic examination of the medicated (methotrexate implant) and nonmedicated devices (placebo) showed no evidence of drug toxicity. Also, no major differences were apparent between the eyes as well as no acute ocular inflammation or infection was evident around the implantation site.

Conclusions: This sustained release implant containing methotrexate proved to be nontoxic histopathologically and well-tolerated in the eyes of normal rabbit.

Introduction

Primary intraocular lymphoma (PIOL) is a term that refers to nonmetastatic malignant lymphoid neoplasia that arises primarily within the eye. It is to be differentiated from intraocular malignant lymphoid infiltrates and tumors that occur metastatically from a primary systemic non-Hodgkin’s lymphoma. An estimated 100 new cases of PIOL were diagnosed in the United States between 2000 and 2005, with a slight male predominance.1,2 PIOL occurs in 3 major clinical forms: (1) primary vitreo-retinal lymphoma (PVRL), (2) primary uveal lymphoma, and (3) PIOL with overlap features (i.e., both vitreoretinal and uveal features).3 PVRL comprises at least 85% of cases of PIOL encountered in the United States. This form of lymphoma represents intraocular involvement by primary central nervous system lymphoma (PCNSL). Approximately 80% of patients found to have PVRL eventually develop malignant lymphomatous lesions in the brain and lymphomatous cellular infiltrations of the cerebrospinal fluid.4 The central nervous system lesions associated with PVRL may precede, occur concurrently with, or follow detection of...
the intraocular lesions. PVRL and PCNSL appear to be independent primary sites and are not considered to be metastatic from one to the other. In studies where adequate tissue has been obtained, immunocytopathologic analysis of PVRL has usually shown the neoplasia to be large cell, B-cell non-Hodgkin’s lymphoma.5 PVRL presents most commonly as an ocular disorder that simulates intermediate or posterior uveitis with pale, finely dispersed intravitreal lymphoid cells and/or geographic creamy yellow accumulations of neoplastic lymphoid cells beneath the retinal pigment epithelium. Bilateral involvement occurs in up to 80% of patients with PVRL.6 This disorder may affect one or both eyes multicentrically but can be grossly asymmetric or sequential rather than concurrent and symmetric.

PVRL provides a therapeutic challenge because of its diverse clinical presentations and variable clinical course. Initial treatment for PVRL depends on whether one or both eyes are involved, whether the brain is also involved at that time, and whether and how prior PCNSL (if any) had been managed. The 2 principal treatment options for PVRL/PCNSL are fractionated external beam radiation therapy to both eyes and the whole brain simultaneously and intravenous chemotherapy (with or without concurrent intrathecal chemotherapy).5,7 In recent years, some eyes with PVRL (especially ones with recurrent intraocular lymphoma after prior intravenous chemotherapy or ocular irradiation but also some eyes of patients without concurrent demonstrable active PCNSL) have been treated by a course of intravitreal injections of methotrexate.8-11 For optimal treatment of PIOL, therapeutic levels of methotrexate need to be maintained in the eye for a prolonged period while avoiding toxic and sub-therapeutic levels. Because the half-life of methotrexate is of the order of only a few hours, frequent injections are generally required to achieve prolonged cytotoxic levels.8,12 These repeated administrations can be associated with ocular complications such as endophthalmitis, cataract, vitreous hemorrhage, and rhegmatogenous retinal detachment.5,10,13,14

A variety of sustained release drug systems, including ones consisting of liposomes, microspheres, or implants made of biodegradable and nonbiodegradable polymers, have been investigated for treatment of eye diseases.15-20 A well-tolerated controlled drug release device has the potential to maintain a sufficient concentration of drug in the eye for a period of time sufficient to eradicate the target disease while avoiding undesirable and toxic peak concentrations of the drug.16,21,22 Sustained-release implants have been developed and approved by the U.S. Food and Drug Administration (FDA) for delivery of prolonged therapeutic levels of ganciclovir in patients with cytomegalovirus retinitis and the acquired immune deficiency syndrome; other devices have been developed and approved for delivery of triamcinolone acetonide in patients with severe or chronic nonmicrobial intraocular inflammation.15,16 Our group designed a new type of biodegradable intraocular implant loaded with methotrexate for intended treatment of selected patients with PIOL. In this article, we describe the design and fabrication of this implant and report our experience with this implant in a normal rabbit eye.

**Materials and Methods**

In this section, we describe the design and fabrication of our biodegradable implants loaded with methotrexate, the implantation procedure employed in our study animals, and the clinical and pathologic studies performed to evaluate the safety of these devices.

**Design and device fabrication**

The process for the fabrication of a biodegradable polymeric implant to potentially deliver methotrexate is based on a micromolding technique from high-aspect-ratio poly (dimethylsiloxane) (PDMS; Sylgard 184; Dow Corning). First, the cycloolefin copolymer was laser drilled and the steel needles were fitted into the holes to create the master structure. Second, the microneedle master structure was coated with PDMS, which was subsequently peeled off to make an inverse mold. This PDMS mold was used to fabricate polymeric microneedle implants by using the solvent cast method. The design of the microneedle implant was adapted from our previous study.24 The 9-needle biodegradable methotrexate implant weighed ~8 mg and was ~2 mm in length, 2 mm in width, and 2.3 mm in height (Fig. 1). The tips of the microneedles were beveled at an angle of about 30° to facilitate their intended penetration through partial thickness sclera and into the underlying uvea.

Poly (d,l-lactide) (PLA) with a high weight-average molecular weight of 90,000 (PLA-90,000) and a low weight-average molecular weight of 6,500 (PLA-6,500) was purchased from Lactel Biodegradable polymers. To prepare the sustained release methotrexate implants, the solution of PLA-90,000/PLA-6,500 (blend ratio; 80/20) and 10% wt of methotrexate (Sigma-Aldrich) dissolved in N-Methylpyrrolidone (HPLC grade) solvent was cast in the PDMS mold. Negative pressure was applied to PDMS mold to load the solution into the needle part, and then the implant was dried for 3 days. To increase the mechanical strength of the implant, the solution in the PDMS mold was heated to glass transition temperature. The placebo implants (polymers without drug) were prepared in a similar fashion. In previously published literature, methotrexate and the biodegradable polymer (PLA) have been used in isolation in a normal rabbit eye. The main goal for this pilot study is to evaluate the safety and toxicity of the biodegradable PLA implant loaded with the methotrexate in a normal rabbit eye.

**Device implantation**

All animals were treated in accordance with the Institutional Animal Care and Use Committee protocol (University of Cincinnati) for the use of animals in the current research. In this pilot study, we used 3 New Zealand white rabbits.
weighing 2–3 kg each. An implant containing methotrexate was placed surgically in a deep lamellar scleral pocket in the right eye, and a placebo device was placed in a comparable deep lamellar scleral pocket in the left eye of each rabbit using the surgical method described below. Our hypothesis is that there will not be any significant differences between the right eyes that received the methotrexate implants versus the left eyes that received the placebo implants. All implantation procedures were performed by the same author (Z.M.C.).

Before anesthesia administration, both pupils of the animals were dilated using two drops of phenylephrine hydrochloride 2.5% and 2 drops of tropicamide 1%. The rabbits were anesthetized with a mixture of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg). After anesthesia, a 24-gauge angiocatheter was inserted into the marginal ear vein to facilitate antibiotic administration; the pulse, temperature, and respiration rate were monitored to ensure sufficient anesthesia and satisfactory vital signs. The procedure started with the aseptic preparation and sterile draping of both eyes. The procedure was essentially the same in both eyes starting with the right eye. An eyelid speculum was inserted between the eyelids to expose the eye. A limited conjunctival peritomy was performed in the superotemporal quadrant, and the subconjunctival connective tissues were dissected down to bare sclera in that quadrant (Fig. 2). Using a surgical blade, a square-shaped fornix-based deep lamellar scleral flap measuring 3.5 × 3.5 mm was created in the superotemporal quadrant of the eye. The center of the lamellar scleral flap was located ~3–3.5 mm from the corneoscleral limbus. The inner lamellar sclera (where the outer lamellar scleral flap was elevated) was thin enough to allow an examination of the dark underlying uveal tissue. The fabricated implant was placed inside the flap with the needles firmly pressed against the inner lamellar sclera. The scleral flap was closed immediately with 8-0 monofilament nylon sutures. The conjunctival peritomy was then closed using 7-0 absorbable sutures.

After the procedure, the animals received postanesthesia care and were returned to their cages after satisfactory recovery had been documented. Besides their usual care, they received topical antibiotic drops in both eyes three times a day for 1 week. After surgical hemostasis, no external bleeding or vitreous hemorrhage in any eye was observed during or after the surgical procedure. Four weeks after the implantation, the animals were killed by an overdose of intravenous sodium pentobarbital and the eyes were enucleated. The enucleated globes were labeled and stored at −80°C in 10% neutral buffered formalin for later sectioning and histopathologic examination.

Histopathology study

The globes were fixed for at least 12 h and subsequently sectioned so as to display as much of the implant site as possible but also including the pupil and optic nerve (P.O. section). The sectioned portion of the specimen was then processed overnight to remove most of the water from the tissue and allow replacement by paraffin. The paraffin mechanically stabilizes the tissue, making possible the cutting of thin sections (4–6 μm thick) for tissue staining with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS). Five microslides were made of each globe, 3 stained with H&E and 2 with PAS (Fig. 3). These slides were evaluated by 2 blinded, independent ophthalmic pathologists.

Results

During the surgery an unanticipated softening of the microneedle implant occurred when the implant came in contact with the scleral tissue that was at body temperature (higher) compared to the refrigerator or storage temperature (lower) of the microneedle implant. No other intraoperative or postoperative unexpected findings and complications were present in either of the studied eyes.

The pertinent histopathological findings were the presence of limited granulomatous inflammation in the intrascleral pocket, and residual implant material in all 3 right eyes examined. In contrast, all 3 left eyes examined presented an empty intrascleral pocket and no inflammation related to the implant. The left eye of rabbit no. 1 presented limited scar tissue around the intrascleral pocket, and the left eye of

![FIG. 2. Implantation procedure employed in the animal experiments. (A) Scleral flap made in the superotemporal quadrant of the eye; (B) implantation of microneedle device in lamellar sclera pocket; and (C) suture closure of the scleral flap and conjunctival peritomy.](image-url)
rabbit no. 3 exhibited rare foreign body giant cells surrounding 1 remaining suture. The inner sclera appeared intact in all cases.

Neither one of the eyes that received methotrexate implants or the eyes that received placebo implants showed any evidence of toxicity caused by the polymeric implant, the methotrexate or by both combined. Histopathological examination showed no appreciable abnormalities of the cornea, anterior chamber, iris, lens, ciliary body, or retina in any eye. Discrete differences were apparent between the right eyes that received the methotrexate implants versus the left eyes that received the placebo implants. Examination of intrascleral pockets (Fig. 3A) showed no traces of implant material in eyes that received placebo implants (without methotrexate in right eyes), whereas for the eyes that received medicated implants (with methotrexate in right eyes), residues of the implant were found inside the intrascleral pockets (Fig. 3B). The arrows in Fig. 3B indicate the locations of the residual implant material.

An unrelated finding was a limited foreign body reaction close to the suture material (indicated by arrows) that was observed in the left eye (treated with placebo) of animal no. 3 (Fig. 3C). No ocular inflammation was evident around the implantation site in the right eyes that received the methotrexate implant. However, limited granulomatous (foreign body giant cells) inflammation was observed in the right eyes around the areas having residual implant (indicated by arrows) in all the rabbits (Fig. 3D). There was no histopathological evidence that the implant microneedles penetrated the inner lamellar sclera. The photomicrographs of the empty intrascleral pocket in the left eye (H&E-20X) and the intrascleral pocket containing implant residue in the right eye (H&E-20X) of animal no. 2 are shown in Figs. 3A and B, respectively. Figure 3C shows the foreign body giant cell reaction to suture material observed in the left eye of animal no. 3 (H&E-20X). The foreign body inflammation observed in the right eye of animal no. 1 (H&E-40X) is shown in Fig. 3D. Since neither one of the eyes showed any evidence of toxicity, and also no major differences were evident between the right eyes versus the left eyes, we can conclude that the sustained release implant with and without methotrexate appeared to be safe in normal rabbit eye. Thus, our hypothesis is validated.

Discussion

The incidence of PIOL, which was previously a rare condition, appears to have increased substantially since the 1970s. Although intravitreal injection therapy using methotrexate (off-label use) is now being used to treat many eyes with PIOL, this method of treatment suffers from uncertainty as to the duration of a satisfactory tumoricidal concentration of drug in the eye, and the necessity of performing multiple intravitreal injections to successfully eradicate intraocular disease. A well-tolerated nontoxic slow release device that delivers prolonged therapeutic methotrexate levels and avoids frequent intravitreal injections would represent a major advance in patient care. In this brief technical report, we describe our experience with a biodegradable microneedle methotrexate implant in normal rabbit eyes for the treatment of PIOL.

Sustained-release devices with microneedles have shown promising results in the field of transdermal drug delivery.25 Guided by the success of microneedles in transdermal drug delivery, we have fabricated a biodegradable microneedle implant to deliver prolonged therapeutic methotrexate levels for the treatment of PIOL. In our previous studies,23,26 we investigated the effect of 2-methoxyestradiol and methotrexate on antiproliferation, apoptosis, and cell cycling in human lymphoma cells and observed that both 2-methoxyestradiol and methotrexate are suitable drugs for treatment of PIOL in humans. Also, we have developed a biodegradable microneedle implant (8.5 mg) loaded with 2-methoxyestradiol (10%, 25%, and 40% wt/wt.). For 10% wt 2-methoxyestradiol-loaded implants, we observed a tri-phasic drug-release profile in the in vitro experiments following the release characteristics of the polymer, due to the diffusion, swelling, and disintegration of the polymeric microneedle. The 10% wt 2-methoxyestradiol implants released the drug completely within 8 weeks, whereas the 25% wt 2-methoxyestradiol

FIG. 3. Histopathologic examination of rabbit eyes 4 weeks after the implantation. Photomicrographs of (A) empty intrascleral pocket in the left eye of animal 2 (H&E-20X); (B) intrascleral pocket containing implant residue (as shown by the arrows) in the right eye of animal 2 (H&E-20X); (C) foreign body giant cell reaction (as indicated by arrow) to suture material in the left eye of animal 3 (H&E-20X); and (D) foreign body inflammation (as indicated by arrows) observed in the right eye of animal 1 (H&E-40X). H&E, hematoxylin and eosin.
implants maintained therapeutic levels (0.1–1 μM) for 90 days.

For the current pilot study, we have used similar polymer combination (blend of high and low molecular poly(D,L-lactide) used in our previous study for preparing implants loaded with 2-methoxyestradiol) for fabricating the microneedle implants loaded with methotrexate (10% wt/wt) and investigated the safety of this implant in normal rabbit eyes. We anticipate that the microneedle methotrexate implant used in this study will release the drug over 8 weeks (similar to the polymer degradation observed for 2-methoxyestradiol implants discussed above). However, since the implant has softened during the implantation due to the relatively higher body temperature of the rabbit, we speculate that the pores and water channels may have formed immediately after implantation connecting the surface to the inside of the implant, which might have accelerated the polymer degradation process. An early rapid release of methotrexate would imply higher concentration of drug inside the vitreous. Under such a scenario it can be argued that ocular tissues in the vicinity of the implant did not have any toxicity despite higher concentration of methotrexate. The cumulative amount of methotrexate released over time for such a scenario was not determined before or in the in vivo experiments.

All implants were inserted successfully into a deep lamellar scleral pocket of the eye in rabbits without any apparent intraoperative or postoperative complications. In the right eyes of all the rabbits (treated with methotrexate), limited granulomatous (foreign body giant cells) inflammation was observed around residual implant material. We speculate that this limited inflammatory reaction is caused by some alteration of the drug or polymer related to drug–polymer interaction, as no inflammation was evident inside the intrascleral pocket of the left eyes treated with placebo implants. Since methotrexate is a hydrophilic (lipophobic) drug, its compatibility with biodegradable polymers is a complex issue. It is possible that a hydrophobic (lipophilic) drug such as 2-methoxyestradiol will not exhibit similar drug–polymer interaction.

Most of the chemotherapeutic drugs injected into the vitreous, in general, have a very short half-life (less than a week), indicative of rapid elimination from the eye. Repeated administrations of intravitreous drugs are usually necessary to maintain prolonged tumoricidal levels in the vitreous. Keeping this scenario in mind, the determination of therapeutic release rate of this biodegradable methotrexate implant and efficacy data for the treatment of PIOL would be needed to prove that implants such as the one described herein have a therapeutic and sustained drug release capability. Further, because drug release implants usually take a few days to achieve therapeutic dose, one can speculate that a sustained release methotrexate implant coupled with a concurrent initial intravitreal injection of drug may be a safe, effective, and alternative treatment for eyes with PIOL. Since the microneedle implants loaded with methotrexate did not perform mechanically in the intended way, our group is currently developing implantable discs, pellets, and spheres that are devoid of any microneedles. We intend to determine the cumulative amount of methotrexate released over time in the in vitro and in vivo studies to evaluate efficacy of our sustained release device.

In summary, the biodegradable microneedle methotrexate implants were inserted successfully into deep lamellar scleral pockets of the rabbits without any intraoperative or postoperative complications. Histopathologic examination of the medicated (methotrexate) and nonmedicated devices (placebo) showed no evidence of drug toxicity. The sustained release implant with and without methotrexate appeared to be safe and well-tolerated in normal rabbit eye.

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