Ultrasonographical assessment of implanted biodegradable device for long-term slow release of methotrexate into the vitreous

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Abstract
Our group has developed a biodegradable drug delivery device (micro-implant) for long-term slow intraocular release of methotrexate (MTX) that can be implanted in the peripheral vitreous. The purpose of this study was to assess the position of the implanted devices and the status of the adjacent vitreous and peripheral retina over time using B-scan ocular ultrasonography (US). In each of the eight New Zealand rabbits used in this study, a chitosan (CS) and poly-lactic acid (PLA)-based micro-implant containing approximately 400 μg of MTX and a placebo micro-implant without MTX were inserted into the peripheral vitreous of the right and left eyes, respective, employing minimally invasive surgery. B-scan US imaging was performed on all of the rabbits immediately after implant insertion and on two rabbits at each of several pre-determined time points post-insertion (post-insertion days 5, 12, 19, and 33) to evaluate the position of the micro-implants and identify any evident morphological changes in the micro-implants and in the peripheral retina and vitreous during treatment. US imaging revealed stable positioning of the PLA-coated CS-based MTX micro-implant and the placebo micro-implant in the respective eyes throughout the study and lack of any changes in size, shape or sonoreactivity of the micro-implants or abnormalities of the peripheral vitreous or retina in any of the study eyes. In summary, US did not show any evident morphological changes in the micro-implants, shifts in post-insertion position of the micro-implants, or identifiable changes in the micro-implants or peripheral vitreous and retina of the study eyes.

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During the past several years, our group has developed and refined a biodegradable long-term slow-release device for intraocular delivery of therapeutic doses of methotrexate (MTX), an antimetabolite chemotherapeutic drug which is used to treat selected vitreoretinal disorders including primary intraocular lymphoma, recalcitrant severe uveitis, and rhegmatogenous retinal detachment complicated by proliferative vitreoretinopathy (Hardwig et al., 2006; Khalatbari D et al., 2003; Riemann C et al., 2015). When MTX is administered by intravitreal injection (Chan and Sen, 2013; Frenkel et al., 2008), this hydrophilic drug is cleared rapidly from the vitreous (intravitreal half-life = approximately 14.3 h) (Palakurthi et al., 2010; Velez et al., 2001).

The chitosan (CS) and poly-lactic acid (PLA)-based micro-implant we developed contains approximately 400 μg of MTX and is able to deliver sustained therapeutic doses of MTX (0.1–1 μM) for more than one month at a steady release rate of between 0.2 and 2 μg/day (Manna et al., 2014). The formulation of the micro-implant, along with the kinetics of MTX release from the micro-implant, has been reported (Manna et al., 2014). The micro-implants are inserted into the peripheral vitreous via a small pars plana incision (Manna et al., 2015).

Our previously reported histopathological and electoretinographic studies of eight rabbits that had one of our MTX-containing micro-implants inserted into one eye and a placebo micro-implant inserted into the other eye showed no histopathological retinal toxicity related to the micro-implants or drug or any clinically evident reduction in electoretinographic responses during a post-implantation period of up to two months (Manna et al., 2015).

While ophthalmoscopic examination can be used to evaluate
the intraocular position and clinical appearance of an implanted intravitreal device, such examination does not provide any independently reviewable evidence of these findings. Fundus photography could potentially document these features, but photographs of the peripheral fundus are difficult to obtain in rabbit eyes. In contrast, ocular ultrasonography is able to image the peripheral retina, peripheral vitreous, and intravitreal foreign bodies (Avitabile et al., 2001; Gonzalez et al., 2001; Kunimatsu et al., 2002; Toni et al., 2010).

The purpose of this paper is to report ultrasonographic findings regarding micro-implant position, morphology of the micro-implants, and appearance of the peripheral vitreous and retina over post-insertion follow-up in rabbit eyes.

Our methodology consisted of a previously described rabbit model in accordance with the Institutional Animal Care and Use Committee protocol (IACUC # 12-09-13-01, University of Cincinnati, Dated: 21 November 2012) (Manna et al., 2015). Eight immune-competent New Zealand white rabbits (weight 2–3 kg) were used in this study. A sterilized PLA-coated CS-MTX micro-implant (containing 400 μg of MTX) and a sterilized placebo micro-implant (containing no drug) was implanted in the right eye and left eye, respectively, in each rabbit employing minimally invasive technique (Manna et al., 2015).

The micro-implants were fabricated as reported in our prior in vitro study (Manna et al., 2014). Low molecular weight CS (M.W:50,000—190,000 and DA 75%) (Sigma Aldrich) was blended with MTX (Letco Medical) in 0.1 N HCl to make a 40% wt/wt drug.

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**Fig. 1.** A) Top view and cross-sectional view of the poly-lactic (PLA)-coated chitosan (CS)-methotrexate (MTX) micro-implant; B) PLA-coated CS-MTX micro-implant just before insertion into the right eye of a rabbit; C) PLA-coated CS-MTX micro-implant being inserted into the right eye of a rabbit; and D) position of the PLA-coated CS-MTX micro-implant with respect to other intraocular features after enucleation of the globe.

**Fig. 2.** Ultrasound images of the micro-implant: A) Post surgery (0 days); B) Post euthanasia (5 days); C) Post euthanasia (12 days); D) Post euthanasia (19 days); and E) Post euthanasia (33 days).
loading mixture. The CS-MTX mixture was injected in a Tygon® tubing (1/16 I.D.) using vacuum. The tygon tubing containing the CS-MTX mixture was bophilized for 2 h at pressure below 1200 mTorr and temperature below –40 °C (Millrock BT48A, Millrock Technology) to obtain CS-MTX fibers. The uncoated CS-MTX micro-implants were obtained by cutting the CS-MTX fibers into desired micro-implant lengths using a surgical knife under an optical microscope.

A 40 mg/ml DL-PLA (inherent viscosity of 1.16 dL/g in CHCl3 @ 30 °C) (Lactel Biodegradable Polymers) in Dichloromethane (Fisher Sci.) coating solution was prepared. The uncoated CS-MTX micro-implants were dip-coated in the PLA solution to obtain a 100 μm lipophilic PLA coating approximately. After an initial overnight drying at room temperature in dark conditions, the PLA-coated CS-MTX micro-implants were vacuum dried for 12 h at 45 °C to ensure evaporation of the dichloromethane from the micro-implant. These micro-implants measured approximately 0.9 mm in cross-sectional diameter and approximately 4.2 mm in length (Fig. 1A). The PLA-coated CS-MTX micro-implant just before insertion into the right eye of a rabbit is shown in Fig. 1B. The PLA-coated CS-MTX micro-implant being inserted into the right eye of a rabbit is shown in Fig. 1C. The position of the PLA-coated CS-MTX micro-implant with respect to other intracocular features after enucleation of the globe is shown in Fig. 1D.

In this study, B-scan ultrasonography (US) scans was performed bilaterally on all rabbits immediately following insertion of the micro-implants and two rabbits were reimaged bilaterally on each of the following post-insertion days: day 5, day 12, day 19, and day 33 using a Linscan 12 MHz veterinary ophthalmic ultrasound unit (Ocuscience LLC, Rolla, Missouri). All scans were performed by the same examiner (ZMC). A drop of 2.5% hypromellose ophthalmic demulcent solution (Conionvisc™, Hub Pharma, CA) was applied to the tip of the ultrasound probe to provide probe-cornea coupling and avoid corneal abrasion. The tip of the ultrasound probe was brought into contact with the topical anesthetized cornea for image acquisition. Both antero-posterior and transverse images of the sector of the peripheral fundus where the micro-implant was located were obtained during each US session. For each rabbit and each eye, the US images were compared. Scans obtained immediately post-insertion of the micro-implant were matched with corresponding images acquired at the specified euthanasia time points. The specific aspects of the images that were assessed in this study were (1) the cross-sectional size and shape of the micro-implant, (2) the position of the micro-implant relative to the crystalline lens and peripheral retina, and (3) the appearance of the peripheral vitreous around the micro-implant and peripheral retina near the micro-implant’s position.

Representative corresponding US images obtained immediately post-insertion of the micro-implants and on the specified post-insertion days are shown in Fig. 2. The PLA-coated CS-MTX micro-implants and placebo micro-implants all appeared stationary in their post-insertion intravitreal position at all time points evaluated in this study. None of them migrated into contact with the crystalline lens or peripheral retina or into the posterior or central vitreous. The size and shape of each of the micro-implants remained stable throughout the study. No evident abnormalities of the peripheral vitreous adjacent to the micro-implant or of the peripheral retina near the micro-implant were identified in any of the evaluated eyes.

This study showed that the intravitreally-inserted biodegradable slow-release MTX-containing micro-implants we developed and evaluated were stationary in their intravitreal position post-insertion and stable in size and shape throughout the period of study. The micro-implants did not cause any significant ultrasonographic changes in the adjacent peripheral vitreous or peripheral retina in any of the study eyes. Taken together with our previously reported findings of no evident histopathological signs of micro-implant or drug-related retinal toxicity and sustained normal electroretinographic responses in these animals, these results suggest that the micro-implants we developed are likely to be safe for implantation in humans.

In spite of these findings, we acknowledge several limitations of our experience. First and foremost, we have studied only a limited number of animals following micro-implant insertion. If we had studied many additional animals, some of them may have shown abnormalities that were not apparent in the tested animals. Second, we have not followed study animals long enough after intravitreal insertion to document complete biodegradation of the micro-implants in the eye. It is possible that toxicity related to terminal release of MTX or to degradation products of the chitosan, poly-lactic acid, or both, would become evident as the micro-implant disintegrates. Third, we have implanted our devices only into healthy vitreous of non-vitrectomized eyes. One could speculate that these micro-implants may or may not remain stable in position in vitrectomized animal eyes.

In summary, this US study confirmed the stability of our fabricated micro-implants following their insertion in the vitreous of rabbit eyes. There was no evidence of any gross morphological changes in the micro-implants or adjacent peripheral vitreous.

References


