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Intravitreal Poly(L-lactide) Microparticles Sustain Retinal and Choroidal Delivery of TG-0054, a Hydrophilic Drug Intended for Neovascular Diseases

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Abstract

While poorly soluble drugs such as corticosteroids sustain drug delivery in the vitreous humor by virtue of slow dissolution, macromolecules such as antibodies and their fragments sustain their levels due to their slow clearance. However, currently there are no approaches to sustain the delivery of well water soluble small molecule drugs in the vitreous. In this study we optimized a PLA microparticle formulation for sustained intravitreal delivery of TG-0054, a well water soluble anti-angiogenic drug that is of potential value in treating choroid neovascularization. After determining the influence of process parameters on particle size and drug loading, spherical microparticles syringeable through a 27 G needle, with a mean diameter of 7.6 μm , 10% w/w TG-0054 loading, sustained in vitro drug release for at least 6 months, and low residual organic solvent content (~ 1 ppb/mg) were prepared. Microparticles as well as drug solution were assessed for their in vivo drug delivery over 3 months following intravitreal injection in New Zealand white rabbits. Drug levels in the microparticle dosed eyes at 3 months were 43.7 ± 16.2 , 243 ± 42.6 , 62.8 ± 22.6 $\mu\text{g/g}$ vitreous, retina, and choroid-RPE, respectively, and similar to levels at one month. Intravitreal injection of plain drug solution resulted in significantly lower amounts of drug in the dosed eye, with the levels being 0.8 ± 0.5 , 2.7 ± 2.8 , and 4.9 ± 4.2 $\mu\text{g/g}$ in vitreous, retina, and choroid-RPE, respectively, at one month, with no detectable drug at three months. Although surface degradation was evident, microparticles maintained their spherical structure during the 6 months in vitro study and the 3 months in vivo study, with the vitreal particle retention at 1 and 3 months being 60% and 27%, respectively. Thus, PLA microparticles capable of sustaining retinal and choroidal delivery of TG-0054 for three to six months were developed.

Keywords

Microparticles; retinal delivery; choroidal delivery; intravitreal injection

Introduction

Diseases affecting posterior segment of the eye including choroidal neovascularization (CNV) associated age related macular degeneration (ARMD), diabetic macular edema (DME), and proliferative diabetic retinopathy (PDR) are leading causes of blindness (1, 2).

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Treating these back of the eye diseases is a major challenge since the conventional routes of drug administration including topical and systemic modes are inefficient in delivering drugs to the back of the eye (3). Topical and systemic routes require high doses for efficacy, which might be associated with systemic side effects of the drug. Intraocular administration of therapeutic agents by intravitreal injections reduces the dose requirement and allows efficient treatment of the back of the eye disorders, as is the case with intravitreally administered antibody fragment against vascular endothelial growth factor (e.g., ranibizumab) and lipophilic corticosteroids (e.g., triamcinolone acetonide, fluocinolone acetonide, and dexamethasone). While ranibizumab is cleared slowly from the vitreous humor due to its large size (4), triamcinolone acetonide (5) persists in the vitreous due to its administration in the form of a suspension that dissolves slowly in the vitreous. For soluble small molecules, the vitreal half-life is in the order of a few hours as opposed to several days for drug suspensions (6, 7). For this reason, soluble small molecule drugs require frequent intravitreal injections to sustain therapeutic levels of the drug. Repeated intravitreal injections can result in severe adverse effects including retinal detachment, endophthalmitis, and cataracts (8). Intravitreal sustained release drug delivery systems offer one approach to minimize injection related as well as drug related systemic toxicities, while reducing the dose required.

Intravitreal administration of a sustained release non-degradable implant (Retisert™) with a total drug dose as small as 0.59 mg sustains the delivery and efficacy of fluocinolone acetonide over approximately 30 months. An alternative to non-degradable implants that potentially require removal after drug release is the use of biodegradable drug delivery systems. For instance, a poly(lactide-co-glycolide) (PLGA) biodegradable implant containing 0.7 mg dexamethasone (Ozurdex™), administered through a 22 G needle to the vitreous, sustains drug delivery and efficacy over six months. Polymeric microparticle formulations potentially allow the use of narrower gauge needles for intravitreal injections, which should avoid humor effusion and entry of pathogens. Further, since low molecular weight, water soluble drugs are not trapped well in lipophilic polymers such as PLGA or PLA, development of a sustained release deliver system for such molecules is of significance in treating diseases of the eye.

TG-0054 (MW: 823.05) is a potent small molecule currently in Phase II clinical trials in cancer patients undergoing stem cell transplant therapy. TG-0054 selectively inhibits the binding of the chemokine (cell-surface) receptor CXCR4 to its ligand, stromal (-cell) - derived factor (SDF)-1 (9). Since CXCR4 activity is implicated in angiogenesis (10), TG-0054 might be capable of inhibiting pathologic angiogenesis associated with PDR and CNV in retina and choroid, respectively, in the back of the eye. A microparticle formulation of TG-0054 that could sustain its release for 3-6 months could therefore be beneficial in sustaining therapeutic levels of the drug in the retina and the choroid following intravitreal administration. Development of an optimum polymeric microparticle formulation depends on several parameters including the physical properties of the drug such as its solubility in aqueous and organic solvents, stability and interaction with the polymer of choice, and in vitro and in vivo release characteristics (11, 12). Microencapsulation of TG-0054, a highly hydrophilic drug into hydrophobic polymer matrix is a key challenge in developing slow release microparticle formulations (13). Microparticle formulations intended for injectable delivery should have optimum syringeability/injectability, allowing easy injection of a consistent amount of drug in vivo (14, 15). One polymer family that has established safety profile in humans is poly(lactide-co-glycolide) and related polymers (16-19). In this series of polymers, as the glycolide content is increased, the polymer degradation half-life reduces, releasing the drug more rapidly. Thus, use of a pure polymer such as poly(L-lactide) (PLA) is likely to sustain the release of the drug for longer period due to its longer degradation

half-life (13). This half-life further depends on polymer molecular weight, with the half-life increasing with an increase in molecular weight(13).

In this study we developed a biodegradable polymeric microparticle formulation of hydrophilic TG-0054 using PLA as the biodegradable polymer. We employed a water-in-oil-in-water ($w_1/o/w_2$) emulsion-solvent-evaporation method with an electrolyte in the aqueous outer phase in order to improve drug loading. We determined the influence of polymer concentration, drug concentration, and power input on drug loading and particle size. Additionally we determined the syringeability, residual solvent content, in vitro drug release, in vivo drug delivery, and morphology of microparticles in vitro and in vivo.

Materials and Methods

Materials

Poly(L-lactide) (i.v. 0.90 – 1.20 dL/gm in CHCl_3 , Part # B6002-2P), TG-0054 (MW. 823.05, TaiGen Biotechnology Co., Ltd, Taiwan), methylene chloride (99.9 %, ACROS, NJ, USA), sodium chloride (Sigma-Aldrich, St. Louis, MO, USA), and poly vinyl alcohol (PVA) (Sigma, P8136 St. Louis, MO) were used as received. Deionized water was used to prepare aqueous media throughout the study. Misonix Soinicator 3000 (Misonix Inc, NY) and Virtis Cyclone IQ2 homogenizer (Virtis Inc, NY) were used for the microparticle preparation.

Preparation TG-0054 loaded PLA microparticles using w/o/w emulsion- evaporation method

Microparticles were prepared by using the water-in-oil-in-water ($w_1/o/w_2$) emulsion followed by solvent evaporation method. PLA (70, 85, or 100 mg) was dissolved in 2 ml of methylene chloride. Primary emulsion was prepared by adding the aqueous TG-0054 solution (32, 36, or 40 mg/0.4 ml water) to the polymer solution followed by probe sonication at 3 W for 30 sec under ice cold conditions. The primary emulsion formed was then added to 20 ml, 2 % PVA solution containing 10 % NaCl and homogenized with various power inputs (sonication – 3 W for 15 or 30 sec; homogenization – 5000 rpm for 5 min), resulting in a secondary emulsion. The $w_1/o/w_2$ emulsion thus formed was stirred with a magnetic stirrer for solvent evaporation at room temperature for 3 hours. Residual amount of organic solvent present in microparticles was removed using a rotary evaporator set at 40 °C for 2 hrs. Microparticles were harvested by centrifugation at 16000g for 30 min. The microparticle pellet obtained was re-dispersed using 20-30 ml of deionized water and again centrifuged to separate the pellet. Finally the microparticle pellet obtained was dispersed in 25 ml of deionized water, and dried by lyophilization (Labconco, Labconco Corporation) for 48 hrs. Thus, various polymer concentrations, drug concentrations, and power inputs during secondary emulsion preparation were assessed for their influence on particle size and drug loading, as described in a subsequent section.

Characterization of microparticles

Determination of particle size—TG-0054 loaded PLA microparticles were characterized for the mean diameter and particle size distribution using dynamic light scattering (NICOMP zeta potential and size analyzer). Samples were prepared in triplicates by dispersing microparticles in filtered deionized water (1 mg in 10 ml).

Determination of drug content—Microparticles (2 mg) loaded with TG-0054 were dissolved in methylene chloride (1 ml) by vortexing the entire content for 30 minutes. Deionized water (5 ml) was added to the above solution, and the TG-0054 present in the solution was extracted by vortexing the entire content for 2 hr. The aqueous layer from the

top was separated after centrifugation for 5 min at 5,000 rpm. The amount of TG-0054 present in the aqueous medium was quantified using a UV spectrophotometer set at 284 nm.

Box-Behnken statistical design to determine the Influence of process parameters on particle size and drug loading in TG-0054-PLA microparticles—

Box-Behnken statistical design (20) was used for determining the influence of process parameters on particle size and drug loading for TG0054 microparticle formulation. Statistical design was generated and evaluated using the Statgraphics Centurion XV software (Statpoint Technologies, Inc. VA). Polymer concentration (3.5 – 5.0%), drug concentration (8-10%), and power input during secondary emulsion preparation (1-26 Watts) were selected as three independent variables (Table 2). The power input during the preparation of primary emulsion was kept constant (25.3 Watts). The dependent variables were particle size and drug loading. The response to the three independent variables can be calculated using a quadratic polynomial equation, described as: $Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3$ Where Y is the predicted response (particle size in μm or drug loading in % w/w), X_1 , X_2 , and X_3 are polymer concentration (% w/v), drug concentration (% w/v), and power input (Watts), respectively. B_0 is a constant, B_1 , B_2 , B_3 are the linear coefficients, B_{12} , B_{13} and B_{23} are the cross product coefficients and B_{11} , B_{22} , and B_{33} are the quadratic coefficients. The software performs a regression analysis and predicts a response.

The range and values for the independent variable of power input were calculated using the methodology described by Roscoe and Velthorst (20), wherein the authors used the following equation to calculate the exact power input used to disrupt soil samples. We used three different sources of power, namely, sonicator (Misonix Sonicator 3000, Misonix, Inc., NY), homogenizer (Virtis Cyclone IQ2 Homogenizer, Virtis, Inc., NY) and a magnetic stirrer (Corning Stirrer, Corning, Inc., NY) for power input. The following equation was used to calculate the power input by the different sources.

$$P_c = \left\{ (m_w c_w + C_{cont}) \frac{\Delta T}{t_s} \right\} + H$$

Wherein P_c is the calculated power in Watts, m_w is the mass of water in grams, c_w is the specific heat of water (4.186 J/g/°C), C_{cont} is the heat capacity of the container (J/°C), ΔT is the temperature input in °C due to the secondary emulsion preparation by sonication or homogenization, t_s is the time of sonication or homogenization (in seconds), and H is the rate of energy loss from the system (J/seconds).

To measure the heat capacity of the container C_{cont} , 50 grams of water was heated to 50°C and immediately transferred to 50 grams of water at room temperature in the container of interest and the resulting temperature of the mixture was noted. Data was put in the following equation to calculate the C_{cont}

$$C_{cont} = \left\{ \frac{m_1 c_w (T_1 - T_2)}{(T_3 - T_2)} \right\} - (m_2 c_w)$$

Where m_1 (50 grams) and m_2 (50 grams) are the weight of the water at T_1 (50°C) and T_2 (26°C), respectively. T_3 (36°C) is the temperature of the mixture. The value of C_w is as above (4.186 J/g/°C). The value for C_{cont} was estimated to be 292.3 J/°C.

The rate of energy loss (H) from the system was calculated by the following equation

$$H = \frac{(m_w c_w) \Delta T}{t_d}$$

Where m_w , c_w , and H are as mentioned above, T is the temperature change of 1°C , and t_d is the time necessary to decrease temperature by 1°C . H was measured by heating 20 ml of 2% w/v aqueous polyvinyl alcohol solution to 50°C and monitoring the fall in temperature. The H vs T relationship could be fit to an exponential equation ($H = 0.0026 e^{0.1642T}$). Using this equation and the actual temperature changes observed with various sonication and homogenization conditions, we determined H for each experimental condition and estimated the power input using the P_c equation mentioned above.

Differential Scanning Calorimetry (DSC)—DSC thermograms of TG-0054, and PLA microparticles with and without TG-0054 were recorded using Perkin Elmer, Diamond DSC with hyper DSC™. DSC curves were recorded between 0°C and 200°C under inert nitrogen atmosphere at a flow rate of 20 ml/min and at a heating rate of $10^\circ\text{C}/\text{min}$.

Determination of residual organic solvent content—Residual methylene chloride (CH_2Cl_2) present in TG-0054-PLA microparticles was analyzed by EPA Method 8260 using gas chromatography-mass spectrometry (GC-MS). The target compound, CH_2Cl_2 , was run at two different concentrations, 30 pg and 100 pg. The vials containing 15 mg of TG-0054-PLA microparticles were placed in an extraction vial, sealed, and the head space of the extraction vial sampled using solid phase micro-extraction (SPME) with a polydimethylsiloxane (PDMS) fiber followed by direct injection into the GC-MS.

Determination of surface morphology of the TG-0054-PLA microparticles—Microparticles were visualized using a scanning electron microscope (SEM) (JEOL, scanning electron microscope) at different magnifications ranging from 1,000X to 10,000X. Samples were analyzed after they were gold sputtered. SEM images of the microparticles were taken before the in vitro or in vivo release studies and at the end of 3 months and 6 months of the study.

Syringeability and injectability of TG-0054-PLA microparticles—A stock of microparticle dispersion at concentrations to be used for in vivo injections (300 mg/ml in 7.4 pH PBS) was prepared. Eppendorf tubes were weighed in triplicates. To each pre-weighed Eppendorf tube, 50 μl of microparticle dispersion was added using a 27 G needle attached to 1 ml syringe. Additionally, using a micropipette (Finnpipette, ThermoFischer Scientific), 50 μl of microparticle suspension was added to pre-weighed Eppendorf tubes. The weight of the microparticle suspension withdrawn was estimated in each group. Amount of TG-0054 present in the tubes was extracted and analyzed using a UV spectrophotometer.

In vitro drug release study

Weighed amount (5 to 10 mg) of TG-0054 loaded microparticles were dispersed in 1 ml of phosphate buffered saline (PBS, pH 7.4) containing 0.05% sodium azide. Sodium azide serves as a preservative to prevent the microbial degradation of the microparticle formulation. These dispersed microparticles were added to a dialysis bag (7Spectra/por®, MWCO 25 kDa) presealed at one end with a clip. After the addition of microparticle dispersion, the other side of the dialysis bag was closed. Dialysis bag containing the dispersion of microparticles was placed in drug release medium (10 ml PBS at pH 7.4 containing 0.05 % sodium azide) in a tube. The tubes were incubated at 37°C while stirring the contents at 200 rpm. At discrete time intervals including 0.5 hr, 1 hr, 2 hr, 4 hr, 8 hr, 16

hr, 1 day, 2 days and every week thereafter, the entire release medium was replaced with fresh medium maintained at 37°C. The dissolution medium removed at each time interval was analyzed using a UV spectrophotometer and the amount of TG-0054 in the release medium was determined. All in vitro studies were carried out in triplicates.

In vivo drug delivery

The in vivo intravitreal delivery of TG-0054 from microparticle formulation was tested in male New Zealand white rabbits and compared with TG-0054 solution. Male New Zealand white rabbits weighing 2-3 kg were assigned to two groups. Group 1 (n = 6) received TG-0054-PLA microparticles intravitreally in one eye. Group 2 (n = 6) received TG-0054 solution intravitreally in both eyes. The rabbits were anaesthetized by intramuscular injection of ketamine : xylazine mixture (50:10 v/v) in the hind limb of rabbits (400 µl/ rabbit). Once the rabbits were in deep anesthesia, betadine solution was applied on eye surface and intravitreal injections were made using a 27G needle. Group 1 animals received 50 microliters of 300 mg microparticles/ml PBS (pH 7.4) [TG-0054-PLA microparticles with 10% drug loading; 15mg microparticles containing 1.5mg TG-0054/ 50µl] in the vitreal cavity of the right eye while the left eye was not dosed. Group 2 animals received 50 microliters of 20 mg TG-0054/ml PBS (pH 7.4) [1 mg/50 µl] in both eyes. After intravitreal injection, gentamicin ointment was applied at the injection site to prevent infections. The animals were regularly monitored for any abnormal signs. Three rabbits from each group were sacrificed at the end of 1 or 3 months post-dosing, in order to compare drug delivery between the formulations at the end of 1 and 3 months. Animals were euthanized by intravenous injection of sodium pentobarbital (150 mg/kg), and both eyes were enucleated and blood was collected. The eyes were immediately frozen in isopentane : dry ice bath and stored at -80 °C until further analysis.

Drug analysis in ocular tissues—Eyes were dissected in frozen condition and different eye tissues were collected in pre-weighed plastic tubes and tissue weight was estimated. To weighed amount of tissue, 500 µl of water: methanol (1:1 V/V) containing 1 µg/ml of d8-TG-0054 as internal standard was added. Tissue samples were briefly vortexed, homogenized by a hand-held homogenizer, followed by sonication for 10 min in a bath sonicator. Samples were heated at 65 °C for 5 min to denature the proteins. The precipitated protein was removed by centrifugation and supernatant was transferred to plastic LC-MS/MS vials and subjected to drug analysis. Extraction recovery was assessed using New Zealand white rabbit vitreous, retina, and choroid-RPE. Standards were prepared in respective tissues.

Characterization of TG-0054 microparticles recovered from the eye following in vivo delivery Drug content analysis

—Microparticles were separated from the vitreous humor by centrifugation and drug content was estimated in isolated microparticles as described previously. Briefly the microparticle pellets recovered from the vitreous humor were weighed and dissolved in 1 ml of methylene chloride. To the above mixture 5 ml of deionized water was added and vortexed for 20 min. The aqueous layer was separated by centrifugation and TG-0054 in the aqueous layer was quantified using LC-MS/MS.

Determination of surface morphology of the microparticles—Microparticles were visualized following gold sputter coating using a scanning electron microscope (SEM) (JEOL, scanning electron microscope) at different magnifications (1,000x to 10,000x). SEM images of the microparticles were taken at 1 and 3 months following in vivo delivery.

Statistical analysis

Statistical comparison for drug levels between different tissues was performed using one way ANOVA followed by Tukey's post-hoc analysis (SPSS 11.0, Chicago, IL). Comparison between drug levels at 1 and 3 months in a given tissue was performed using a Student's t-test. Statistical significance was set at $p < 0.05$.

Results

Power input parameters

Calculation of power input for sonicator and homogenizer indicated a power input of 25.3, 12.7, and 1.41 Watts for sonication at 3W for 30 seconds, sonication at 3W for 15 seconds, and homogenization at 5000 rpm for 5 min, respectively (Table 1). We determined the influence of various drug/polymer concentrations and the above power input conditions during secondary emulsion preparation on particle size and drug loading.

Properties of microparticles

Particle size and drug content—TG-0054-PLA microparticles with mean diameters ranging from 1.5 – 7.1 μm (Table 2) were obtained. TG-0054 loading ranged from 4 to 11.5% w/w.

Influence of process parameters on particle size and drug loading—Figure 1 shows the influence of power input, polymer concentration, and drug concentration on particle size and drug loading. With an increase in power input, the particle size decreased (Fig. 1A). With an increase polymer concentration, there was an increase in particle size (Fig. 1C). Drug loading decreased with an increase in polymer concentration (Fig. 1D) and increased with an increase in power input (Fig. 1F). When both power input and polymer concentrations were varied, they countered the effect of each other on particle size (Fig. 1B). For additional in vitro and in vivo studies, particles were prepared at 5% w/v polymer concentration in methylene chloride, 10% w/v drug concentration in water (W_1 phase), and 1.41W power input with homogenizer during the preparation of the secondary emulsion.

Differential scanning calorimetry and residual solvent content—PLA blank microparticles and those loaded with TG-0054 exhibited a melt temperature at $\sim 170^\circ\text{C}$ that is characteristic of PLA (Figure 2). A broad peak for TG-0054 was observed at $120\text{--}130^\circ\text{C}$. Due to the lack of a distinct, sharp peak, TG-0054 can be considered as amorphous. This broad peak was not evident in the drug loaded microparticles, possibly due to a homogeneous dispersion of TG-0054 within the PLA matrix.

The particles were subjected to residual solvent analysis to detect methylene chloride (CH_2Cl_2) using selected ion monitoring. The concentration dependent increase in peak was observed for the CH_2Cl_2 standards at 30 pg and 100 pg, respectively (Figures 3 B and 3C). While no peak for CH_2Cl_2 was observed in the blank sample (Figure 3A), the amount of CH_2Cl_2 detected in 15 mg of microparticles was ~ 1 ppb (parts per billion) (Figure 3D). US FDA guideline for residual CH_2Cl_2 in pharmaceutical formulations is 600 ppm (parts per million). The residual amount of CH_2Cl_2 present in TG-0054-PLA microparticles is well within the acceptable limits of the FDA.

Syringeability—Drug extraction from microparticles delivered by 27G needle demonstrated a drug recovery of $89.17 \pm 1.37\%$. Drug extraction from microparticles delivered by micropipette demonstrated a drug recovery of $91.96 \pm 5.0\%$. There was no significant difference in these measures as well as the weights of the suspensions dispensed.

In vitro drug release

For in vitro release and in vivo delivery studies, microparticles of size 7.6 μm , poly dispersity index of 0.1 and actual drug loading of 10% w/w were used. Within 24 hours, an initial burst release of 23% was observed for TG-0054 - PLA microparticles (Figure 4). Beyond this, the drug was released slowly, with the release sustained during the entire 6 month study.

Surface morphology of the microparticles

Scanning electron microscopic pictures taken before the release studies suggested that microparticles formed were spherical and had almost smooth surfaces (Figure 5A). Following 3 and 6 months of in vitro release, the particles appeared to have reduced in size (Figures 5B and 5C). However, the particles were still spherical. Similar results were observed for microparticles following 1 (Figures 6A and 6B) and 3 months (Figures 6C and 6D) of in vivo release. In vivo, the particles exhibited greater number of surface deformations including pits, when compared to particles assessed after in vitro release.

In vivo drug delivery

Extraction recovery of TG-0054 from retina and choroid-RPE ranged from 65-70%. Extraction recovery from the vitreous humor was 80-90%. A peak corresponding to intact TG-0054 was observed following LC-MS-MS analysis of the retina samples obtained at 3 months after dosing with microparticles, as evident in Figure 7. Intact microparticles still carrying TG-0054 were present in the vitreous humor at the end of one and three months of in vivo release (Figure 6 and Table 3). About 60 and 27% of dosed microparticle mass could be recovered from the vitreous humor at the end of 1 and 3 months, respectively (Table 3). The drug concentrations in the particles (μg drug/mg particles) recovered at 1 and 3 months were 44 and 17% of the starting concentrations, respectively. Following intravitreal administration of TG-0054 microparticles, a mean retinal concentration of 244.14 ± 95.28 $\mu\text{g/g}$ TG-0054 was detected at the end of one month and the levels were sustained at similar levels at the end of three months (Figure 8A, Table 4). Significant drug levels were also detectable in the vitreous humor and the choroid-RPE at 1 month. These levels were slightly elevated at 3 months. Contralateral eye received measureable quantities of drug in the microparticle group.

In the eyes dosed with the 1 mg drug solution, ~90-fold lower retinal levels of the drug were observed at the end of one month (2.7 ± 2.7 $\mu\text{g/g}$ of retina), when compared to the microparticle group containing 1.5 mg drug. In the solution group, TG-0054 was not detectable in any of the tissues at the end of three months (Figure 8C, Table 4). These results suggest that TG-0054 microparticles are able to sustain the release of stable TG-0054 in vivo for at least three months.

We detected and isolated microparticles from the vitreous humor of animals sacrificed at 1 and 3 months. However, we did not observe any visible particle mass in other tissues. Vitreous humor drug levels shown in Figure 8 and Table 4 correspond to the free drug in the vitreous humor. Presuming that tissues contained the release drug and the particles mass isolated from the vitreous humor contained encapsulated drug, we provided approximate mass balance for the amounts of drug in various tissues (Table 5). In the tissues quantified (retina, choroid-RPE and vitreous), at least 3.7 and 5.2% of the dose was present at 1 and 3 months, respectively, in the dosed eye. Drug remaining in the particles present in vitreous humor was at least 26 and 4.5% at the end of 1 and 3 months, respectively. Drug levels assessed in the dosed eye of solution group and contralateral eyes of microparticle group represented about 0.1% of the dose at 1 month. While solution group had no detectable levels the eye tissues at 3 months, microparticle group exhibited about 0.1% drug dose in the

contralateral eye tissues. The actual drug amounts and percent dose remaining in the eye can be somewhat higher than what is depicted in Table 5 since we did not analyze several other tissues of the eye such as optic nerve, lens, iris-ciliary body, aqueous humor, etc. Also, the tissue weights shown in Table 5 may be underestimates of the actual tissue weights.

Discussion

We successfully developed a sustained release, poly(L-lactide) based biodegradable microparticle formulation for TG-0054, a potent CXCR4 antagonist, that is undergoing clinical trials for cancer patients undergoing stem cell transplant therapy and holds promise for treating neovascular disorders of the eye. The microparticle formulation exhibited ultra low residual organic solvent content. The particles had good syringeability and could be administered intravitreally through a small gauge needle. The microparticles sustained in vitro drug release for six months and in vivo drug delivery for at least 3 months.

Pilot solubility studies suggested that TG-0054 was a highly hydrophilic molecule with water solubility greater than 100 mg/ml and with little or no solubility in organic solvents. Given its high aqueous solubility, water in oil in water ($w_1/o/w_2$) emulsion-evaporation technique was chosen to prepare the microparticles. However, the hydrophilic nature of TG-0054 resulted in very low drug loading due to leaching of the drug from the inner aqueous phase to the outer continuous phase during the process of formulation. Our pilot solubility studies also indicated a reduction in the solubility of TG-0054 by about 50% when the aqueous medium contained sodium chloride (10% w/v). Use of an aqueous medium with reduced drug solubility as the external w_2 phase increases drug loading in microparticles (21). With sodium chloride in the external phase and additional modification to the process parameters, we could achieve TG-0054 loading up to 11.5% (Table 2), which was 10-15-fold higher when compared to microparticle prepared in the absence of electrolyte in the outer continuous phase.

TG-0054 microparticle formulation parameters were characterized by Box-Behnken statistical design to determine the influence of process variables including polymer concentration, drug concentration, and power input during the preparation of secondary emulsion on particle size and drug loading (Figure 1). With an increase in power input, the particle size decreased, possibly due to a decrease in the emulsion droplet size. With an increase in polymer concentration, the particle size increased, possibly due to the formation of large emulsion droplets by more viscous polymer solution. Drug loading decreased with an increase in polymer concentration, possibly due to the inability of the aqueous inner core to be dispersed well within a more viscous oily phase. Interestingly, with an increase in power input, there was an increase in drug loading. We speculate that this may be an outcome of enhanced dispersion induced between the inner aqueous phase and the oily phase.

Microparticle formulation intended for in vivo use should exhibit acceptable syringeability and injectability for uniform dose delivery. We observed that about 90% of the drug in 50 μ l volume could be syringed and injected consistently with a 27G needle, similar to a micropipette. This demonstrated that the microparticle formulation had good syringeability.

Thermal analysis of the microparticles by differential scanning calorimeter helps determine the physical nature of the polymer and the drug in the preparation(22). The process of microparticle preparation can contribute to a reduced crystallinity of both the polymer and the drug. From the DSC study it was observed that PLA microparticles with and without TG-0054 demonstrated an endothermic peak at around 170 °C (Figure 2), characteristic of a crystalline PLA. However, pure TG-0054 demonstrated a broad peak at 120 –130°C

suggesting that the drug is amorphous. This peak was lost after its encapsulation into PLA microparticles, possibly due to homogeneous distribution of the drug in the PLA matrix of microparticles.

Low residual organic solvent in formulations is essential to avoid in vivo toxicity of organic solvents. Low levels of residual organic solvents also ensure integrity of polymeric microparticles during storage. The US FDA guidelines limit the residual organic solvents content in formulations. TG-0054-PLA microparticles demonstrated very low residual organic solvent levels at ~1 ppb, well within the 600 ppm limit for methylene chloride used in the preparation of microparticles (Figure 3).

Prepared TG-0054-PLA microparticles were intended to slowly release TG-0054 for 3 to 6 months. In vitro release studies are essential in predicting the in vivo release patterns of the prepared formulations and are essential tools to screen formulations. In vitro release studies were carried out at physiological temperature (37 °C) and pH 7.4 simulating physiologic conditions. A high initial burst release of the drug from microparticles could lead to release of drug in toxic doses as well as a loss of a major amount of drug that will not be available for later release. Controlling the initial burst release from microparticle formulations is very critical for achieving sustained drug release for desired periods of time(23). Microparticle formulations of hydrophilic drugs are often associated with high burst release as the drug remains on the surface as a phase separate from the hydrophobic polymer matrix at the end of the double emulsion method of particle formulation. Moritera et al. (24) observed about ~30-80% burst release within a day and ~70-98% total drug release within 7 days for 5-fluorouracil from PLA microparticles. Unlike this previous study, we employed a high molecular weight PLA. Our particles, demonstrated very low burst release of 23% (Figure 4), even though the drug is very hydrophilic with an aqueous solubility greater than 100 mg/ml. The low burst release might be because the drug was distributed well throughout the polymer matrix in a molecular dispersion. The second phase of drug release characterized as a slow release phase is diffusion limited and influenced by the hydration and swelling of the polymer matrix(23). Drug release in this phase is controlled by polymer properties including polymer molecular weight, the type of the polymer, and the crystallinity of the polymer. Drug release from microparticles is typically related inversely to the molecular weight and lactic acid content of the polymers. Polymers comprising of the L isomer of lactic acid that are crystalline in nature, release the drug more slowly than polymers containing both the D and L isoforms that are more amorphous in nature. Given the hydrophilic nature of TG-0054, we decided to incorporate it in L-PLA with a high molecular weight in order to prepare sustained release formulations that allow low frequency injections to the eye. As expected, microparticles formulated with L-PLA demonstrated sustained drug release for several months, with about 70% of the total drug released by the end of six months (Figure 4).

Microparticles formed were spherical and retained their shape during in vitro as well as in vivo release (Figures 5 and 6). TG-0054-PLA microparticles resulted in the delivery of intact drug to the retina (Figure 7) and choroid-RPE for at least 3 months (Figure 8, Table 4). The tissue concentrations of the released drug in the dosed eye were in the order: retina >> choroid-RPE > choroid-RPE. High drug levels in retina in the dosed eye are consistent with the proximity of the dosage form, which was placed in the vitreous humor adjacent to the retina. Although the amount of drug in retina was not greater than what was solubilized in the vitreous (Table 5), the concentrations were higher in the retina. Either drug binding/partitioning in the retina or some tissue uptake of microparticles may account for this observation. Preferential drug binding/partitioning in the retina may be a key cause for this observation since contralateral also exhibited high retinal levels compared to vitreous humor at 1 and 3 months (Table 4). Further, retinal levels were greater than choroid-RPE levels in the contralateral eye at 1 month. Also, binding of TG-0054 to the choroid-RPE may not be

substantial or slow since drugs with high pigment binding preferentially and readily accumulate in the choroid-RPE (25). Slow accumulation in choroid-RPE is consistent with higher levels observed in this tissue at 3 months compared to 1 month in both dosed and contralateral eyes. The drug was largely localized to the dosed eye, with only a small fraction (~0.1%) of the dose detected in the contralateral, undosed eye tissues (Table 5). Entry into the contralateral can occur either via systemic circulation or via anatomical pathways that connect both eyes. In the contralateral eye, although the dose measured remained constant at about 0.1% at 1 and 3 months, the vitreous levels were below detection limit at 3 months, while the choroid-RPE levels were significantly higher at 3 months when compared to 1 month. The reason for this shift in concentrations from vitreous to choroid-RPE at 3 months in the contralateral eye is unclear. When administered as a drug solution, the drug levels were not detected in any of the ocular tissues at 3 months. At 1 month, although the drug levels were detectable with the solution dosage form, the levels were much lower, with the retinal levels being 90-fold lower compared to the microparticle group. Thus, microparticle formulation is critical for maintaining therapeutic levels in the eye for prolonged periods.

PLA and PLGA microparticles are biocompatible and result in biocompatible products, namely, lactic acid and glycolic acid, as a result of hydrolytic degradation of the polymer. These products are readily metabolized or easily eliminated from the body. Both PLA and PLGA microparticles encapsulating various therapeutic agents have been administered intravitreally or periorcularly to deliver therapeutic agents to the posterior segment of the eye. It has been observed that low molecular weight PLA microparticles encapsulating 5FU, a hydrophilic drug, was able to sustain its release in the vitreous for 1 week or less, with the particles disappearing completely by 48 ± 5.2 days following intravitreal injection (24). PLGA (85:15) celecoxib microparticles administered periorcularly were able to sustain release of celecoxib and offer therapeutic effects for at least two months (26, 27). PLA-PEG microparticles encapsulating a TAT-EGFP protein was able to deliver and sustain retinal therapeutic levels up to nine weeks following sub-retinal injection (28). Following the intravitreal injection of 15 mg of PLA microparticles encapsulating 1.5 mg of TG-0054, we were able to recover 60 % particle mass at one month and 27% of the particle mass at the end of three months from the vitreous humor (Table 3). The drug concentrations remaining in the particles the end of 1 and 3 months in vivo were 44 and 17%, respectively (Table 3). This suggests a more rapid drug release in vivo, since an estimated ~40% of the drug was still retained in microparticles at the end of 3 months during in vitro release study (Figure 4). The surface of microparticles isolated from in vivo at 3 months appeared to have undergone greater amount of degradation (Figure 6), consistent with greater release rate in vivo.

Conclusions

We prepared biodegradable PLA microparticles of highly hydrophilic drug TG-0054 with a drug loading up to 11.5% and mean particle size up to 7.6 μm and demonstrated their ability to sustain in vitro and in vivo release of intact TG-0054 for 3-6 months. This microparticle formulation could be potentially used for sustained intravitreal delivery of TG-0054 for treating neovascular disorders of the eye including choroid neovascularization and proliferative diabetic retinopathy.

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References

1. Tolentino MJ. Current molecular understanding and future treatment strategies for pathologic ocular neovascularization. *Curr Mol Med*. 2009; 9:973–81. [PubMed: 19925410]
2. Sapieha P, Hamel D, Shao Z, Rivera JC, Zaniolo K, Joyal JS, Chemtob S. Proliferative retinopathies: angiogenesis that blinds. *Int J Biochem Cell Biol*. 2010; 42:5–12. [PubMed: 19836461]
3. Kompella UB, Kadam RS, Lee VHL. Recent advances in ophthalmic drug delivery. *Therapeutic Delivery*. 2010; 1:435–456. [PubMed: 21399724]
4. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ. Pharmacokinetics of intravitreal ranibizumab (Lucentis). *Ophthalmology*. 2007; 114:2179–82. [PubMed: 18054637]
5. Audren F, Tod M, Massin P, Benosman R, Haouchine B, Erginay A, Caulin C, Gaudric A, Bergmann JF. Pharmacokinetic-pharmacodynamic modeling of the effect of triamcinolone acetonide on central macular thickness in patients with diabetic macular edema. *Invest Ophthalmol Vis Sci*. 2004; 45:3435–41. [PubMed: 15452046]
6. Durairaj C, Shah JC, Senapati S, Kompella UB. Prediction of vitreal half-life based on drug physicochemical properties: quantitative structure-pharmacokinetic relationships (QSPKR). *Pharm Res*. 2009; 26:1236–60. [PubMed: 18841448]
7. Durairaj C, Kim SJ, Edelhauser HF, Shah JC, Kompella UB. Influence of dosage form on the intravitreal pharmacokinetics of diclofenac. *Invest Ophthalmol Vis Sci*. 2009; 50:4887–97. [PubMed: 19516015]
8. Sampatand KM, Garg SJ. Complications of intravitreal injections. *Curr Opin Ophthalmol*. 2010; 21:178–83. [PubMed: 20375895]
9. TaiGen. TaiGen Biotechnology. <http://www.taigenbiotech.com.tw/news.html#4>
10. Liang Z, Brooks J, Willard M, Liang K, Yoon Y, Kang S, Shim H. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. *Biochem Biophys Res Commun*. 2007; 359:716–22. [PubMed: 17559806]
11. Ionomopoulou SM, Kallitsis JK, Voyiatzis GA. Incorporation of small molecular weight active agents into polymeric components. *Recent Pat Drug Deliv Formul*. 2008; 2:94–107. [PubMed: 19075901]
12. Wischkeand C, Schwendeman SP. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *Int J Pharm*. 2008; 364:298–327. [PubMed: 18621492]
13. Vardeand NK, Pack DW. Microspheres for controlled release drug delivery. *Expert Opin Biol Ther*. 2004; 4:35–51. [PubMed: 14680467]
14. Deng JS, Li L, Tian Y, Ginsburg E, Widman M, Myers A. In vitro characterization of polyorthoester microparticles containing bupivacaine. *Pharm Dev Technol*. 2003; 8:31–8. [PubMed: 12665195]
15. Puthliand S, Vavia PR. Stability studies of microparticulate system with piroxicam as model drug. *AAPS PharmSciTech*. 2009; 10:872–80. [PubMed: 19568938]
16. Okadaand H, Toguchi H. Biodegradable microspheres in drug delivery. *Crit Rev Ther Drug Carrier Syst*. 1995; 12:1–99. [PubMed: 8521523]
17. Herrero-Vanrelland R, Refojo MF. Biodegradable microspheres for vitreoretinal drug delivery. *Adv Drug Deliv Rev*. 2001; 52:5–16. [PubMed: 11672871]
18. Kimuraand H, Ogura Y. Biodegradable polymers for ocular drug delivery. *Ophthalmologica*. 2001; 215:143–55. [PubMed: 11340382]
19. Shiveand MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 1997; 28:5–24. [PubMed: 10837562]
20. Roscoe BP, Velthorst R, J E. Dispersion of soil aggregates by varied amount of ultrasonic energy in fractionation of organic matter of clay Latosol: carbon, nitrogen and ^{13}C distribution in particle size fractions. *European Journal of Soil Science*. 2000; 51:445–454.
21. Yeh MK, Chen JL, Chiang CH. In vivo and in vitro characteristics for insulin-loaded PLA microparticles prepared by w/o/w solvent evaporation method with electrolytes in the continuous phase. *J Microencapsul*. 2004; 21:719–28. [PubMed: 15799222]

22. Martin TM, Bandi N, Shulz R, Roberts CB, Kompella UB. Preparation of budesonide and budesonide-PLA microparticles using supercritical fluid precipitation technology. *AAPS PharmSciTech*. 2002; 3:E18. [PubMed: 12916933]
23. Allison SD. Analysis of initial burst in PLGA microparticles. *Expert Opin Drug Deliv*. 2008; 5:615–28. [PubMed: 18532918]
24. Moritera T, Ogura Y, Honda Y, Wada R, Hyon SH, Ikada Y. Microspheres of biodegradable polymers as a drug-delivery system in the vitreous. *Invest Ophthalmol Vis Sci*. 1991; 32:1785–90. [PubMed: 2032801]
25. Kadamand RS, Kompella UB. Influence of lipophilicity on drug partitioning into sclera, choroid-retinal pigment epithelium, retina, trabecular meshwork, and optic nerve. *J Pharmacol Exp Ther*. 332:1107–20. [PubMed: 19926800]
26. Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci*. 2006; 47:1149–60. [PubMed: 16505053]
27. Ayalasomayajulaand SP, Kompella UB. Subconjunctivally administered celecoxib-PLGA microparticles sustain retinal drug levels and alleviate diabetes-induced oxidative stress in a rat model. *Eur J Pharmacol*. 2005; 511:191–8. [PubMed: 15792788]
28. Rafat M, Cleroux CA, Fong WG, Baker AN, Leonard BC, O'Connor MD, Tsilfidis C. PEG-PLA microparticles for encapsulation and delivery of Tat-EGFP to retinal cells. *Biomaterials*. 2010; 31:3414–21. [PubMed: 20149443]

Figure 1.

Influence of formulation variables on particle size and drug loading for TG-0054-poly(L-lactide) microparticles as determined by Box-Behnken statistical design. Statistical design was generated using Statgraphics Centurion XV and the relationship between independent variables (power input and polymer concentration) and dependent variables (particle size and drug loading) was evaluated.

Figure 2.

Differential scanning calorimetry (DSC) thermograms of poly(L-lactide) (PLA) blank microparticles (MP), TG-0054, and TG-0054-PLA microparticles. Thermograms were recorded between 0 and 200°C under inert nitrogen atmosphere at a flow rate of 20 mL/min and at a heating rate of 10 °C/ min using Perkin Elmer, Diamond DSC with hyper DSC™.

Figure 3. Residual methylene chloride (CH_2Cl_2) content in TG-0054-poly(L-lactide) microparticles. Residual solvent analysis for methylene chloride was performed using GC-MS for A) blank; B) CH_2Cl_2 standard, 30 pg; C) CH_2Cl_2 standard, 100 pg; and D) poly(L-lactide) microparticles loaded with TG-0054.

Figure 4. In-vitro drug release from TG-0054-poly(L-lactide) microparticles. Three batches of microparticles with 10 % w/w drug loading were assessed. Data is expressed as mean $\pm$ s.d. for $n = 3$.

Figure 5.

Size and surface morphology of microparticles before and during in vitro release studies. A) Poly(L-lactide) microparticles loaded with TG-0054, assessed before drug release study. B) Appearance of microparticles after 3 months of in vitro drug release. C) Appearance of microparticles after 6 months of in vitro drug release. Pictures were obtained using a scanning electron microscope (SEM) (JEOL) at magnifications 1,000-10,000X.

Figure 6.

Size and surface morphology of microparticles after in vivo drug release in the vitreous humor of New Zealand white rabbit eye. A and B) Appearance of TG-0054-poly(L-lactide) microparticles after 1 month of in vivo drug release. Each row represents a different rabbit. C and D) Appearance of TG-0054-poly(L-lactide) microparticles after 3 months of in vivo drug release. Each row represents a different rabbit. Pictures were obtained using a scanning electron microscope (SEM) (JEOL) at magnifications 1,000-10,000X.

Figure 7. Representative LC-MS/MS chromatogram showing intact TG-0054 and internal standard d8-TG-0054 in the retina samples isolated at 3 months post-dosing from the eyes of New Zealand white rabbits.

Figure 8.

Intravitreally administered poly(L-lactide) microparticles sustain TG-0054 drug delivery to the back of the eye tissues during the three month study period. Tissue levels were quantified using an LC-MS/MS method. For the solution group, which received the drug in both eyes, drug levels were not detected at 3 months. Data is presented as mean $\pm$ s.d. for $n = 3$ for microparticle groups and $n = 6$ for solution groups. * $P < 0.01$ compared to vitreous and choroid-RPE. † $P < 0.01$ compared with 3 months.

Table 1

Calculated power for potential sources of energy input to secondary emulsion.

Method	Intensity and duration	Power input (Watts)
Sonication	3 watts for 30 seconds	25.30
	3 watts for 15 seconds	12.70
Homogenization	10000 rpm for 10 minutes	3.5
	10000 rpm for 5 minutes	2.71
	10000 rpm for 2 minutes	1.71
	5000 rpm for 5 minutes	1.41
Teflon stir bar	1000 rpm for 3 hours	0.18

Table 2

Box-Behnken design to determine the influence of polymer concentration, drug concentration, and power input on drug loading and particle size of poly(L-lactic acid)-TG-0054 microparticles. Actual power inputs close to 1, 13.5, and 26 were chosen for the study based on calculations in Table 1.

Formulation Number	Independent variables				Dependent variables	
	Polymer concentration (%)	Drug concentration (%)	Power input (Watts)	Actual power input (Watts)	Drug loading (%)	Particle size (µm)
1.	4.25	9	26	25.3	5.2	2.4
2.	4.25	8	26	25.3	5.1	1.5
3.	3.5	8	13.5	12.7	11.5	6.5
4.	5	9	1	1.41	6.0	5.8
5.	3.5	9	26	25.3	5.5	5.4
6.	4.25	9	1	1.41	8.0	7.1
7.	4.25	9	13.5	12.7	8.2	5.7
8.	5	10	13.5	12.7	4.0	6.0
9.	5	9	26	25.3	5.1	2.2
10.	3.5	9	1	1.41	5.3	4.0
11.	5	8	13.5	12.7	5.8	6.1
12.	4.25	10	26	25.3	7.7	5.0
13.	3.5	10	13.5	12.7	7.8	1.6
14.	4.25	8	1	1.41	8.2	4.8
15.	4.25	10	1	1.41	7.0	5.0

Amount of TG-0054 in microparticles isolated from rabbit eyes at the end of one and three months. For the 3 month study, only 2 rabbits are reported due to sample loss for rabbit 3.

Table 3

Months	Sample name	Amount of microparticles recovered (mg)	Amount of microparticles used for drug extraction (mg)	Amount of TG-0054 recovered in the particle mass used for extraction (µg)	Concentration of TG-0054 in recovered particles (µg/mg particle)
1 month (15 mg particles with 100 µg drug/mg particles)	Rabbit-1- Microparticles	9.8	9.8	501.80	51.20
	Rabbit-2- Microparticles	8.5	6.2	299.30	48.27
	Rabbit-3- Microparticles	8.6	5.9	184.0	31.18
	Mean ± SD (% Dose)	8.97 ± 0.723 (60%)	7.3 ± 2.17	328.36 ± 160.88	43.55 ± 10.81 (44%)
3 months (15 mg particles with 100 µg drug/mg particles)	Rabbit-1- Microparticles	3.54	1.54	24.69	16.03
	Rabbit-2- Microparticles	4.56	2.56	44.20	17.27
	Mean	4.05 (27%)	2.05	34.45	16.65 (17%)

Table 4

Tissue distribution of TG-0054 in male New Zealand white rabbit ocular tissues after intravitreal injection of microparticle suspension (1.5 mg drug dose) or free drug solution (1 mg dose). Data is expressed as mean $\pm$ SD for n = 3. ND indicates “not detected”.

Tissue name	Tissue Concentrations ($\mu\text{g/g}$ of tissue)			
	Microparticles		Solution	
	1 Month	3 Months	1 Month	3 Months
Retina (Dosed eye)	244.1 $\pm$ 95.3	242.9 $\pm$ 42.6	2.7 $\pm$ 2.8	ND
Retina (Undosed eye)	13.8 $\pm$ 4.2	12.4 $\pm$ 3.3	ND	ND
CRPE (Dosed eye)	33.4 $\pm$ 6.3	62.8 $\pm$ 22.6	4.9 $\pm$ 4.2	ND
CRPE (Undosed eye)	1.28 $\pm$ 0.22	18.9 $\pm$ 0.74	ND	ND
Vitreous (Dosed eye)	28.1 $\pm$ 10.8	43.7 $\pm$ 16.2	0.82 $\pm$ 0.53	ND
Vitreous (Undosed eye)	0.72 $\pm$ 0.26	ND	ND	ND

Table 5

Amount of released drug in retina, choroid-RPE (CRPE), and vitreous humor and amount left in microparticles recovered from the vitreous humor at the end of one month and three months. For retina, choroid-RPE, and vitreous, tissue weights are approximations since mean weights of tissues isolated for drug analysis are reported. Actual tissue weights may be higher due to incomplete isolation of some tissues. Mean tissue weights reported in this table were multiplied by the tissue concentrations reported in Table 4 to obtain total drug amounts in various tissues. Drug doses in microparticle and solution groups were 1.5 and 1 mg, respectively. Data is expressed as mean for n = 3. ND indicates “not detected”.

Sample name	Average Tissue weight (mg)	Microparticles Group		Solution Group	
		Amount of drug at 1 month (µg)	Amount of drug at 3 months (µg)	Amount of drug at 1 month (µg)	Amount of drug at 3 months (µg)
Retina (Dosed eye)	60	14.64	14.57	0.162	ND
Retina (Undosed eye)	60	0.83	0.74	ND	ND
CRPE (Dosed eye)	45	1.50	2.83	0.221	ND
CRPE (Undosed eye)	45	0.058	0.85	ND	ND
Vitreous (Dosed eye)	1400	39.34	61.2	1.15	ND
Vitreous (Undosed eye)	1400	1.01	ND	ND	ND
Microparticles Recovered (%Drug Dose)	-	390 (26%)	67.43 (4.5%)		-
Total drug recovered in dosed eye (Retina + CRPE + Vitreous) (%Drug dose)	-	55.48(3.7%)	78.6 (5.2%)	1.53 (0.10%)	ND
Total drug recovered in undosed eye (Retina + CRPE + Vitreous) (%Drug dose)	-	1.90(0.13%)	1.59(0.11%)	ND	ND
Total drug recovered in dosed eye Retina, CRPE, and Vitreous (Free + Encapsulated) (%Drug dose)	-	445.5 (29.7%)	146.0 (9.7%)	-	ND