INTRODUCTION

Primary intraocular lymphoma (PIOL) is a term that refers to non-metastatic malignant lymphoid neoplasia that arises primarily within the eye. An estimated 100 new cases of PIOL occurred in the United States from 2002 to 2005 with a slight male predominance.\(^1,2\) PIOL occurs in three major clinical forms: (1) primary vitreoretinal lymphoma (PVRL), (2) primary uveal lymphoma, and (3) PIOL with both vitreoretinal and uveal features.\(^3\) PVRL comprises approximately 85% of cases of PIOL encountered in the United States. It represents intraocular...
involvement by primary central nervous system lymphoma (PCNSL). Approximately 80% of patients who have PVRL eventually develop malignant lymphomatous lesions in the brain. 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 most commonly presents as a lesion that simulates intermediate or posterior uveitis with pale, finely dispersed intravitreal lymphoid cells and/or geographic accumulations of neoplastic lymphoid cells beneath the retinal pigment epithelium. Some patients also develop pale yellow retinal infiltrates with associated retinal hemorrhage that resemble foci of microbial retinitis. PVRL involves both eyes in approximately 80% of cases.6 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 two principal treatment options for PVRL/PCNSL are fractionated external beam radiation therapy and intravenous (with or without intrathecal) chemotherapy.

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. The in vitro cytotoxic activity of methotrexate for 63 different cell lines has been evaluated and the therapeutic levels of methotrexate range from 0.1 μM to 1 μM, with a mean IC50 of 0.32 μM.7,8 Different research groups have used the induction-consolidation-maintenance (I-C-M) injection protocols using methotrexate, with some changes in the dosing intervals for the treatment of PIOL. In the largest series of such cases yet reported, an average of 13 injections or less (over a 2-month period) were needed for the eyes to be cleared of malignant cells and retinal infiltrates.9

The drug release kinetics from biodegradable implants have been studied as a function of blending ratios of the high and low molecular weight polymers, and also various design parameters, such as shape, size, and surface area of the implants, were investigated.10–19 Most of the drugs exhibit a tri-phasic drug release profile from biodegradable implants. At the beginning, drugs that are deposited at the surface of the implant are released through initial burst (1st phase). This is followed by a slow and sustained release (2nd phase) of drug attributed to the diffusion of drug through the polymer. Finally, the rapid release of the drug or the late burst (3rd phase) is caused by the swelling and disintegration of the polymeric implant. Ideally, a sustained release device should have a lower initial burst and deliver drug such that the therapeutic levels are achieved for 3–6 months. Recent studies have shown that the first burst can be reduced and the second burst can be prevented for implants prepared with a blend of high and low molecular weight polymers.10 Our group has developed a biodegradable micro-needle implant loaded with 2-methoxyestradiol (25% wt/wt) and achieved a drug release profile without any significant burst following an initial burst, by using a blend of high and low molecular weight polyactic acid.20 No studies have been reported to date of an intravitreal sustained-release device containing methotrexate for treatment of PIOL.

Considering the narrow therapeutic range of methotrexate, it is critical to have prior knowledge of drug distribution and elimination characteristics in order to develop effective treatment protocols for PIOL. The distribution of methotrexate in rabbit and human eyes is significantly influenced by its rate of elimination through retina and Schlemm’s canal. The value of retinal permeability of methotrexate is not available in the published literature. Thus, in this study, we have numerically calculated the permeability of methotrexate across the retina for rabbit and human eyes by making use of the results of the previous in vivo and clinical studies, respectively.5,21 The kinetics of methotrexate were then investigated inside the human eye following the standard induction-consolidation-maintenance (I-C-M) injection protocols. The duration time for which the therapeutic methotrexate levels were maintained in the human eye following different injection doses was calculated and is reported in this manuscript. Keeping in mind that repeated injections of methotrexate for 2 months have induced considerable remission of tumors, a 90-day intravitreal implant was modeled and its mean or average therapeutic release rate was determined in order to have sustained cytotoxic methotrexate levels for 3 months in the vitreous of the human eye.

Sustained-Release-Implantable Devices

A variety of sustained-release drug delivery systems, such as liposomes, microspheres, and implantable devices made of biodegradable or non-biodegradable polymers, have been investigated by previous authors.10–12 Over the last two decades, such devices have been approved by the U.S. FDA for delivery of prolonged therapeutic levels of ganciclovir in patients with cytomegalovirus retinitis and acquired immune deficiency syndrome, and also other devices have been developed for the treatment of uveitis, proliferative vitreoretinopathy, and macular edema.13–16
METHODOLOGY

In this section, the geometry of the computational eye models, conservation equations, and the boundary conditions used for the numerical simulations are explained. Also, various model parameters and the solution methodology employed for evaluating the convective-diffusive transport of methotrexate within rabbit and human eyes are discussed in detail.

Model Development and Conservation Equations

The 3-dimensional model of the rabbit eye was adapted from our previous studies and a human eye model was constructed close to physiological dimensions.\textsuperscript{22–27} The detailed explanation of the model construction was provided in our previous articles.\textsuperscript{25,26} The twelve-compartment eye models consist of sclera, retina-choroid, vitreous (assumed to be gelatinous), lens, Schlemm’s canal, ciliary process, hyaloid membrane, posterior chamber, iris, anterior chamber, cornea, and a drug source at a desired location. The cross sectional view of the 3D rabbit and human eye models are shown in Figure 1. The most significant differences between a rabbit eye and a human eye are the size of lens, volume of vitreous humor, and the flow dynamics inside posterior and anterior chambers. The volume of the vitreous humor in rabbit and human eyes is 1.5 ml and 4 ml, respectively.\textsuperscript{8,23,24}

The unique physiological retinal permeability of any drug can be estimated numerically, if the exact initial location of the drug source (injection or implantation site) and the distribution of the drug are known from the in vivo studies. In this study, we have determined the retinal permeability of methotrexate computationally by developing numerical eye models matching with the experimental conditions. The location and volume of the injection site, and the total mass of methotrexate used for the model simulations corresponded with the previously reported in vivo and clinical data,\textsuperscript{8,21} which is shown in Table 1. Half of the eye models were constructed for both rabbits and humans with the unique symmetry plane passing through the middle of the drug site and other eye compartments. With the calculated retinal permeability value, kinetics of methotrexate was then analyzed in a human eye following an intravitreal injection and intravitreal implant. In the clinical studies, the methotrexate was intravitreally injected into the human subjects at the level of pars plana. Thus, in this study, the drug source was modeled closer to the hyaloid membrane for simulating the kinetics of methotrexate in a human eye. Based on the data from previously published literature, an injection volume of 100 µL was chosen for simulating different injection doses, whereas the 8.5 mg implant was modeled for evaluating the therapeutic release rate of a 90-day intravitreal implant.\textsuperscript{9,18,19,21,28,29} The drug site was represented as a cylinder in our numerical models for both injection and implant (i.e., the injected drug was assumed to take the shape of a cylinder in the vitreous).

![Diagram of eye models](https://example.com/diagram)

**FIGURE 1** Cross sectional view of human and rabbit eye models. (A) Twelve compartments of the eye models. (B) Dimensions of the eye models.
The distribution and elimination characteristics of any drug administered into the eye are significantly affected by both convection and diffusion. The diffusion of the drug in the eye is driven by the concentration gradient and the convection is due to the steady permeation of vitreous humor into the posterior segment of the eye. The flows of aqueous and vitreous humor were assumed to be steady and independent of drug concentration. The coupled convective-diffusive transport of methotrexate in the eye was evaluated by solving the nonlinear Navier-Stokes equations [Eqs. (1) and (2)]. The distribution of methotrexate was then calculated by coupling the species mass (methotrexate) conservation equation [Eq. (3)] with the calculated flow field (Fidap; Ansys Inc., USA, version 8.6):

\begin{align}
\nabla \cdot (U) &= 0 \quad (1) \\
U \cdot \Delta U &= -\nabla P + \mu \nabla^2 U \quad (2) \\
\frac{\partial C}{\partial t} + U \cdot \nabla C &= D \nabla^2 C \quad (3)
\end{align}

where \( P \) is pressure (g/cm\(^2\) or µPa), \( U \) is velocity vector (cm/s), \( \rho \) and \( \mu \) are the density (g/cm\(^3\)) and viscosity (g/cm-s), respectively, of aqueous and vitreous humor, and \( C \) and \( D \) are the concentration (g/ml or µM) and diffusivity (cm\(^2\)/s) of the drug, respectively.

The initial and boundary conditions used for the model simulations were well explained in our previous literature.\(^{25,26}\) Briefly, the ciliary process was modeled as a fluid source, which generates aqueous humor at a constant flow rate of 2.2 µL/min in a rabbit eye and 2.5 µL/min in a human eye.\(^{26,27}\) The vitreous outflow was modeled as 0.1 µL/min and 0.14 µL/min for rabbit and human eyes, respectively.\(^{22,27}\) The pressure at the outer surface of the sclera was set to zero gauge pressure and the upstream pressure was calculated based on the prescribed flow rate as boundary condition. The choroid was treated as a perfect sink and the drug passing through it was assumed to be cleared by the vast network of blood capillaries. So, the drug concentration at the outer surface of the retina-choroid was set to zero. Also, a zero species gradient boundary condition was imposed at the outer surface of the Schlemm’s canal, at all the symmetry surfaces, and also at surfaces that are assumed to be impermeable to the drug (iris, lens, and cornea). Intravitreal injection of methotrexate with various doses was modeled by specifying a corresponding initial concentration of the drug throughout the cylindrical injection volume. In contrast, for an intravitreal implant with different release rates, an appropriate amount of drug was delivered with a constant flux at the outer surface of the drug source over a period of 90 days. Elsewhere in the model, the initial species concentration was set to zero for both injection and implant.

### Model Parameters and Solution Methodology

The fluid properties of the aqueous and vitreous humor were nearly identical to that of water; hence, a viscosity of 6.9 × 10\(^{-3}\) g/cm-s and a density of 1 g/cm\(^3\) were used for the computations.\(^{25,26}\) Since the diffusivity value of methotrexate was not available from published literature, the diffusivity of methotrexate for different compartments in the eye model was estimated [Eq. (4)] from diffusion coefficients of ganciclovir that were previously published.\(^{30}\) Using the Wilke-Chang correlation and assuming that the molar volume of each species at its normal boiling point is proportional to its molecular weight leads to the following expression:

\[
D_{\text{MTX}} = \left( \frac{MW_{\text{GCV}}}{MW_{\text{MTX}}} \right)^{0.6} D_{\text{GCV}}
\]

where \( D \) and \( MW \) are the diffusivity and molecular weight, respectively, of methotrexate and ganciclovir. The diffusivity values of methotrexate were determined as 7 × 10\(^{-6}\) cm\(^2\)/s in the vitreous and 7.1 × 10\(^{-6}\) cm\(^2\)/s in aqueous humor (posterior and anterior chambers).

The retinal permeability value of methotrexate was the only unknown parameter in the model and numerical simulations were conducted by varying its value from 1 × 10\(^{-4}\) cm/s to 1 × 10\(^{-7}\) cm/s. The numerically obtained data was then compared with the \textit{in vivo} and clinical data to determine the retinal permeability value of methotrexate for rabbit and human eyes.\(^{8,21}\) Using the calculated retinal permeability of methotrexate, numerical simulations were performed to analyze the kinetics of methotrexate in a human eye following the existing I-C-M protocols. The intra-vitreal injections of methotrexate were simulated in

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### TABLE 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Injection location</th>
<th>Administered amount</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit eye</td>
<td>3 mm posterior to limbus</td>
<td>400 µg in 32 µL</td>
<td>Velez et al.(^{8})</td>
</tr>
<tr>
<td>Human eye</td>
<td>3 mm posterior to limbus</td>
<td>400 µg in 100 µL</td>
<td>de Smet et al.(^{21})</td>
</tr>
</tbody>
</table>
the human eye according to the standard protocol (de Smet et al.) of twice-weekly injections for 4 weeks (induction phase), followed by weekly consolidation injections for 1 month (consolidation phase), and followed by monthly injections for 9 months to 1 year (maintenance phase). The above protocol has been modified at Hadassah University Hospital (Frenkel et al.) such that the consolidation injections were given for 2 months instead of 1 month of the treatment period. The kinetics of methotrexate following the protocols used by de Smet et al. and Frenkel et al. were evaluated. Furthermore, the release rate of an intravitreal implant that would maintain therapeutic levels of methotrexate for 3 months in the vitreous of a human eye was estimated.

RESULTS

In this section, we first present the retinal permeability value of methotrexate for rabbit and human eyes based on numerical results and then investigate its kinetics in a human eye following the standard I-C-M protocols that are currently being used for the treatment of PIOL. Also, the mean release rate of a 90-day methotrexate implant that needs to be achieved in the in vitro experiments for maintaining therapeutic levels in the vitreous is discussed in this manuscript. In the current study, we have considered the therapeutic range of methotrexate as 0.1 µM–1 µM.

Retinal Permeability and Half Life of Methotrexate in Rabbit and Human Eyes

The best fit data obtained between the simulated methotrexate vitreous concentrations and the experimentally determined concentrations published by Velez et al. and de Smet et al. for rabbit and human eyes, respectively, are shown in Figure 2. For the selected material, properties and boundary conditions, the calculated numerical concentrations agreed well with the in vivo and clinical data of methotrexate for rabbit and human eyes. This validation provides strong support for the numerical methodology employed in the current study. The retinal permeability values of methotrexate obtained from the validation of numerical models with the experimental data (Figure 2) were 1.1 × 10⁻⁵ cm/s and 9.25 × 10⁻⁶ cm/s for rabbit and human eyes, respectively.

A linear relation on a semi-logarithmic plot (Figure 2) suggests a first order elimination process for methotrexate. The half life of methotrexate following an intravitreal injection was determined by using the slopes (Kₜ, defined as the fraction of the drug in the eye eliminated per unit time) of mean vitreous concentration curve with time. The half-life (t½ = 0.693/Kₜ) of methotrexate in the vitreous was determined as 7.1 hr for a rabbit eye and 14.3 hr for a human eye. The difference between half-lives of methotrexate in rabbit and human eyes could be attributed to the differences in their

FIGURE 2 Comparison of the model simulated vitreous concentrations with the available in vivo and clinical data. (A) Numerical eye models were matched with the experimental conditions (injection location, volume, and dose). (B) Comparison of simulated methotrexate vitreous concentrations and the experimentally determined concentrations published by Velez et al. and de Smet et al. Exponential fit for the in vivo data of rabbit and human eyes were represented as grey dotted lines.
retinal permeability values and volume of the vitreous humor as well as its perfusion which, in turn, affected the drug clearance.

Elimination of Methotrexate in Rabbit and Human Eyes Following an Intravitreal Injection

The percentage cumulative amount of methotrexate cleared through choroid and Schlemm’s canal of rabbit and human eyes following an intravitreal injection is shown in Figure 3. The total amount of methotrexate cleared through choroid following an injection was calculated to be 13.6% for a rabbit eye and 7.1% for a human eye. The remaining 86.4% and 92.9% of methotrexate was washed out by the aqueous flow through the Schlemm’s canal for rabbit and human eyes, respectively. The above result suggests that a greater amount of methotrexate is cleared through choroid for a rabbit eye when compared to a human eye. This increase in clearance of methotrexate through choroid of the rabbit eye, when compared to a human eye, can be attributed to its high retinal permeability value. Thus, the retinal permeability of a drug has significant effect on its elimination characteristics, and the higher the permeability, the greater the elimination will be through the choroid.

Methotrexate Concentration in the Human Eye Following Standard Induction-Consolidation-Maintenance (I-C-M) Protocols

The mean vitreous concentration of methotrexate in a human eye for the existing I-C-M regimens in which methotrexate was re-injected at different time intervals is shown in Figure 4. The treatment protocols used by de Smet et al. and Frankel et al. were simulated for 5 months of the treatment period and are shown in the Figures 4A and 4B, respectively. The duration for which methotrexate concentration levels are less than 0.001 µM in the maintenance phase has been represented as dotted lines in Figures 4A and 4B. The methotrexate concentration profile is similar in the induction phase for both the protocols, as the dosing interval and injection dose (400 µg) used were identical in both cases.

From Figure 4A it can be noticed that the methotrexate levels drop below 0.001 µM just after 2 months of the treatment following the protocol used by de Smet et al. Whereas, in the case of protocol used by Frenkel et al., the drop in methotrexate levels to 0.001 µM, occur well after 3 months from the start of the treatment (Figure 4B). The peak concentration of methotrexate achieved during all three phases of treatment following the dosing interval that was selected in the above discussed standard regimens was approximately 269 µM or 122 µg/mL. Given that the dosage above 0.1 µM is considered tumoricidal, it can be stated from Figures 4A and 4B that the therapeutic methotrexate levels are consistently achieved only in the induction phase of the treatment and are nearly maintained during the consolidation phase. The methotrexate levels drop below the therapeutic range during the maintenance phase, following the above mentioned protocols.

Kinetics of Methotrexate in the Human Eye Following Different Doses of Intravitreal Injection

In order to determine the appropriate injection dose for the treatment of PIOL using the I-C-M protocols, kinetics of methotrexate following different injection doses needs evaluation. Hence, the variation of the mean vitreous concentration of methotrexate in a human eye with different doses of intravitreal injection was compared and is shown in Figure 5. The peak methotrexate concentration achieved in the vitreous following different injection doses, and the duration of time for which the methotrexate levels were within the therapeutic range, were calculated and are shown in Table 2. The average time for which methotrexate levels inside the vitreous were within therapeutic range following various injection doses was determined as 45 ± 4 hr. The peak concentration of methotrexate in the vitreous following various injection doses is shown in Table 2. It is important to avoid high concentrations of methotrexate that are toxic to normal tissues in the eye. From these results, one could not only select an appropriate injection dose, but also estimate the dosing...
Investigation of Kinetics of Methotrexate

FIGURE 4 Model-simulated kinetics of methotrexate in a human eye following the existing Induction-Consolidation-Maintenance (I-C-M) protocols. (A) Protocol used by de Smet et al.²⁹ (B) Protocol used by Frenkel et al.⁹

FIGURE 5 Mean vitreous concentration of methotrexate in a human eye following different doses of intravitreal injection.

TABLE 2 Duration of time therapeutic levels of methotrexate were achieved in the vitreous of a human eye following different intravitreal injection doses

<table>
<thead>
<tr>
<th>Intravitreal injection dose of methotrexate (µg)</th>
<th>200 µg</th>
<th>400 µg</th>
<th>600 µg</th>
<th>800 µg</th>
<th>1500 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak methotrexate concentration achieved (µM)</td>
<td>132.7</td>
<td>264.8</td>
<td>396.5</td>
<td>529.4</td>
<td>982.3</td>
</tr>
<tr>
<td>Time (hr) for methotrexate levels (&gt;1 µM)</td>
<td>102</td>
<td>114</td>
<td>125</td>
<td>132</td>
<td>143</td>
</tr>
<tr>
<td>Time (hr) for methotrexate levels (0.1–1 µM)</td>
<td>46</td>
<td>49</td>
<td>47</td>
<td>44</td>
<td>41</td>
</tr>
</tbody>
</table>
interval for developing therapeutic protocols for PIOL using serial injections of methotrexate.

**Therapeutic Release Rate of a 90-day Sustained-Release Methotrexate Implant**

The overall drug release profile can be controlled in the *in vitro* experiments by many factors including polymer microstructure, copolymer ratio, drug content, and implant design. Using an effective sustained release device, therapeutic drug levels can be achieved in vitreous for 3–6 months, thus avoiding multiple surgeries. As a concluding part of this study, we have numerically calculated drug concentration in the eye based on a new generation implant having an optimal and a predetermined methotrexate-release rate that is within the therapeutic range. The temporal distribution of methotrexate inside the vitreous of a human eye following a 90-day implant with different release rates is shown in Figure 6. It can be noticed from Figure 6 that in order to achieve tumoricidal methotrexate levels in the vitreous for 3 months, a sustained-release implant with a mean release rate of 0.2–2 µg/day should be designed for therapeutic treatment of PIOL. For our calculations, we have assumed that the drug will be released from the implant uniformly over time.

**DISCUSSION**

PIOL provides a diagnostic and therapeutic challenge because of its non-specific ocular symptoms, diverse clinical picture, nonspecific presentation, and variable clinical course. The incidence of PIOL, which was previously a rare condition, has increased dramatically since the 1970s. The response of tumors to the intravitreal injections of methotrexate is encouraging from the studies conducted by different research groups. Even though injection therapy gained widespread acceptance because of the complete remission of tumors, other alternatives need to be considered as intravitreal injection is associated with severe complications in some patients. At times during these treatments, the tolerance of the patients towards the injections was very poor. For such patients, the treatment period was reduced to 2 months; however, with an uncertainty of tumor recurrence. Hence, in the present study, we have numerically analyzed the kinetics of methotrexate in a human eye following the standard I-C-M injection protocols and investigated the scope for reducing the number of injections during the treatment.

The permeability of methotrexate across the retina has a significant impact on its clearance from the vitreous. It is a cumbersome process to measure the permeability of retinal tissues to various drugs through *in vivo* experiments. Thus, in this study, we have numerically calculated and determined the retinal permeability of methotrexate for albino rabbit and human eyes (Figure 2) by matching the calculated vitreous concentrations with the *in vivo* data (mean or volume averaged methotrexate concentration in entire vitreous) and clinical data (concentration of methotrexate in the extracted sample) that has been previously published. The calculated retinal permeability of methotrexate in a human eye (pigmented mammal) was lower than that in the albino rabbit eye. This observation is consistent with a previous study, in which the retinal elimination rate constant (normalized for retinal surface area, distribution volume, and anatomic volume) of ganciclovir was slightly greater in a rabbit eye (0.017 cm⁻² hr⁻¹) as compared to the human eye (0.015 cm⁻² hr⁻¹), thus indicating a higher retinal permeability in rabbits. However, in this study, the binding of methotrexate to ocular tissues, its metabolism, and active transport process have not been incorporated in the numerical method. All the above mentioned factors could affect the retinal permeability of methotrexate and in turn affects the methotrexate distribution in the eye.

Previous studies have investigated the inward and outward retinal permeability and attributed an active efflux mechanism for higher outward permeability. This higher value of the permeability in the outward direction is because of the active pumping by the retinal pigment epithelium, which is known to pump water from the retinal space into the choroidal circulation. Since the retinal permeability is iterated and calculated to match the measured concentration data, the reaction kinetics for binding, association, dissociation, and other active transport are, in a way, indirectly included in the
retinal permeability value. In other words, the value of retinal permeability is a lumped or bulk parameter that includes the effect of reaction kinetics and active transport in an indirect manner.

Most of the drugs that are intravitreally administered will be eliminated through anterior and posterior routes. The clearance of methotrexate in rabbit and human eyes following an intravitreal injection was investigated in this study (Figure 3). It was observed that because of high retinal permeability of methotrexate in the rabbit eye when compared to that of a human eye, there is a greater clearance of methotrexate through the choroid in a rabbit eye as opposed to that in a human eye. Previous numerical studies have shown that increasing the retinal permeability increases the rate of clearance of the drug from the eye, thus, resulting in a lower half life.24,25 Also, it is apparent that if the drug source was modeled close to the retina or at the center of the eye, the amount of methotrexate washed away by aqueous humor will be reduced.

Half-lives of most of the drugs that are injected into the vitreous cavity are predominantly low because of the continuous clearance of the drug across the blood eye barriers, aqueous humor turnover, and uveal blood flow. Since the half life of methotrexate is of the order of only a few hours, frequent injections are required to maintain cytotoxic levels, which are often associated with ocular complications, such as vitreous hemorrhage, endophthalmitis, and retinal detachment. Initial clinical experiments have shown that tumoricidal methotrexate levels can be maintained in the vitreous for up to 3–5 days following an intravitreal injection (400 µg), suggesting a rapid elimination of the drug.21 Hence, in order to maintain tumoricidal methotrexate levels consistently for a longer duration (2–3 months), repeated administrations are necessary. Based on this result, I-C-M protocols have been developed such that twice-weekly, weekly, and monthly injections were given as induction, consolidation, and maintenance phases, respectively. Different research groups have used the I-C-M injection protocols with some changes in the dosing intervals.9,29,33 In a study conducted on an Asian patient,33 twice-weekly injections of methotrexate were given for 3 weeks as compared to the standard 4 weeks of the induction phase.

In the present study, we have simulated the kinetics of methotrexate in a human eye following the protocols used by de Smet et al.29 and Frenkel et al.9 (Figures 4A, 4B), as it is not feasible to experimentally measure the concentration levels of methotrexate at different time points during the treatment period. Following the above mentioned I-C-M protocols with an injection dose of 400 µg, cytotoxic levels of methotrexate were not achieved consistently in the vitreous for part of the consolidation and maintenance phases of the treatment. We have calculated the mean vitreous concentration of methotrexate in a human eye for various injection doses not only to analyze the scope of using different injection doses for I-C-M protocols but also to reduce the number of injections during the treatment. It can be observed (Figure 5) that tumoricidal methotrexate levels (>1 µM) can still be achieved in the induction phase by using a lower injection dose of 200 µg instead of 400 µg. By using an injection dose of 200 µg in the induction phase of the treatment, the peak concentration of methotrexate can be reduced by 50% as compared to an injection dose of 400 µg.

It is evident from Figure 5 that even by increasing the injection dose (>400 µg), it might not be possible to reduce the number of injections significantly during the treatment, as the methotrexate is rapidly eliminated from the vitreous. By increasing the injection dose, higher methotrexate levels (>0.1 µM) can be achieved for a few additional days when compared to the lower injection doses, but it could lead to higher toxicity to normal retinal tissues. The other alternative is to vary the dosing interval so that cytotoxic methotrexate levels are achieved during the treatment period with a minimum number of injections. It can be noticed from Figures 4A and 4B that the number of injections needed for the treatment could be significantly reduced by giving weekly injections in the induction phase instead of twice-weekly injections. From our numerical calculations (Figures 4A, 4B), we have evaluated that the tumoricidal levels of methotrexate are reasonably achieved in the consolidation phase following weekly injections (400 µg). Thus, the possibility of having induction and consolidation phases with the weekly injections (with a dose ≥400 µg) for the first 2–3 months of the treatment period, can be considered in future experiments. The results of this study, when used in combination with the toxicological studies, could provide more information about the appropriate injection dose and the dosing interval that needs to be developed for the therapeutic treatment of PIOL.

Considering the tolerance of the patient and the surgical procedure involved in injection therapy, development of a new treatment modality is necessary. Various sustained-release devices have been investigated to achieve therapeutic drug levels in the eye while avoiding toxic or sub-therapeutic levels and some of these studies have reached a clinical phase. In a study conducted by Whitcup et al.,40 sustained-release methotrexate implants were developed in vitro using polyvinyl alcohol for the treatment of PIOL. However, it was reported that the implants were designed to maintain therapeutic levels only for 2 weeks to 1 month. Considering that the cytotoxic methotrexate levels need to be achieved for at least
REFERENCES


by intravitreal injection and controlled release implant. 


