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A technique for drug surrogate diffusion coefficient measurement by intravitreal injection

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ABSTRACT

The aim of this work is to develop a technique to accurately measure the diffusion coefficient of drugs and drug surrogates in the vitreous humor using MRI. Fresh bovine eyes were used for drug diffusion study in the vitreous, and Gd-DTPA was chosen as a drug surrogate/contrast agent to visualize the diffusion process by MRI. Experiments were conducted by injecting 30 μl of the Gd-DTPA with a concentration of 20 mM/l in 0.9% saline in the vitreous of the whole bovine eye. MRI images were acquired at regular intervals for 1.5–2 h, and Gd-DTPA concentration was determined from the MRI signal intensity. At each time point, concentration contours were constructed and a least-squares best fit to the corresponding theoretical contours, based on a cylindrical bolus model, was performed. The best fit at different time points resulted in fairly consistent diffusion coefficient values. With the surrogate injection technique perfected, highly-symmetric distribution of Gd-DTPA was observed, allowing for spherically symmetric mathematical models, with adjustments for ellipticity as needed, for the diffusion coefficient analysis. The analysis yielded an average diffusion coefficient value of $(3.040 \pm 0.274) \times 10^{-6}$ cm^2/s. The 3-D MRI visualization together with careful symmetric injection of the surrogate/contrast agent has provided a quantitative tool for the accurate measurement of the diffusion coefficient. With symmetric injection and corresponding diffusion theory based on the point-source model with adjustments for nonzero bolus size and asymmetry, strong agreement of theory with experiments has been achieved.

1. Introduction

Drug delivery to the posterior segment of the eye is a challenging task because of the various physiological and anatomical barriers which affect the drug concentration distribution [1]. Low drug concentrations are insufficient to treat the retinal disease and high concentrations can be toxic, depending on the specific drug and its toxicity. It is therefore imperative that unnecessary drug distribution in healthy tissues be minimized by every viable means. While intravitreal delivery allows high concentrations of drug in the retina, the surgical procedure carries risks of side effects that include cataract, retinal detachment, and endophthalmitis [2–4]. Nevertheless, the use of intravitreal corticosteroids for DME (Diabetic Macular Edema) and intravitreal injections of anti-vascular endothelial growth factor (VEGF) for CNV (Choroidal Neovascularization) have revolutionized the treatment of these diseases.

Delivering drug to the posterior segment of the eye requires detailed information about the transport mechanisms and major diffusion barriers present in the eye. Nevertheless, with the advances that have been made in the area of drug delivery, several open questions relating to the feasibility, maintenance of drug levels within a desired optimum concentration range and achievement of more effective therapies still remain. Most of the drugs used to treat vitreoretinal diseases have many limitations and side effects. Therefore, it is critical to predict drug concentration gradients in the vitreous and the accompanying transport mechanisms (diffusion and convection) in order to control drug delivery rate to achieve maximum effectiveness. While the scientific expertise of pharmacologists and biochemists has led to the development of various successful drugs, the focused distribution to the target areas requires an understanding of the fluid mechanics and transport phenomena. Accurate diffusion coefficient measurements are necessary for development of algorithms for the prediction of transport rates of different compounds for potential application

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Nomenclature

a	half-height of cylindrical bolus
b	radius of cylindrical bolus
c	drug surrogate concentration
$C_{empirical,i}$	measured concentration at various locations and times
$C_{model,i}$	calculated concentration at measured locations
c_0	initial bolus concentration
D	diffusion coefficient
Fo	Dt/R^2, Fourier number
M	molar amount of deposited drug surrogate
r	radial coordinate (spherical)
r_0	bolus radius
R	spherical model shell radius
SD	standard deviation
t	time
x,y,z	Cartesian coordinates

in ocular drug delivery. The overall drug transport is based on both diffusion and convection [5], and the latter can be quite important for large-molecular weight drugs. Convection is also very significant in the case of a liquefied vitreous in which the high mobility of the liquid facilitates such transport. Pioneering studies on the diffusion properties in ocular tissue have been conducted by Maurice and coworkers [6–8], with the use of fluorescein. In a live vitreous, convection and diffusion usually happen together and often the combined drug transport rate is measured. With such measurements, it is convenient to refer to an effective diffusion coefficient. However, the two processes are driven by separate mechanisms, and we would like to reinforce the physics whereby these processes are treated as separate. It is of course well known that diffusion happens due to a concentration gradient while convection is controlled by the water flow that carries the solute (see e.g., Vafai [9], Khanafer and Vafai [10], Mukundakrishnan and Ayyaswamy [11] and Siggers and Ethier [5]). Perfusion in tissue plays an important role for both thermal [12,13] and solutal transport. While both thermal and solutal convection can be quite substantial in fully liquid regions of the eye such as the aqueous [13], a young vitreous being in a fully gel state does not have as much free movement of the liquid except by Darcy-type convection. Nevertheless, we are presently interested in pure diffusive transport of the solute. The proper mathematical modeling requires knowledge of the diffusion coefficient D, and an accurate measurement will entail the complete stoppage of the convection, or very accurate accounting of the convection-driven transport to be subtracted from the overall transport. The latter can be quite complicated since the fluid velocity varies all over the vitreous humor. To accurately measure D, it is much easier if convective transport is stopped (as is the case in the ex vivo eye). For a comprehensive mathematical model, the value of D will be used only to account for the diffusion and at the same time, convection will be included as a separate added-on process. Therefore, we are taking an approach to the full problem by isolating the diffusion process from the convection in the ex vivo bovine model. It is important to point out that the predictive models backed by sound experiments will indeed provide the drug delivery rate to the retina as a function of the input parameters.

Given the complexity of the transport processes in the eye, among the available avenues for scientific and translational progress necessarily includes the mathematical modeling alongside with careful experimentation. MRI visualization has provided a great deal of insight into the transport processes following intravitreal injection (see e.g., Li et al. [14,15]). In the last few decades, there has been considerable effort with the numerical modeling of ocular transport processes [16–22]. Investigations have also been conducted on the effect of saccadic motion on convective transport which is quite substantial if the vitreous is liquefied [23–27]. Fundamental to such calculations are accurate measurement of the transport properties of the vitreous, in particular the diffusion coefficient. This is the focus of the present work, and we have developed a technique that delivers the value of the diffusion coefficient through an inverse-problem solution from the concentration distribution data at various values of lapsed time after the injection of the surrogate.

The approach taken is one in which we inject the surrogate as a spike, maintaining as much spherical symmetry as possible, and use a model with like symmetry to invert the concentration data to the diffusion coefficient value. Understandably, drug delivery is not typically done in this manner but the reason for this approach is to take advantage of as much symmetry as achievable to minimize the mathematical complexity. We are applying a procedure as follows:

1. Inject surrogate in the middle of the ex vivo eye, and obtain the concentration distribution by MRI visualization for various time points for that eye.
2. Develop an analytical expression for the distribution for an initial bolus shape (ellipsoidal) for unknown diffusion coefficient.
3. Least-squares fit the experimental concentration contours at different time values with the theoretical ones, and deduce the value of D based on the best fit.

The effectiveness and the accuracy of this procedure lies in the bolus injection being close to spherical symmetry since the theory is based on the relative simplicity (and analytical tractability) of a spherically-symmetric initial injection. These aspects are discussed in Methods and Results sections. Initially, the point source model with spherically symmetric distribution was adopted, and a value of the diffusion coefficient was obtained by the above methodology (items 1 and 3). It was then realized that the fit on the contours could be improved, and the overall standard deviation reduced by bringing the model closer to the actual injection. However, an estimate of D was obtained and used to obtain numerical values of the concentration distribution for various models considered to analyze the deviation from sphericity, nonzero bolus size and wall effects of the eyeball (see Section 2.3).

2. Methods and materials

To understand the approach, we first examine the distribution resulting from a single spike of a drug (or a drug surrogate) in an infinite medium,

$$c(r,t) = \frac{M}{8(\pi D t)^{\frac{3}{2}}} \exp\left(-\frac{r^2}{4Dt}\right), \tag{1}$$

where M is the molar amount of the drug deposited, r is the radial distance from the point of injection, D is the diffusion coefficient, t is the time, and $c(r,t)$ is the concentration. This distribution is based on complete spherical symmetry of the injection, and that the injected volume is small enough to be regarded as a point source. With our injection technique, we have come very close to this realization by carrying out the injection at a very slow rate (3 μl/m) and using a stereotactic stand described in Fig. 1.