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Barrier analysis of periocular drug delivery to the posterior segment

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ABSTRACT

Periocular administration is a potential way of delivering drugs to their targets in posterior eye segment (vitreous, neural retina, retinal pigment epithelium (RPE), choroid). Purpose of this study was to evaluate the role of the barriers in periocular drug delivery. Permeation of FITC-dextrans and oligonucleotides in the bovine sclera was assessed with and without Pluronic gel in the donor compartment. Computational model for subconjunctival drug delivery to the choroid and neural retina/vitreous was built based on clearance concept. Kinetic parameters for small hydrophilic and lipophilic drug molecules, and a macromolecule were obtained from published *ex vivo* and *in vivo* animal experiments. High negative charge field of oligonucleotides slows down their permeation in the sclera. Pluronic does not provide adequate rate control to modify posterior segment drug delivery. Theoretical calculations for subconjunctival drug administration indicated that local clearance by the blood flow and lymphatics removes most of the drug dose which is in accordance with experimental results. Calculations suggested that choroidal blood flow removes most of the drug that has reached the choroid, but this requires experimental verification. Calculations at steady state using the same subconconjunctival input rate showed that the choroidal and vitreal concentrations of the macromolecule is 2–3 orders of magnitude higher than that of small molecules. The evaluation of the roles of the barriers augments to design new drug delivery strategies for posterior segment of the eye.

1. Introduction

Posterior segment of the eye is an important target of drug treatment in ophthalmology. Neural retina, choroid, and vitreous are affected in many ophthalmic diseases. For example, age-related macular degeneration (AMD), glaucoma, diabetic retinopathy, and various forms of retinitis pigmentosa are damaging the posterior eye segment, where they may lead to impaired vision and even blindness. AMD is affecting tens of millions inhabitants in Europe, and the overall numbers worldwide are even higher [1].

The clinical and experimental drugs for the posterior segment treatments include both small and large molecules (such as siRNA, antibodies, growth factors, DNA), but their delivery to the posterior segment of the eye is problematic. Topical eye drops do not deliver drug effectively to the retina and choroid: the concentration in those tissues is circa 10^5 times lower than in the tear fluid [2]. The systemic drug administration is not a viable alternative either. Only very small fraction of the systemic dose reaches the eye due to the blood-retina barrier that limits the drug access to the posterior tissues of the eye [3].

Intravitreal injections and implants deliver drugs effectively to the retina and choroid. For example, intravitreal antibodies are widely used in the treatment of the wet form of AMD. Intravitreal drug administration is invasive and potentially risky, and may cause severe adverse effects such as endophthalmitis and retinal detachment. Even though the complications are rare, the number of adverse reactions will inevitably increase when millions of patients will be treated using repeated intravitreal drugs for years or decades. Alternative modes of drug delivery and improved long-acting formulations are needed [4].

Periocular route is less invasive than intravitreal administration and it provides higher retinal and vitreal drug bioavailability (about 0.01–0.1%) compared to the eye drops (about 0.001% or less) [2,5]. Periocular drug delivery can be accomplished as injections or implantation of the drug to the sub-conjunctival, sub-tenon or parabulbar space. The drug must permeate across several barriers to reach the target sites in the choroid, RPE or neural retina [3]. The physical barriers include sclera, choroid and RPE, whereas the lymphatic flow in the conjunctiva and episclera, and the blood flow of the conjunctiva and choroid constitute the physiological barriers.

Periocular barriers for drug delivery have been investigated using ocular membranes *ex vivo* (sclera, RPE) and drug distribution studies

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in animals *in vivo*. Conrad and Robinson [6] showed that subconjunctival drug permeation takes place primarily via drug distribution through the sclera into the posterior ocular tissues. Spilling of the drug to the lacrimal fluid and subsequent corneal absorption did not contribute significantly. Ahmed and Patton [7] demonstrated that transscleral permeation of timolol and inulin delivered the drug to the uvea and posterior tissues, but not to the aqueous humour. Injected small molecules are eliminated rapidly from the subconjunctival administration site, presumably via conjunctival and episcleral blood and lymphatic flow [8,9], but the elimination of proteins is 1–2 orders of magnitude slower [10]. The role of blood and lymphatic flow was proven in experiments with post-mortem rabbits showing slower drug elimination from subconjunctival site and significantly improved delivery to the posterior segment [8]. Sclera is permeable to hydrophilic compounds, even macromolecules [11–14], but the permeability in the RPE is 1–2 orders of magnitude lower than in the sclera [15]. Selective block of the choroidal blood flow did not have significant influence on the retinal and subconjunctival drug concentrations suggesting minor barrier role for choroidal blood flow in posterior segment drug delivery [16]. Conjunctival and episcleral blood and lymphatic flows are considered to be the main limiting factors in posterior segment drug distribution after subconjunctival drug administration, whereas anatomic barriers and choroidal blood flow are claimed to be less important [8,16]. The assessment of the relative roles of these barriers is not easy, because they form a complex interacting system. In addition, roles of the barriers are probably dependent on drug properties and this aspect has not been discussed in the literature.

Pharmacokinetic models may be helpful in evaluation of the complex interplay between the anatomical and physiological barriers of periocular drug delivery. Lee and Robinson presented simplified three-compartment models with first-order rate constants [17,18]. More complex models have been built and parameters solved by curve fitting [19,20]. Gabhann et al. [21] presented a complex model for periocular administration of green fluorescent protein (GFP), but also this model is partly based on fitted values, mouse data have been used, and only GFP was used as the drug. These models are not entirely physiologically based and the influence of drug on the roles of the barriers has not been taken into account.

In this study, we extended our previous work [22], investigated scleral permeability, and built a physiological compartmental model to describe drug administration after periocular drug administration. The model reveals the relative contributions of the anatomical and physiological barriers for small molecules and macromolecules.

2. Materials and methods

2.1. Materials

5(6)-Carboxyfluorescein, FITC-dextrans and poloxamer 407 (Pluronic F127) were obtained from Sigma-Aldrich Chemie, Germany. The FITC-dextrans (mean molecular weight) were 4 kDa (4300), 10 kDa (9500), 20 kDa (20200), 40 kDa (38000), and 70 kDa (77000).

Phosphorothioate oligonucleotides were synthesized using ASM 800 DNA/RNA synthesizer (BIOSSET Ltd, Russia) employing the standard coupling protocols of the manufacturer, solid phase support material (US II, Glen Research Corp., USA), fast sulfurizing agent (Glen Research Corp.) and phosphoramidite monomers (dA–CE-, dC–CE-, dG–CE-, dT–CE- from Sigma-Aldrich). Fluorescein phosphoramidite (Glen Research Corp.) was used to attach fluorescent label to the 5' terminus of oligonucleotides. Oligonucleotides were cleaved from the solid support with 4 M ammonium in methanol for 40 min., the support was rinsed with 32% water ammonia and final deprotection was carried out for 36 h at room temperature combined water/methanol/ammonia solution. The solvents were evaporated, the residue dissolved in 0.5 ml of water and then compounds were purified by ion exchange HPLC (Tosoh Bioscience DEAE-2SW; 4.6 mm × 25.0 cm, 5 μm column; from 0 to 50% B in 30 min. linear gradient [A – 0.1 M sodium acetate in 20% acetonitrile , pH = 8; B – 0.1 M sodium acetate and 0.4 M sodium perchlorate in 20% acetonitrile]). Peaks corresponding to the target compounds were collected. After desalting using Sephadex G-25 1 × 30 cm column the structure of compounds were confirmed with mass spectrometry. Three oligonucleotide sequences were synthesized: 12-nt (5'-GTT CCA TTC ATA-3'), 24-nt (5'-ACC TGG GAC ATC GTT CCA TTC ATA-3'), and 36-nt (5'-ACC TGG GAC ATC GTT CCA TTC ATA GTT CCA TTC ATA-3').

Poloxamer solution (20% m/m) was prepared by dissolving the poloxamer in 0.9% saline (4 °C). The solution was autoclaved. The permeating molecules were dissolved at 100 μM to the poloxamer solution that was stored at 4 °C before the experiment.

Bovine sclera was obtained from slaughterhouse (Atria, Kuopio, Finland). The sclera was dissected freshly from the eyes, and then frozen at −20 C until used.

2.2. Release and permeation experiments

Piece of sclera (1.54 cm^2) was attached to the horizontal diffusion chamber apical side facing up. After ensuring that no leaking was taking place, 500 μl of 20% permeant solution (100 μM) in buffer or in poloxamer was pipetted on the sclera. Samples of 200 μl were withdrawn periodically from the donor and receiver compartments. Blank buffer was added as replacement. The experiments were carried out at 37 °C and the receiver chamber solution was mixed at 150 min^{-1}.

Concentrations of permeant molecules in the donor and receiver phases were determined by fluorescence (excitation at 485 nm, emission at 535 nm) with multi-label plate reader (Viktor^2 1420, Wallac). Apparent permeability coefficients were calculated using pseudo steady state approach from the linear part of the permeated drug quantity vs time plots.

2.3. Compartmental pharmacokinetic model

The pharmacokinetic model was an extension of our previous model [22] (Fig. 1). The model was built with Stella software (version 7.0.3, Isee Systems, Lebanon, NH, USA). The drug is administered to the compartment 'Drug depot' that is located at the scleral surface in the sub-conjunctival space or more posteriorly at the scleral surface periocularly. Part of the drug dose is eliminated from the depot and enters the capillaries and lymphatics (J_{10}). The remaining drug enters the sclera-choroid border at the rate determined by the scleral permeability (J_{12}). Thereafter, the drug diffuses across the extravascular choroid to the inner choroid (J_{23}) , where the choroidal vasculature clears part of the drug (J_{30}) , and the rest may permeate across the retinal pigment epithelium to the neural retina and vitreous (J_{34}). Finally, the drug is eliminated from the vitreous (J_{40}). In the case of controlled release formulation, the drug is released at constant rate to the 'subconjunctival drug depot' compartment

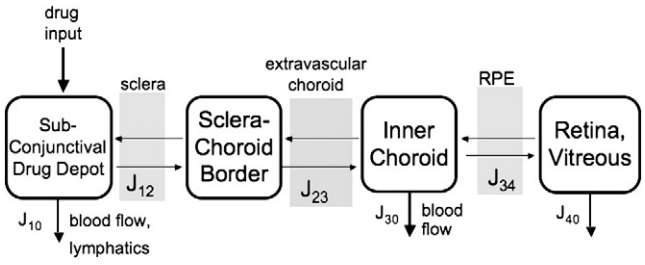

Fig. 1. Compartmental model of periocular drug delivery to the posterior eye segment. 'Subconjunctival drug depot' is the drug reservoir in the subconjunctival (or periocular) site after injection or implantation. 'Retina, vitreous' compartment includes the vitreous humor and the neural retina. The drug transfer rates are described as fluxes (J).