

EFFECTIVE TRANSSCLERAL DELIVERY OF TWO RETINAL ANTI-ANGIOGENIC MOLECULES

Carboxyamido-triazole (CAI) and 2-Methoxyestradiol (2ME₂)

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Purpose: To evaluate the human transscleral diffusion and intravitreal delivery of carboxyamido-triazole (CAI) and 2-Methoxyestradiol (2ME₂).

Methods: The transscleral diffusion of two retinal antiangiogenic molecules, CAI and 2ME₂, was measured in vitro to assess their potential transscleral delivery. Varying concentrations and different solvents of CAI and 2ME₂ were placed in the upper compartment of a two-chamber acrylic perfusion apparatus, on the episcleral side of the sclera obtained from human donor eyes. Samples were taken from the lower compartment (uveal side) for up to 24 hours and measured by high performance liquid chromatography.

Results: All three solutions that contained CAI efficiently diffused through the sclera with permeability constants that ranged from 2.8 to 5.5 × 10⁻⁶ cm/s. The scleral permeability constant derived for 2ME₂ was 9.96 × 10⁻⁶ cm/s. The permeability constants obtained for both CAI and 2ME₂ are similar to each other as well as to permeability constants measured for other small molecules such as fluorescein and dexamethasone fluorescein.

Conclusion: Both CAI and 2ME₂ traverse the sclera efficiently. These data combined with the reported inhibition of posterior segment neovascularization observed with these two molecules demonstrates that CAI and 2ME₂ are good candidate molecules to treat posterior segment neovascularization by local delivery.

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Intraocular neovascularization is often associated with significant pathology or vision loss, while the angiogenic process can be helpful in other crucial organs such as myocardium.¹⁻⁵ Therefore, one chal-

lenge in the treatment of neovascularization secondary to age-related macular degeneration (ARMD), diabetic retinopathy (DR), or retinal vein occlusions (RVO) is to deliver effective antiangiogenic therapy to the posterior segment of the eye without disturbing physiologic angiogenesis in other critical organ sys-

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tems. Towards this end, we have investigated the local delivery by transscleral diffusion of carboxyamido-triazole (CAI)^{6–10} and 2-Methoxyestradiol (2ME₂),^{11–14} two potent low molecular weight antiangiogenic factors.

Drug delivery to the posterior segment of the eye can be accomplished by four different methods: 1) systemic delivery, 2) topical drops, 3) intraocular, or 4) intravitreal.¹⁵ The intravitreal half-life of a compound and the specific neovascular process to be treated are major determinants for the required frequency and/or duration of intravitreal injections. Topical administration to the ocular surface often does not influence posterior segment disease.^{16–18} Multiple intravitreal injections can cause scleral necrosis, and are difficult to tolerate. However, intravitreal sustained release devices for many agents deliver a prolonged and acceptable drug concentration to the posterior segment of the eye.¹⁹ If the sclera is relatively permeable to a drug, then multiple schemes can be developed to deliver a sustained, high concentration of that agent to the posterior segment, while avoiding potential complications of intraocular surgery such as cataract formation, glaucoma, retinal detachment, and endophthalmitis.^{15,20–22}

Carboxyamido-triazole (CAI) is a drug that has been shown to have antiangiogenic effects in mouse and human cancers.^{9,23–28} CAI was originally developed as a coccidiostat, but was subsequently shown to have potent antiproliferative and antimetastatic effects in many animal models.^{27,29} CAI decreases cellular calcium by inhibition of nonvoltage gated calcium channels, the predominant calcium channel type present on vascular endothelial cells, which inhibits endothelial cellular proliferation and division.^{10,30–32} In addition, CAI antagonizes many of the effects mediated by VEGF at a biochemical level, such that IP3 metabolism is altered, and MMP-2, c-fos, and VL30 gene expression are inhibited by CAI.³³ In a mouse model of retinal neovascularization, CAI significantly inhibited new blood vessel growth when given before the formation of abnormal new blood vessels, and when given after formation of these neovascular fronds, and promoted their regression.²⁹ Although CAI appears relatively well tolerated when administered orally in Phase I–III clinical trials, the systemic side effects of a given compound are usually minimized with a local drug delivery system.^{23–25,34,35}

Another candidate molecule for treating pathologic ocular neovascularization is 2ME₂, a naturally occurring metabolite of the endogenous estrogen hormone estradiol-17 β .¹² Small quantities of 2ME₂ are found physiologically in human blood and urine.^{12,36} This molecule was found to disturb mitosis in Chinese hamster ovary cell line.³⁷ 2ME₂ inhibits tubulin for-

mation, and incubation of 2ME₂ with MCF-7 and HeLa cells deranges their cell cycle.³⁸ This molecule also causes apoptosis in a number of vascular endothelial and tumor cell lines.^{36,39,40} Indeed, 2ME₂ induces apoptosis via the extrinsic pathway and upregulation of death receptor 5.⁴¹ In vivo, 2ME₂ has been shown to inhibit tumors and is undergoing multiple clinical trials for the treatment of human cancer.¹¹ Finally, 2ME₂ also is an angiogenic inhibitor of a number of processes that include chick chorioallantoic membrane model, rat pituitary tumor angiogenesis, follicular growth, and choroidal neovascularization.^{14,42,43}

Therefore, the purpose of this study was to investigate the potential of two relatively small, nontoxic, and antiangiogenic molecules for local delivery into the eye via the transscleral route, and to assess the possibility for incorporation in local ocular delivery systems in future experiments. Since compounds of similar molecular weight, such as fluorescein (MW = 332) and rhodamine (MW = 479), have been shown to diffuse across the sclera with permeability constants in the range of $2 - 5 \times 10^{-6}$ cm/s,^{15,20,21} a hypothesis was formed that CAI and 2ME₂ would diffuse across the sclera with a similar permeability constant. The following data show that both CAI and 2ME₂ may be used effectively for periocular delivery to achieve physiologic concentrations of these molecules to the posterior segment of the eye.

Methods

Scleral Dissection and Preparation of the Two-chamber Perfusion Apparatus

Scleral tissue was obtained from 23 human donor eyes (Georgia Eye Bank, Atlanta) and was stored in moist chambers for 2 to 6 days postmortem. The mean age at time of death (\pm SE) for the donor tissue used in this study was 52.4 ± 4.4 years. Scleral tissue was dissected from the superotemporal quadrant of each eye to minimize associated structures that can modify scleral permeability such as vortex veins, posterior ciliary arteries, and anterior ciliary vessels. Uveal tissue and episclera were carefully removed using a cotton-tip applicator. The sclera was mounted horizontally in a two-chamber acrylic perfusion apparatus (Figure 1). The uveal surface was apposed to the lower flow-through hemichamber (volume 500 μ L) containing balanced salt solution (BSS, Alcon laboratories, Ft. Worth, TX). The test solution was applied to the episcleral surface. Since CAI has a 423 Da molecular weight and the molecule is hydrophobic with a solubility of 1 to 10 μ mol/L in water, three different test solutions that contained carboxyamido-triazole

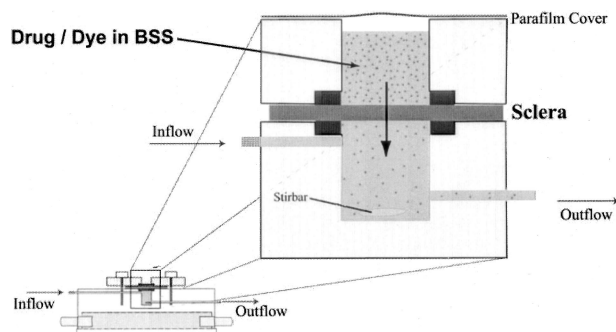


Fig. 1. In vitro perfusion chamber set-up. Human sclera was placed horizontally between two compartments. The upper compartment (the episcleral side) served as a donor depot. Transscleral diffusion of CAI and 2ME₂ was measured by collecting fractions from the lower compartment (uveal side), which was perfused with balanced salt solution at a rate of 0.03 mL/min. During the experiment a transscleral pressure of 15 mmHg was maintained by the height of the water column of the outflow tubing. Temperature was kept at 37°C by a circulating water bath. The donor side was kept static, whereas the receptor side was perfused at a slow rate and mixed by a magnetic stir bar.

were evaluated: CAI in 20% DMSO (80% BSS vol/vol) at a concentration (mean \pm SE) of 15.2 ± 3.0 $\mu\text{mol/L}$ (solution A), CAI in 10% DMSO (90% BSS) at a concentration of 4.1 ± 0.4 $\mu\text{mol/L}$ (solution B), and CAI in 10% ethanol (90% BSS) at a concentration of 3.3 ± 0.2 $\mu\text{mol/L}$ (solution C). The maximum solubility of 2ME₂, with a molecular weight of 302 Da, is 12 $\mu\text{mol/L}$ in water. The studied solution of 2ME₂ was 100 $\mu\text{mol/L}$ in 1% DMSO (99% BSS vol/vol). CAI was obtained from the National Institutes of Health, Bethesda, MD, and 2ME₂ was obtained from EntreMed, Inc., Rockville, MD.

The BSS in the lower hemichamber was perfused at a rate of 0.03 mL/min. The perfusion apparatus was placed on a magnetic stir plate and mixing in the lower hemichamber was achieved using a stir bar. The upper hemichamber containing the test compound was covered with parafilm and sealed with silicone grease to prevent evaporation. This also provided a flexible seal so that transscleral pressure would not be altered. The temperature of the apparatus was maintained at 37°C by a water jacket with circulating water bath.

A physiologic pressure of 15 mmHg was applied across the sclera by raising the height of the outflow tube of the receptor (lower) chamber to 22 cm. A pressure transducer that was connected to the lower hemichamber verified the pressure. For all three solutions that contained carboxyamido-triazole 100 μL was applied to the episcleral side and samples of perfusate were collected at the uveal side of the sclera at time zero and at 30-minute intervals for the duration of 8 hours. The scleral permeability to CAI in a 10% ethanol solution was also evaluated over a longer period of 24 hours of which samples were taken

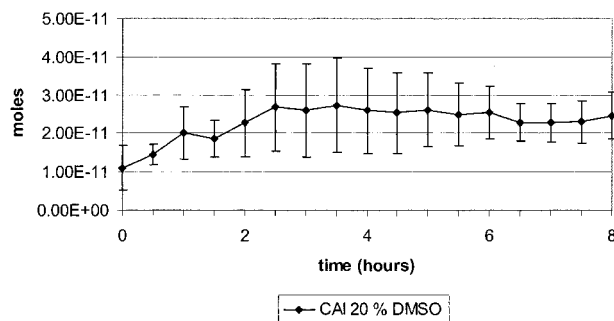


Fig. 2. Transscleral diffusion of a (mean \pm SE) 15.2 ± 3.0 $\mu\text{mol/L}$ CAI solution in 20% DMSO (80% balanced salt solution [BSS] vol/vol). Moles of CAI measured in the receiver chamber (n = 4) as a function of time during BSS perfusion (0.03 mL/min) through the receiver (uveal) chamber. Each point represents the mean \pm SE.

hourly. Scleral permeability to 2ME₂ (100 $\mu\text{mol/L}$, 1% DMSO) was also evaluated over a 24-hour period with hourly sampling intervals. For the 24-hour experiments 500 μL of the test solution was added to the upper hemichamber to minimize depletion of the drug on the donor side.

High-performance Liquid Chromatography Analysis

From each sample obtained from the transscleral diffusion experiments an aliquot of 100 μL was taken and the concentrations of CAI were monitored by reverse-phase high-performance liquid chromatography (HPLC). Results were calculated on the basis of peak areas. Samples were analyzed by using a Hewlett Packard HP1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a UV detector, an auto-sampler, a pump, and a HP Kayak workstation that controls the operation of HPLC and analyzes the data. A Zorbak Rx-C8 column (4.6 \times 250 mm) was used for separation and detection was set at 260 nm. The flow rate employed was 1.0 mL/min with mobile phase of 55% acetonitrile and 45% water. The injection volume was 100 μL and retention time of CAI was approximately 0.8 minutes. The injection volume for 2ME₂ was also 100 μL . The 2ME₂ samples were similarly assayed using a Hewlett Packard HP 1,100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a Diode Array detector, an autosampler, a quaternary pump, and a HP Kayak workstation which controlled the operation of HPLC and analyzed the data. A Beckman Ultrasphere C-18 column (250 \times 4.6 mm) (Beckman Coulter, Inc., Fullerton, CA) was used for separation and detection was set at 205 nm. The flow rate was 1.0 mL/min with mobile phase of 53% of water, 43% of acetonitrile, and 0.1% trifluoroacetic acid by volume. The retention time of 2ME₂ was approximately 7.3 minutes. The concentra-

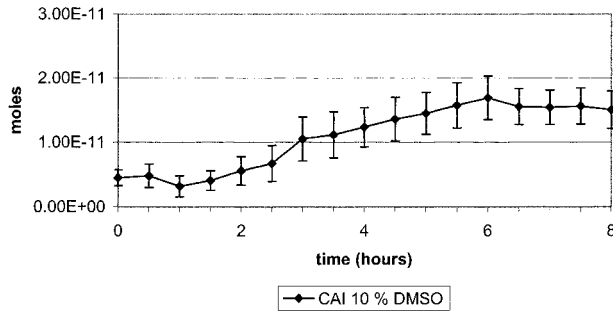


Fig. 3. Transscleral diffusion of a (mean ± SE) 4.1 ± 0.4 μmol/L CAI solution in 10% DMSO (90% balanced salt solution [BSS]). Moles of CAI measured in the receiver chamber (n = 7) as a function of time during BSS perfusion (0.03 mL/min) through the receiver (uveal) chamber. Each point represents the mean ± SE.

tion of 2ME₂ was calculated against a six-point calibration curve constructed over the 2ME₂ concentration from 0.09 μg/mL to 23.2 μg/mL with correlation coefficient of 0.9994.

Calculation of CAI and 2ME2 Transscleral Permeability

Steady state permeability constants were calculated from the data as follows:

$$K_{trans} = [R_{total}/(t)(A)] \times 1/[D]$$

Where R_{total} is the amount of drug in the receiver

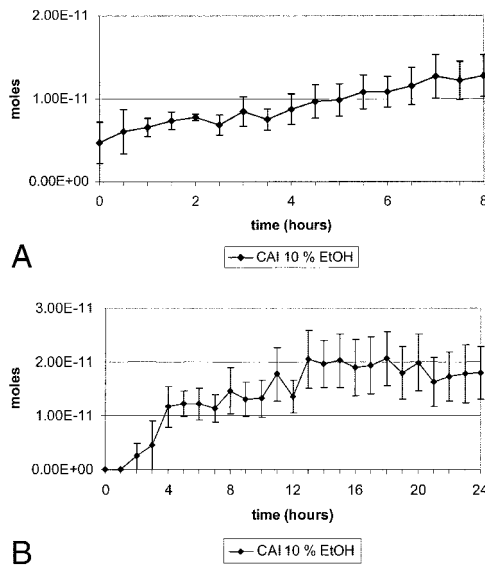


Fig. 4. A, B, Transscleral diffusion of a (mean ± SE) 3.3 ± 0.2 μmol/L CAI solution in 10% ethanol (90% balanced salt solution [BSS]) measured over an 8-hour period (n = 8) and over a 24-hour period (n = 4). Moles of CAI measured in the receiver chamber as a function of time during BSS perfusion (0.03 mL/min) through the receiver (uveal) chamber. Each point represents the mean ± SE.

effluent per collected fraction, and t is the fraction collection time (in seconds). A is the area of exposed sclera (in square centimeters). This value— $R_{total}/(t)(A)$ —is equal to the flux across the tissue. D is the concentration of drug in the donor hemichamber. Permeability thus represents the steady state flux normalized by donor concentration. The area of exposed sclera was 0.385 cm². Mean steady state permeability values (±SE) were calculated from four to eight experiments performed for each compound.

Electron Microscopy of Human Sclera

At the end of the transscleral diffusion experiments with CAI (DMSO 10%, 20%, and ethanol 10%) and 2ME₂ (1% DMSO) the scleral tissue was fixed in 2.5% glutaraldehyde in 0.1 moles/L sodium cacodylate buffer and prepared for transmission electron microscopy (TEM). The scleral tissue was bisected and postfixed in 2% osmium tetroxide for 2 hours. Small pieces of the sclera were embedded in low-viscosity epoxy medium, thin sectioned, stained with uranyl acetate and lead citrate, and viewed with a JEOL 100 CX transmission electron microscope. The micrographs were taken at ×2,850.

Results

CAI Transscleral Delivery

Figures 2 through 4 show the diffusion through the sclera (in moles) of CAI in the three different solutions over a period of 8 to 24 hours. CAI diffused across the sclera effectively in all three solutions. Solution A (15.2 μmol/L dissolved in 20% DMSO) reached a semisteady state in approximately 5 hours (see Figure 2), and the permeability constant was measured (mean ± SE) at $2.8 \pm 0.8 \times 10^{-6}$ cm/s

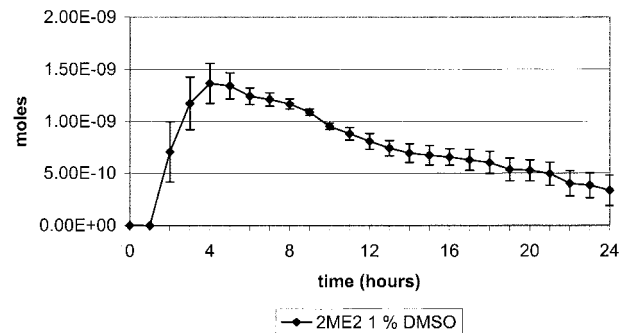


Fig. 5. Transscleral diffusion of a (mean ± SE) 91.7 ± 5.5 μmol/L 2ME₂ solution in 1% DMSO (99% balanced salt solution [BSS] vol/vol). Moles of 2ME₂ measured in the receiver chamber (n = 4) as a function of time during BSS perfusion (0.03 mL/min) through the receiver (uveal) chamber. Each point represents the mean ± SE.

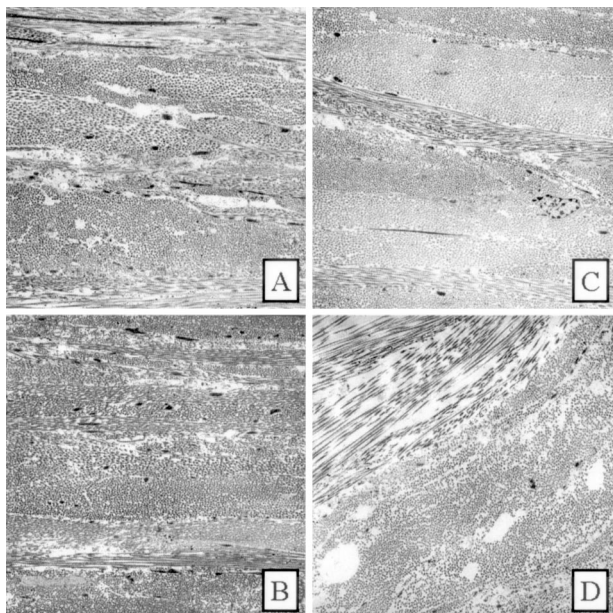


Fig. 6. A-D, Transmission electron micrographs (TEM) of the human sclera after 24 hours of exposure to CAI in 10% DMSO placed on the episcleral surface (A). B and C show TEM photomicrographs of the human sclera after 24 hours of exposure to CAI in 10% EtOH and 20% DMSO, respectively placed on the episcleral surface. D depicts a TEM of human sclera after 24 hours of exposure to 2ME₂ in 1% DMSO. Although mild hydration was observed after 2ME₂ incubation, the scleral ultrastructure shows a normal array of collagen bundles, both longitudinal and transverse, with variable-diameter fibrils forming the lamellae, and few fibroblasts present between the lamellae. DMSO and EtOH solutions did not alter the normal lamellar structure for any of the solutions ($\times 2,850$).

($n = 4$). The permeability constant for solution B ($4.1 \mu\text{mol/L}$ in 10% DMSO) was measured at $5.5 \pm 1.0 \times 10^{-6} \text{ cm/s}$ (see Figure 3), and steady state was reached in 6 hours ($n = 7$). For solution C ($3.3 \mu\text{mol/L}$ in 10% EtOH) no apparent steady state was observed over the duration of the 8-hour experiment (Figure 4A, $n = 8$). An additional series of experiments was performed, extending the sampling period to 24 hours. In these experiments a semisteady state flux was observed at 13 hours (Figure 4B). The permeability constant was calculated as $4.16 \pm 1.1 \times 10^{-6} \text{ cm/s}$ ($n = 4$). The values, representing semisteady state permeability for all three solutions, appeared comparable, and indeed were not significantly different from each other with analysis of variance testing: P value = 0.2385. Transmission electron microscopy (TEM) showed a normal ultrastructure within the sclera for the experiments with CAI in all three test solutions. Figure 6, A and B, illustrates the ultrastructure of the sclera after 24 hours of exposure to CAI in 10% DMSO and 10% EtOH. The sclera shows a densely packed, intramingle arrangement of collagen bundles. There are fibroblasts, longitudinal and transverse collagen lamellae, with

variable-diameter fibrils forming the lamellae. The collagen lamellae are normally arranged, and more irregular than observed and expected in the cornea. Similar scleral appearance is also retained after incubation with CAI in 20% DMSO (Figure 6C). With all vehicles of DMSO and EtOH in BSS the collagen structure was normal after the 24-hour perfusion experiment.

2ME₂ Possesses Favorable Transscleral Delivery Characteristics

Figure 4 shows the diffusion through the sclera (in moles) of 2ME₂ in a $100 \mu\text{mol/L}$ solution of 1% DMSO over a 24-hour period. 2ME₂ was also able to diffuse across the sclera effectively over this time period and reached steady state at 4 hours. The permeability constant was measured (mean \pm SE) at $9.96 \pm 0.11 \times 10^{-6} \text{ cm/s}$ ($n = 4$) (see Figure 5). TEM showed a normal scleral ultrastructure after the diffusion studies with 2ME₂ over a 24-hour period. After 24 hours of exposure to 2ME₂, mild scleral edema was observed, but the collagen fibrils appeared intact and normal (Figure 6D).

Discussion

Current Treatment and Clinical Trials of Antiangiogenic Molecules for Posterior Segment Ocular Neovascularization

Current approved treatment of both DR and ARMD relies on either threshold or subthreshold laser energy. Threshold laser is inherently destructive and usually causes paracentral and central scotomata in patients with ARMD, and limits peripheral and central vision in patients with DR.⁴⁴⁻⁴⁸ Ocular photodynamic therapy (OPT) is approved for the treatment of predominantly classic choroidal neovascularization (CNVM) underneath the foveal center. Although OPT is an effective treatment for classic subfoveal CNVM, the results are far from ideal in that most patients require three or more sessions of OPT to eradicate the CNVM, and it only reduces the incidence of severe vision loss three lines by 15% over a 1-year time period. In addition, there is only a 5.0% incidence of a two line or greater vision improvement over this time compared to 2.4% observed with the natural history of the disease.⁴⁹⁻⁵⁸ Many groups have studied transpupillary thermoplasty (TTT) to treat subfoveal CNVM, but most results demonstrate similar limited or less efficacy compared to OPT.⁵⁹⁻⁶³ There are four antiangiogenic molecules that have completed enrolling patients for Phase II clinical trials, have recently been approved, or are under investigation in Phase III.

There is an approximate 25% incidence of two line or greater visual improvement observed after treatment of subfoveal CNVM with only one out of four of these molecules, which is clearly superior to the current laser treatment modalities. The first of these molecules are protein kinase C inhibitors.⁶⁴ These molecules target a retinal enriched enzyme and are orally administered. The second and third molecules bind with high affinity to VEGF, thereby blocking the action of this potent angiogenic growth factor.⁶⁵ One mechanism of VEGF binding utilizes a modified oligonucleotide, aptamer (Macugen), while the other uses a fragment of a high affinity antibody.⁶⁵ Both currently are administered by intravitreal injections at a frequency in the range of every 6 weeks. Finally, anecortave acetate is an antiangiogenic steroid derivative that is administered every 6 months by juxtasceral injection.⁶⁶ The oral protein kinase C inhibitors have been used predominantly in the treatment of DR, while the aptamer, blocking antibody (RhuFab), and anecortave acetate are under testing to treat subfoveal CNVM in ARMD. Only one of these molecules relies upon transscleral delivery. The molecular weights of the other molecules (Macugen ~10,000; RhuFab ~48,000) and their predicted range of transscleral permeability are orders of magnitude lower.

Antiangiogenic Effects and Relative Nontoxicity of CAI and 2ME₂

Since anecortave acetate is the only candidate molecule in later clinical trial stages that relies on transscleral delivery, we have been interested in developing other antiangiogenic molecules that also traverse the sclera effectively to treat posterior segment neovascularization. To design an ideal candidate molecule for the treatment of posterior segment neovascularization, one should consider several criteria. First, it must be relatively nontoxic; second, it must have reasonable bioavailability in ocular tissues; third, it should have some physiologic basis to be studied; and fourth, it must not adversely interfere with other physiologic neovascular responses such as wound healing and coronary vascular remodeling.

CAI exerts potent antiproliferative and antimetastatic effects in many animal models of cancer.^{27,29} Moreover, CAI mediates much of the antimetastatic and antiproliferative effects by blocking tumor angiogenesis. More recently, CAI has been shown to inhibit formation of abnormal new vessels as well as to cause regression of existing vascular fronds in a mouse model of retinal neovascularization.²⁹ CAI has undergone testing in multiple Phase I oncology trials and several Phase II trials and one Phase III trial is under-

way for the treatment of refractory solid cancerous tumors so that over 500 patients have had CAI administered systemically.^{23–25,34,35,67–69} Long-term administration of up to 3 years has shown it to be well tolerated. Major side effects requiring dose modification are rare and include mild and reversible cerebellar ataxia in 1%, rapidly reversible sensory peripheral neuropathy in 5%, and exacerbation of depression in 1% to 3% of patients.^{23–25,34,35,67–69} The healing from simple lacerations to emergency surgery has not been altered while CAI is administered, which implies that CAI does not affect the physiologic neovascularization associated with wound healing. In addition, CAI intravitreal implants have been designed that possess favorable delivery profiles of 5.92 ± 1.29 to 19.56 ± 6.74 $\mu\text{g/d}$ for up to 19 months.⁷⁰ Pharmacokinetic modeling predicts that these implants would achieve steady state concentrations in the vitreous ranging from 0.58 to 1.93 $\mu\text{mol/L}$. When these devices were implanted into rabbits no anatomic toxicity was found on retinal examination, and no electroretinographic abnormalities were observed over a 7-month period.⁷⁰

Similarly, 2ME₂ appears both relatively efficacious and nontoxic. This molecule inhibits angiogenesis in a variety of animal models. Intravitreal implants release 2ME₂ at 1.72–6.04 $\mu\text{g/day}$, and these implants are effective inhibitors of CNV in a rabbit model.⁴³ 2ME₂ is an endogenous metabolite of estrogen. Hence, there are relatively few side effects of systemically administered 2ME₂, and no Grade IV toxicity events have been reported in multiple Phase I studies.¹⁴ Thus, both CAI and 2ME₂ appeared to meet the criteria of relative bioefficacy and nontoxicity, therefore we decided to study these molecules further to determine whether they could be optimally delivered to the posterior segment without systemic administration or mechanically penetrating the eye.

In vitro Transscleral Diffusion in Scleral Sections From Human Donor Eyes

The permeability of human sclera to CAI and 2ME₂ was measured as K_{trans} , a compound-specific value for diffusion through the sclera. It represents the steady state flux normalized by donor concentration. Scleral permeability is dependent on the characteristics of the solute (molecular radius and weight, the solubility, and the configuration of the molecule).^{17,18,71} Another determining factor is the thickness and the hydration of the sclera.¹⁷ Finally, other factors that could influence the permeability are the pressure gradient across the sclera (the intraocular pressure), the solvent used to dissolve the drug, and the vehicle in which it is administered to the eye.¹⁸ K_{trans} values for CAI in the

Table 1. Relative Trans-scleral Permeabilities of Various Compounds*

Drug/Molecule Name	Molecular Wt.	K_{trans} (cm/s)	Reference
Polymyxin B-BODIPY	1800	$3.90 \pm 0.59 \times 10^{-7}$	75
Vancomycin-BODIPY	1723	$6.66 \pm 1.46 \times 10^{-7}$	75
Dexamethasone-fluorescein	841	$1.64 \pm 0.17 \times 10^{-6}$	2
Rhodamine	479	$1.86 \pm 0.39 \times 10^{-6}$	2
Penicillin-BODIPY	661	$1.89 \pm 0.21 \times 10^{-6}$	75
Methotrexate-fluorescein	979	$3.36 \pm 0.62 \times 10^{-6}$	2
CAI	423	$2.8\text{--}5.5 \pm 1.1 \times 10^{-6}$	—
Fluorescein	332	$5.21 \pm 0.71 \times 10^{-6}$	2
Dexamethasone	392	$8.94 \pm 1.5 \times 10^{-6}$	7
Carboxyfluorescein	317	$9.93 \pm 3.5 \times 10^{-6}$	7
2ME ₂	302	$9.96 \pm 1.1 \times 10^{-6}$	—
Carboplatin	371	$27.0 \pm 1.7 \times 10^{-6}$	12
Water (H ₂ O)	18	$51.8 \pm 18 \times 10^{-6}$	7

* Human scleral permeability, expressed as K_{trans} (cm/sec) for different drugs and molecules, as reported in the literature and in this study. All values represent the mean of *n* experiments \pm SE. The transscleral diffusion of CAI and 2-ME₂ are compared with other compounds with a similar molecular weight. K_{trans} values for both CAI and 2-ME₂ were in the same range as other compounds with similar molecular weight.

three test solutions ranged from 2.76 to 5.49×10^{-6} cm/s, and K_{trans} for 2ME₂ was measured at 9.96×10^{-6} cm/s, which is comparable to solutes with similar molecular weights (rhodamine 6G, fluorescein, dexamethasone) (Table 1).^{17,20,72–74} The results of these experiments show that both CAI and 2ME₂ successfully diffuse across the human sclera in vitro without causing any anatomic derangement of the sclera by transmission electron microscopy analysis.

Local Ocular Bioavailability of CAI and 2ME₂

Since both CAI and 2ME₂ are relatively small molecules with molecular weights of 423 Da and 302 Da, respectively, we postulated that they would diffuse across the sclera efficiently. Indeed, in our model the transscleral diffusion kinetics of CAI and 2ME₂ were similar to other small molecules such as fluorescein and dexamethasone-fluorescein.²⁰ Dexamethasone has been detected at 13 ng/mL in the vitreous cavity following a single 5 mg peribulbar injection in humans.^{73,74} This results in a vitreous concentration of approximately 0.10 to 0.13 μ mol/L assuming a vitreous volume of 4 to 5 mL. Delivery to the subretinal space is 10-fold higher following subconjunctival injection.⁷² The serum half-life of dexamethasone (18 to 36 hours) is significantly shorter than that of CAI (111 hours) and comparable to 2ME₂ (10 hours). Physiologic tissue concentrations of CAI and 2ME₂ are in the 1 to 10 μ mol/L range so that it appears reasonable to postulate based on the scleral permeability and prolonged serum half-life of CAI that it can be effectively delivered transsclerally to the subretinal space and the vitreous cavity in physiologic concentrations.^{23,43,67} The release rates of CAI and 2ME₂ from sustained

release devices are also favorable. One potential drawback of the intravitreal implants that were constructed with CAI was that some demonstrated rapid expansion because of the hygroscopic nature of CAI.⁷⁰ This rapid expansion may be alleviated by developing a non-aqueous reservoir for the CAI pellet. Because both CAI and 2ME₂ diffuse across the sclera efficiently, it is possible to formulate a periocular delivery system for these molecules. Various periocular systems could be employed that include drug suspension, fibrin glue formulations, as well as an implant designed with a nonaqueous reservoir.

Conclusion

Based on the current studies, the previous demonstration of angiogenic inhibition in the posterior segment of the eye in animal models, and the observed relative nontoxicity, both CAI and 2ME₂ fulfill the four criteria established above for candidate compounds to treat posterior segment neovascularization. Therefore, both of these molecules warrant further study and development of periocular delivery systems to treat common blinding diseases such as DR and ARMD.

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