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Targeting Retinal and Choroid Neovascularization Using the Small Molecule Inhibitor Carboxyamidotriazole

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Abstract

Neovascular ocular diseases as exemplified by proliferative diabetic retinopathy (PDR), exudative age-related macular degeneration (AMD), and retinopathy of prematurity (ROP) are severe diseases affecting all age groups in the US. We asked whether a small molecule, carboxyamidotriazole (CAI) known for its anti-angiogenic and anti-tumor effects and its ability to be administered orally in humans, could have anti-angiogenic effects in ocular *in vitro* and *in vivo* angiogenesis models. The anti-proliferative effects of CAI were examined by BrdU incorporation using human retinal and dermal endothelial cells and human pigment epithelial cells. The effect of CAI was determined using the Matrigel tube formation assay. The mouse model of choroidal neovascularization (CNV) initiated by laser rupture of Bruch's membrane was used to quantify *in vivo* effects of aqueous beta-hydroxypropyl cyclodextrin (bHPCD) formulations of CAI on neovascularization. The pharmacokinetics (PK) of CAI after intravitreal administration bHPCD-CAI were studied undertaken in rabbit. The intravitreal toxicology of bHPCD-CAI was also examined in rat ocular tissue. We observed that CAI treatment of human endothelial cells decreased cell proliferation in a dose dependent manner. In the *in vivo* tests bHPCD-CAI treatment reduced choroidal neovascular lesion volume, also in a dose-dependent manner. The intravitreal PK of bHPCD-CAI demonstrated that highly efficacious concentrations of CAI are reached in the vitreous compartment. No ocular toxicology was observed with intravitreal injection of CAI. These studies support the potential of developing intravitreal CAI in an bHPCD ocular formulation for treatment of proliferative retinopathies in humans.

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Keywords

angiogenesis; therapy; choroidal neovascularization; endothelial cell; animal model; carboxyamidotriazole

1. Introduction

Posterior segment neovascular ocular diseases, as exemplified by proliferative diabetic retinopathy (PDR), exudative age-related macular degeneration (AMD) and retinopathy of prematurity (ROP), are a growing health threat which require new effective therapies. Retinal neovascularization associated with PDR is the leading cause of blindness in working age adults and choroidal neovascularization (CNV) is responsible for 200,000 new cases of exudative AMD each year in the US rendering this neovascular pathology the leading cause of legal blindness in non-third world nations. Pathological angiogenesis associated with ROP is the major cause of blindness in children under the age of seven [1], and retinal vascular occlusions (RVO) are slightly less prevalent but equally debilitating.

The administration of VEGF inhibitors (VEGF-i) into the vitreous is very effective in treatment of ocular angiogenesis. The VEGF antibody ranibizumab (Lucentis®) was FDA approved for CNV-AMD in 2005 [2, 3]. Despite this breakthrough, new drug therapies are still needed as long-term clinical trials using Lucentis® indicate that a significant population of AMD patients has an incomplete response to VEGF-i. This is not surprising as other angiogenic and inflammatory mediators contribute to the pathogenesis of CNV besides VEGF. Moreover, repeated long term ocular injection with current VEGF-i is not a sustainable therapeutic approach for the younger PDR patient population. Finally, since VEGF has beneficial functional effects, including neuroprotective properties and maintenance of the choriocapillaris, long term anti-VEGF therapy may have inherent risks [4].

Therefore, new therapies that interfere with non-VEGF signaling pathways associated with angiogenesis and vascular leakage would represent a significant advance in disease treatment. Herein, we report pre-clinical data for carboxyamidotriazole (CAI), a small molecule which has the potential to address unmet clinical needs and has a novel mechanism of action. CAI, chemically known as 5-amino-[4-(4-chlorobenzoyl)-3,5-dichlorobenzyl]-1,2,3-triazole-4-carboxamide (Figure 1), was originally discovered to be an effective anti-parasitic compound *in vitro* and *in vivo* [5, 6]. Subsequently, CAI at a concentration of 1-10 μ M has been shown to have antitumor and antiangiogenic activity which is linked to blocking cellular calcium flux [7]. While the exact “calcium channel” molecular target of CAI has still not been elucidated, recent work has indicated that CAI selectively blocks non-voltage gated capacitative Ca²⁺ entry (CCE) into cells as well as release of calcium into the cytoplasm from intracellular calcium stores [8]. CAI also inhibited capillary expansion in the chick chorioallantoic membrane assay [9]. In studies with bovine choroidal endothelial capillary (CEC) cells, CAI inhibited serum- and basic fibroblast growth factor (FGF-2)-induced proliferation and cell attachment onto laminin and also inhibited VEGF- and FGF-2-stimulated secretion of matrix metalloprotease 2 [2]. *In vivo*, systemic dosing of CAI in mice bearing the Lewis lung carcinoma decreased the number of vascularized metastases while leaving the total number of metastases unaffected [10]. Based on this compelling anti-angiogenic profile, the NCI advanced CAI into clinical trials for diverse refractory cancers as an oral therapy [11, 12]. Unfortunately, the overall outcome of the trials was unfavorable due to variable oral bioavailability and high plasma protein binding that narrowed the therapeutic window in clinical trials so that the development of oral CAI as chemotherapy was discontinued.

The potential for CAI as an ocular antiangiogenic therapy has been substantiated previously via oral dosing in the mouse OIR model [13]. However, the translation of this finding to clinical ocular disease application has been precluded to date by the side effects observed in cancer patients with oral CAI combined with the development of local VEGFi that have significantly fewer systemic side effects, compared to both systemic CAI and VEGFi. Therefore to similarly diminish CAI systemic side effects, we have developed a high yield local ocular formulation. In the current study we report the first results of CAI as a local ocular therapy via intravitreal administration of novel beta-hydroxypropyl cyclodextrin (bHPCD) based injectable CAI formulations (bHPCD-CAI). We demonstrate that intravitreal administration of bHPCD-CAI is highly efficacious in the mouse CNV model and has a safety and pharmacokinetic profile in rats and rabbit models supporting ultimate clinical application in humans.

2. Materials and methods

2.0 CAI Formulations and Dosing

For *in vitro* experiments serial dilutions were performed on a DMSO stock solution of CAI which has a molecular weight of MW= 424.7 (Figure 1). For *in vivo* experiments buffered aqueous bHPCD-CAI formulations devoid of organic solvents were prepared using the hydroxypropyl- β -cyclodextrin (bHPCD) as a complexing agent. Homogeneous solution bHPCD-CAI formulations with CAI concentrations up to 4.5 mg/mL were prepared by varying the concentration of bHPCD up to 20%. Higher CAI load suspension bHPCD-CAI formulations with total CAI load of 15 mg/mL or 30 mg/mL were prepared using 10% bHPCD which is generally regarded as safe. The particulate phase of these bHPCD-CAI suspension formulations consists solely of CAI and the free solution fraction of CAI was 1.5 mg/mL. Thus, for example, the 30 mg/mL bHPCD-CAI suspension formulation consists of 1.5 mg/mL of CAI in solution and 28.5 mg/mL of CAI in the suspended particulate phase.

2.1 Animals

All animal procedures used were in agreement with the NIH Guide for the Care and Use of Laboratory Animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with institutional guidelines and were approved by the University of Florida Institutional Animal Care and Use Committee.

Female New Zealand white rabbits were purchased from Charles Rivers Laboratories (Wilmington, MA). Female C57Bl/6J mice were obtained from Jackson Laboratory (Bar Harbor, MA). Long Evans rats were obtained from an in-house breeding colony at the Atlanta VA Medical Center.

2.2 Proliferation assays

Human retinal endothelial cells (HRECs) and human retinal pigment epithelial cells (HRPE) were isolated and maintained as previously described [14, 15]. Human microvascular endothelial cells (HMEC) were purchased from Lonza (Walkersville, MD). Cells were seeded in 96-well dishes at a density of 25,000 cells/well and allowed to recover for 24 hours prior to being placed in low serum medium (2% FBS) or 10% FBS for an additional 24 hours. Cells were exposed to varying concentrations of CAI (10^{-8} M; 10^{-7} M; 10^{-6} ; and 10^{-5} M). Reduced serum medium, vehicle, and serum-free media were used as controls. BrdU incorporation was measured using a BrdU Cell Proliferation Assay (Calbiochem, Madison, WI) per-manufacturer's protocol. Each experimental group consisted of eight samples and the experiments were repeated three times to ensure reproducibility. The data were expressed as "percent" relative to the control values.

2.3 Matrigel tube formation assay

HRECs (20,000) were plated on Matrigel precoated plates (BD Biosciences, San Jose, CA) either treated with CAI in media with 5% FBS or left untreated. CAI concentrations of 10^{-7} , 10^{-6} and 10^{-5} M were used. The cells were observed for 24 hours and tube formation was photographed at 24 hours using an inverted Zeiss Microscope (Carl-Zeiss, Thornwood, NY).

2.4 Laser-induced CNV model and CNV quantitation

Bruch's membrane in the murine eye was ruptured by three discrete applications of an OcuLight GLx 532 nm ophthalmic laser (Iridex, Mountain View, CA) coupled to a Haag-Streit slit lamp, delivering a 50 μ m spot at a power of 150 mW for 100 ms [16]. Immediately following laser rupture, the mice received a 1.0 μ L injection into the vitreous of either bHPCD CAI solution or suspension bHPCD-CAI formulations. A second intravitreal injection was performed one week later. CNV was evaluated by volume measurement as previously reported [16]. The three lesion volumes for each animal were then averaged and treated as an n of 1 for statistical analysis. Changes in lesion volume among treatment groups were determined by averaging the mean lesion volume for all animals in a treatment group, and reported as mean $\pm$ standard error from the mean. Differences in lesion volume with a *p* value less than or equal to 0.05 were considered significant.

2.5 Rabbit intravitreal PK

A solid phase extraction (SPE) bioanalytical method was developed for accurate and sensitive quantitation of CAI in ocular tissues by HPLC-MS-MS analysis. This method employed a tetradeuterated CAI synthesized by RFE Pharma as an internal standard and gave a limit of quantitation (LOQ) of 1 ng/mL. Rabbits were injected intravitreally with 30 μ L of 15.0 mg/mL bHPCD-CAI suspension formulation. The control eyes received an equal volume (30 μ L) of the vehicle. Drug quality control was ensured by the specificity of the synthetic pathway and verified by HPLC and NMR. The vitreous from three rabbits were examined per time point.

2.6 Toxicology studies using bHPCD-CAI suspension formulation

Rats were injected intravitreally with 5.0 μ L of 30.0 mg/mL bHPCD-CAI suspension formulation using a 32 gauge needle with a 10 μ L Hamilton syringe. The control eyes received an equal volume of the bHPCD formulation vehicle or balanced salt solution (BSS, Alcon Laboratories, Inc; Fort Worth, TX) (n=3 eyes/group). Each vial was prepared as a single use vial and was discarded after use to exclude potential contamination. Fundus exam was performed prior to injection to ensure that no pathology was present and then immediately after the injection to exclude possible disruption of the tissue due to the procedure itself. Fundus exams were performed using a fundus camera (RetCam II, Clarity Medical Systems, Pleasanton, CA) every two weeks until 8 weeks when rats were euthanized. Eyes were processed and embedded in plastic resin (Embed 812/DER 736; Electron Microscopy Sciences, Hatfield, PA). Superior-inferior sections, 0.5 μ m thick, were cut and subsequently stained with toluidine blue.

2.7 Assessing retinal function with the electroretinogram

At 1, 2, and 4 weeks post-injection, ERG recordings were performed, as previously described [17]. Rats were dark-adapted overnight and then prepared under dim red lights. Following anesthesia with ketamine (60 mg/Kg) and xylazine (7.5 mg/Kg), corneas were anesthetized with 1% tetracaine and pupils were dilated with 1% tropicamide and 1.5% cyclopentolate. The rats were placed on a homeothermic heating pad in a Faraday cage. A needle electrode placed in the tail served as ground while a needle electrode placed in the

cheek served as the reference. The recording electrode was a thin silver-impregnated nylon fiber that contacts the cornea through a layer of methylcellulose. Single flashes of increasing intensity (-3.0 to $2.1 \log \text{cd s/m}^2$) were presented to the rat while the response were acquired by a signal averaging system with low and high filter settings set at 1 and 1500 Hz, respectively (UTAS 2000; LKC Systems, Gaithersburg, MD). Interstimulus interval increased from 5 seconds to 60 seconds as flash intensity increased. Following dark-adapted recordings, the eyes were light adapted for ten minutes with a background light ($30 \log \text{cd/m}^2$). Four flashes of increasing intensity (-0.82 to $1.88 \log \text{cd s/m}^2$) were superimposed on this background light at a frequency of 2.1 Hz in order to isolate cone driven responses. At the end of the recording session rats were given yohimbine (2.1 mg/Kg) and returned to a recovery cage.

ERG waveforms were analyzed by measuring the a-wave from baseline to the trough of the first negative peak; b-wave from the trough of the a-wave, or baseline when the a-wave was not visible, to the peak of the large positive wave. Amplitude and latency were analyzed using two-way repeated ANOVAs (SigmaStat 3.5, Chicago, IL) [18].

2.7 Statistical analysis

Unless otherwise noted, statistical analysis was performed using Student's *t*-test (Excel; Microsoft, Redmond, WA). $P > 0.001$ is signified by an asterisk on the graphs.

3. Results

3.1 In vitro assays

3.1.1 Proliferation as measured by BrdU incorporation—CAI exposure reduced BrdU incorporation in HREC (Figure 2A), HRPE (Figure 2B) and HMEC (Figure 2C), all in a dose-dependant manner. CAI effectively inhibited proliferation in all three cell lines. The addition of 10% serum to the CAI cultures resulted in an increase in the projected IC_{50} of CAI (Figure 3).

3.1.2 Tube formation on Matrigel—HREC plated with their full media formed extensive tube networks (Figure 4A), while HREC in serum free media did not form any tubes at all (Figure 4B). HREC exposed to CAI demonstrated a regression in micro-vessel tube formation (Figure 4C-E). This decrease of tube formation was observed after incubation of $1\text{-}10 \mu\text{m}$ CAI in 5% FBS.

3.2 In vivo assays

3.2.1 CNV induced by laser rupture of Bruch's membrane—The mean CNV volume of mice injected with the bHPCD-CAI solution and suspension formulations and the bHPCD-CAI preparation was evaluated over a two-week time course of lesion development and regression. Three different concentrations (0.5 mg/mL , 1.5 mg/mL and 4.5 mg/mL) of bHPCD-CAI solution formulations all showed significant reduction in the CNV lesion size compared to the vehicle treated animals (Figure 5). The maximal effect of 75% reduction in CNV volume was achieved at the concentration of 4.5 mg/mL dose. A 30 mg/mL CAI suspension of bHPCD-CAI (10% of CAI) in solution inhibited CNV volume similarly to the 1.5 and 4.5 mg/mL solution doses as expected.

3.2.2 Retention of CAI in vitreous—Figure 6 shows the solid-phase quantification of CAI in vitreous after administration of bHPCD-CAI suspension formulation over time. The vitreous of the injected eye showed that CAI concentrations between $5\text{-}200 \mu\text{M}$ were maintained over the first 24 hours, and then $1 \mu\text{M}$ concentrations were still present up to 1

week post administration of CAI (data not shown). These concentrations are consistent with the efficacy observed in the CNV model.

3.2.3 Ocular toxicity studies—The intravitreal injection of bHPCD-CAI (30 mg/mL) suspension formulation was not associated with any ocular toxicity (Figure 7). The histological appearance of the retina was normal after intravitreal injection of bHPCD formulation vehicle, PBS, and the bHPCD-CAI formulation compared to the opposite untreated eyes. No changes in retinal thickness were observed (data not shown). No vacuoles, vessel leakage, or disorganization of retinal layers were seen and there were no regional changes of the superior retina where the drug was most concentrated after intravitreal injection, compared to the inferior retina.

3.2.4 Retinal function testing—Retinal function was normal after bHPCD-CAI treatment in the rat eye. As expected following intravitreal injections, there was a slight decrease in ERG amplitudes for both vehicle and bHPCD-CAI injected eyes compared to the opposite eyes (Repeated ANOVA $F(8, 44) = 3.8, p=0.005$; Figure 8C). BSS injections enhanced the ERG response at 1 week post-treatment (Figure 8). At 2 weeks, the responses were similar between the three treatment groups. At four weeks after injections, the CA-injected eyes were slightly greater in amplitude than the opposite eyes (Figure 8). Light-adapted ERG b-wave responses showed similar trends across time (data not shown).

4. Discussion

Herein, we report the ocular pharmacology and safety of an intravitreal injectable bHPCD formulation of the anti-angiogenic CAI molecule. The results indicate that intravitreal bHPCD-CAI for posterior segment neovascular disease (eg. AMD, DR) can substantially widen the narrow therapeutic window encountered with orally administered CAI in cancer patients. The anti-angiogenic activity of the CAI molecule in human retinal endothelial cell (HREC) angiogenesis models confirms expected efficacy in the relevant target ocular tissue. To establish ocular clinical utility for CAI we developed the first aqueous formulations devoid of toxic organic solvents based on bHPCD as a complexing agent for the otherwise insoluble CAI molecule. These novel bHPCD-CAI formulations potentially decrease neovascular size in the CNV model and do not display ocular tissue toxicity after intravitreal injection. Taken together with the ocular pharmacokinetic data, the results indicate that intravitreal bHPCD-CAI has the potential to overcome the dose and efficacy limitations encountered with oral CAI as a systemic cancer therapy. Furthermore, the anti-angiogenic mechanism of action of the CAI molecule based on inhibition of intracellular calcium mediated signaling is complementary to current VEGF-i therapies. As a result bHPCD-CAI has the potential both to improve overall treatment outcomes in patient with posterior segment neovascular disease (eg. AMD, DR).

Our *in vitro* results in ocular HREC cellular assay systems are consistent with an intrinsically effective CAI concentration in the single digit micromolar range established previously in a range of *in vitro* angiogenesis models. Based on CAI's *in vitro* single digit micromolar intrinsic anti-angiogenic potency, the NCI developed an oral CAI capsule and dosing regimens in patients to achieve projected efficacious single digit micromolar CAI plasma concentrations. However, while low single digit micromolar plasma CAI concentrations could be achieved with oral CAI, these plasma concentrations suffered from a high degree of both inter-patient and intra-patient variability. Unfortunately, the use of higher doses of oral CAI to target higher concentrations across all patients was circumscribed by non-linear pharmacokinetics and systemic side effects. Furthermore, in a retrospective analysis, Figg *et al.*, pointed out the importance of targeting higher plasma concentrations of CAI *in vivo* than those anticipated to be intrinsically efficacious from *in*

vitro studies. To illustrate this Figg *et al.* showed that the effective concentration of CAI in the rat aorta assay is raised by an order of magnitude when the assay is conducted in the presence of human serum [19, 20]. The development of oral CAI for advanced metastatic cancer has been terminated by the NCI due to the narrow therapeutic window of the orally administered molecule as a monotherapy for advanced cancers. Nevertheless, the understanding of the limitations with oral CAI has proved invaluable in informing the development of a locally administered injectable dose form of CAI for ocular disease as described below.

The clinical oral CAI cancer experience demonstrated the need for an improved dosage form with improved pharmacokinetic consistency and performance to enable application of CAI for pathological ocular angiogenesis. Furthermore, our need for higher concentrations of CAI to achieve HREC assay efficacy in the presence of serum (Figure 3) pointed to the potential need for similar higher CAI concentrations in ocular tissue for robust *in vivo* and clinical efficacy. Therefore, we set out to develop an ocular intravitreal injectable dose form of CAI which could yield >10 μ M concentrations and evaluate its efficacy and safety in ocular animal models. The preparation of a clinically viable injectable dose form of CAI has previously encountered difficulties due to poor aqueous solubility of the molecule and the previous need for toxic organic cosolvents. However, by complexing CAI with hydroxypropyl-beta-cyclodextrin, we have been able to prepare simple isotonic aqueous bHPCD-CAI formulations that are clinically viable and safe. By varying the bHPCD concentration used to complex the CAI molecule, we have prepared both homogeneous solution and suspension CAI formulations with 4.5 mg/mL and 30mg/mL respective total CAI concentrations. For the suspension bHPCD-CAI formulations, we have limited the bHPCD concentration to a generally accepted safe level of 10% which allows for a *ca.* 1.5 mg/mL concentration of CAI in the solution phase. A bHPCD-CAI suspension formulation is our envisaged ultimate clinical ocular injectable dose form. In addition to providing immediately effective ocular tissue concentrations of CAI from the free solution fraction (as we demonstrate below) suspension, bHPCD-CAI can also provide sustained efficacy via slow release from the particulate phase which acts as a depot drug reservoir. This advantage has precedence in the ocular application of triamcinalone, Kenalog® or Triesence®, a high concentration (40 mg/mL) suspension formulation of the corticosteroid triamcinolone. Intravitreal Kenalog® provides for initial drug release and thereafter sustained dissipation of depot particles over several months [21]. Due to its high clinical efficacy, intravitreal triamcinalone has become an “off-label” standard of care therapy for diabetic macular edema.

Our results in the mouse CNV laser lesion model (Figure 5) with intravitreal bHPCD-CAI formulations demonstrate that robust efficacy can be achieved. The dose response observed with the solution bHPCD-CAI formulations (0.5, 1.5 and 4.5mg/mL CAI concentrations, indicates a maximal effect is already achieved with 1.5mg/mL CAI. Also, the efficacy of 30 mg/mL bHPCD-CAI suspension formulation is comparable to the maximal efficacy achieved with solution bHPCD-CAI. This result is consistent with a comparable *ca.* 1.5 mg/mL solution phase concentration of CAI in the 30 mg/mL bHPCD-CAI suspension formulation. Thus, the free solution phase concentration of CAI is the key driver of efficacy in this short term 2-week CNV model. The maximal efficacy of bHPCD-CAI in the CNV model of 75% neovascular volume reduction compares favorably with the efficacy of other anti-angiogenic molecules and mechanisms in rodent CNV models [22-26]. This intravitreal bHPCD-CAI study complements our previous report describing the ocular anti-angiogenic efficacy of CAI after oral administration in the mouse OIR model [13]. In this model high dose oral CAI (100 mg/Kg) inhibited formation of abnormal new vessels by over 90%. The *in vivo* ocular model efficacy results for CAI are consistent with the intrinsic *in vivo* anti-

angiogenic efficacy of CAI previously established in cancer tumor xenograft models of lung cancer and melanoma.

Based on the positive efficacy data, we next evaluated the ocular pharmacokinetics and safety of bHPCD-CAI suspension formulations following intravitreal injection in rabbits and rats respectively. The pharmacokinetic study of intravitreal injected 15mg/mL suspension bHPCD-CAI was performed with a 30 μ L dose volume in the rabbit (Figure 6). Maximal vitreous concentrations of >100 μ M CAI were achieved in the first few hours while concentrations of >10 μ M were maintained after 8hrs with 1-2 μ M concentrations were observed at the 1-week time point. This result supports the ability to achieve highly efficacious CAI concentrations in target retinal and choroidal target tissues of >10 μ M in a significant transient time period. In addition, the maintenance of single digit micromolar levels of CAI after one week in the vitreous indicates that the particulate phase of the formulation provides a sustained release of CAI to the ocular tissues. The maintenance of CAI levels in ocular tissue well beyond the 1-week time point is further postulated due to the observation of particulate CAI in the rat intravitreal safety study at the 1 and 2-month timepoints post-injection (data not shown). Thus, the pharmacokinetic study indicates that intravitreal administration of suspension bHPCD-CAI provides a highly effective transient concentration of >10 μ M CAI to the target ocular tissues, consistent with the efficacy observed in the CNV model. In addition, the anticipated prolonged release from the particulate phase of bHPCD-CAI yields sustained lower concentrations of CAI. This may serve to maintain a therapeutic effect for bHPCD-CAI for two months in clinical practice where currently intravitreal injections are typically administered at a 4-6 week interval.

In the rat intravitreal ocular safety study with 30 mg/mL suspension formulation bHPCD-CAI no ocular pathology was observed. The histopathology was normal after intravitreal injection of the 10% bHPCD vehicle as well as the bHPCD-CAI formulation compared to the opposite untreated eyes. No changes in retinal thickness were observed that would indicate edema or retinal degeneration. In addition, no vacuoles, vessel leakage, or disorganization of retinal layers was seen, and there were no regional changes of the superior retina. Thus, high dose intravitreal bHPCD-CAI suspension formulation is safe with respect to ocular anatomy after single administration. In addition, ERG studies with the same bHPCD-CAI formulation in the rat indicated that retinal function is normal (Figure 8). Finally, fundus photography demonstrated that particulate CAI dissipated slowly but was still present 1-2 months after bHPCD-CAI injection consistent with the pharmacokinetic results described above (data not shown).

In summary, our studies have shown that intravitreal suspension bHPCD-CAI is effective in the CNV model, has an ocular pharmacokinetic profile that may be superior to the standard 4-6 week administration interval and is well tolerated in the rat based on both anatomic and physiologic testing. Thus, intravitreal bHPCD-CAI warrants further characterization with regard to safety and efficacy in primate animal models and ultimate clinical investigation in patients who suffer from common posterior segment neovascular diseases that include exudative AMD, PDR, ROP, and RVO.

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Figure 1.
5-amino-[4-(4-chlorobenzoyl)-3,5-dichlorobenzyl]-1,2,3-triazole-4-carboxamide commonly named carboxyamidotriazole (CAI).

Figure 2.

CAI inhibits proliferation of human retinal endothelial cells (2A), retinal pigment epithelial cells (2B) and human dermal microvascular endothelial cells (2C). The graph shows the mean values plus/minus standard deviations. Asterisks denote statistically significant differences, with P values of 0.001 and lower.

Figure 3.

The addition of serum reduces the inhibitory effect of CAI on RPE proliferation by approximately 10 fold. The graph shows the mean values plus/minus standard deviations. Asterisks denote statistically significant differences between concentrations, with *P* values of 0.001 and lower.

Figure 4. CAI reduces HREC tube formation on Matrigel. A) Normal growth medium (+ control). B) Serum-free medium (– control). C), D) and E) normal medium with 10^{-7} , 10^{-6} , and 10^{-5} M CAI, respectively. All images are typical of four independent studies

Figure 5.

Effect of intravitreal CAI on CNV lesion volume. CNV was induced by laser rupture of Bruch's membrane. **A)** bHPCD-CAI solution at doses indicated was given intravitreally in 1 μ L volume on Day 0 (at time of laser), 7 and 14. Evaluation occurred 21 days after injury. **B)** Effect of intravitreal bHPCD-CAI suspension formulation on CNV lesion volume. There was no statistical difference among bHPCD-CAI-treated groups indicating that maximal CNV inhibitory effect is achieved at the lowest dose tested. All bars are mean $\pm$ SEM, $n = 5$. Individual t-tests were performed between each condition.

Figure 6. Quantitation of CAI in vitreous over time after administration of bHPCD-CAI suspension formulation. Note that high levels remain 24 hr after initial injection. Even after 1 week, micromolar concentrations can still be detected in the vitreous. Data points are mean $\pm$ SD of 3 measures, each measure repeated for vitreous from 3 eyes. Analysis shows a half-life of 31 hr.

Figure 7.

Histological section from the superior retina, 0.5 mm from the optic nerve from eyes injected with vehicle (A) or bHPCD-CAI (B). GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, PRL: photoreceptor layer. The scale bar indicates 50 microns.

Figure 8.

Normal retinal function from bHPCD-CAI injected eyes compared to vehicle and BSS injected eyes and opposite, uninjected eyes. (A) Representative waveforms at the three timepoints post-injection to a 2.1 log cd-s/m² flash show increased amplitude in the BSS-injected eye at 1 week. By 4 weeks, the bHPCD-CAI eye has slightly larger amplitudes. (B) Average amplitude values for each treatment group across flash intensity at 4 weeks post-injection revealed no significant changes for dark-adapted a- and b-waves. (C) Ratio of the dark-adapted a- and b-wave amplitude to a 2.1 log cd s/m² flash in the treated/untreated eye for each treatment group across the three timepoints. There were significant decreases in amplitude in the bHPCD-CAI and vehicle-treated eyes at 1 week post-injection (Repeated ANOVA $F(8, 44) = 3.8$, $p=0.005$) that returned to normal levels by 2 weeks. A trend for greater amplitude responses in the bHPCD-CAI injected eyes was observed at 4 weeks post-injection. Error bars represent standard error of the mean.