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Trans-scleral delivery of polyamine analogs for ocular neovascularization ☆

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Abstract

Periocular injections of the polyamine analog CGC-11144 three times a week causes regression of choroidal neovascularization. This regimen was selected to maximize chances of success for proof of concept, but is not ideal for clinical application. In this study we explored other regimens for periocular delivery of CGC-11144, and 2 other polyamine analogs, CGC-11047 and CGC-11093. A single periocular injection of 200 μg of CGC-11144, 2 mg of CGC-11047, or 1.5 mg of CGC-11093 caused significant suppression and regression of laser-induced choroidal neovascularization. An injection of 2 mg of CGC-11047 or 1.5 mg of CGC-11093 one or two weeks before, but not 3 weeks before, rupture of Bruch's membrane also caused significant suppression. Periocular injection of polyamine analogs also caused strong inhibition of retinal or subretinal neovascularization in mice with oxygen-induced ischemic retinopathy or *Rhodopsin promoter/VEGF* transgenic mice, respectively. These data suggest that periocular injection of one of 3 different polyamine analogs inhibits retinal or choroidal neovascularization and a single injection provides inhibitory activity for at least 2 to 3 weeks, which could provide the basis for a feasible treatment regimen for clinical trials.

Keywords: age-related macular degeneration; angiogenesis; choroidal neovascularization; endothelial cells; polyamines; proliferative retinopathies

1. Introduction

Polyamines are essential for cell growth and inhibition of their biosynthesis or administration of polyamine analogs that mimic natural polyamines but that have altered function, blocks cell proliferation (Gerner and Meyskens, 2004; Marton and Pegg, 1995; Thomas and Thomas, 2001). Therefore, agents that interfere with polyamine metabolism and function are potentially useful in treatments for diseases in which there is excessive proliferation of cells. In addition to disrupting proliferation, polyamine antagonists differentially induce apoptosis in cycling cells, thereby eliminating them (Schipper et al., 2000). Although non-cycling cells are less sensitive to the effects of polyamine antagonists, at high doses quiescent cells may also undergo apoptosis. Therefore, dosage and mode of delivery are important parameters that must be optimized for therapeutic utilization of polyamine antagonists.

We have recently demonstrated that 2 intraocular injections of 20 μg of the polyamine analog CGC-11144 induces apoptosis of vascular cells participating in choroidal neovascularization (CNV) resulting in partial regression of CNV (Lima e Silva et al., 2005). However, intraocular injections of CGC-11144 also induce apoptosis in retinal neurons causing retinal damage and reducing retinal function as assessed

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by electroretinograms (ERGs). Theoretically, it should be possible to identify an intraocular dose of CGC-11144 that induces apoptosis of proliferating vascular cells while not causing retinal damage, but dosing errors might have grave consequences. In contrast, an alternative mode of delivery of CGC-11144, periocular injection, was found to be safe and effective. Starting 7 days after rupture of Bruch's membrane, a time point when CNV is well-established, 3 periocular injections of 200 μg of CGC-11144 between days 7 and 14 resulted in a significant decrease in the area of CNV with no alteration in ERGs or retinal structure. This provides proof of concept that periocular delivery of CGC-11144 may be useful for treatment of patients with CNV. However, since it is impractical to perform periocular injections 3 times a week in patients, we explored alternative regimens. We also tested 2 related polyamine analogs, CGC-11047 and CGC-11093 (Frydman et al., 2003; Reddy et al., 1998; Valasinas et al., 2003), for their effect on CNV and assessed the effect of periocular injection of each of these polyamine analogs in models of retinal and subretinal neovascularization.

2. Materials and methods

2.1. Drugs

The decamine, CGC-11144 and the tetra amines CGC-11047 and CGC-11093 were synthesized as previously described (Reddy et al., 1998; Valasinas et al., 2001, 2003). The agents were diluted in phosphate-buffered saline for injections.

2.2. Preventive treatment of laser-induced CNV

Mice were treated in accordance with the recommendations of the Association for Research in Vision and Ophthalmology and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Laser photocoagulation-induced rupture of Bruch's membrane was used to generate CNV (Tobe et al., 1998b). Briefly, 6 to 8 week old female C57BL/6J mice were anesthetized with ketamine hydrochloride (100 mg/kg body weight) and the pupils were dilated with 1% tropicamide (Alcon Labs, Inc., Forth Worth, TX). Three burns of 532 nm diode laser photocoagulation (75 μm spot size, 0.1 s duration, 120 mW) were delivered to each retina using the slit lamp delivery system of an OcuLight GL Photocoagulator (Iridex, Mountain View, CA) and a hand held cover slide as a contact lens. Burns were performed in the 9, 12, and 3 o'clock positions of the posterior pole of the retina. Production of a bubble at the time of laser, which indicates rupture of Bruch's membrane, is an important factor in obtaining CNV (Tobe et al., 1998b), so only burns in which a bubble was produced were included in the study.

For periocular injections, mice were anesthetized with 100 mg/kg body weight of ketamine hydrochloride and a drop of 0.5% proparacaine hydrochloride was placed on the eye. Under a dissecting microscope a disposable 30-gauge needle connected to a Hamilton syringe was inserted under the conjunctiva at the equator of the eye. Elevation of the conjunctiva was visualized as 5 μl was injected allowing confirmation that the injection was successful. Mice received a periocular injection of 5 μl of vehicle alone or vehicle containing 200 μg of CGC11144, 2 mg of CGC-11047, or 1.5 mg CGC-11093 immediately after laser or 1, 2, or 3 weeks before laser. Two weeks after rupture of Bruch's membrane, mice were perfused with fluorescein-labeled dextran (2×10^6 average mw, Sigma, St. Louis, MO) and choroidal flat mounts were prepared as previously described (Nambu et al., 2003). Briefly, the eyes were removed, fixed for 1 h in 10% phosphate-buffered formalin, and the cornea and lens were removed. The entire retina was carefully dissected from the eyecup, radial cuts were made from the edge of the eyecup to the equator in all 4 quadrants, and it was flat-mounted in Aquamount. Flat-mounts were examined by fluorescence microscopy using an Axioskop microscope (Zeiss, Thornwood, NY) and images were digitized using a 3 CCD color video camera (IK-TU40A, Toshiba, Tokyo, Japan) and a frame grabber. Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) was used to measure the area of each CNV lesion. Statistical comparisons were made using analysis of variance (ANOVA) with Dunnett's correction for multiple comparisons or by unpaired t-test when experimental eyes were compared only to control eyes.

2.3. Treatment of mice with established CNV

Adult female C57BL/6 mice had laser-induced rupture of Bruch's membrane at 3 locations in each eye and after one week several of the mice were perfused with fluorescein-labeled dextran and had measurement of the baseline amount of CNV. At the same time point, one week after rupture of Bruch's membrane, all remaining mice were given a single 5 μl periocular injection in one eye containing one of the following: 200 μg of CGC-11144, 2 mg of CGC-11047, 1.5 mg of CGC-11093, or vehicle alone. Seven days after the treatment (14 days after laser), the mice were perfused with fluorescein-labeled dextran and the area of CNV at rupture sites was measured on choroidal flat mounts.

2.4. Transgenic mice with increased expression of VEGF in photoreceptors

Transgenic mice in which the *Rhodopsin* promoter drives expression of $VEGF_{165}$ in photoreceptors have onset of VEGF expression at postnatal day (P) 7 and within a few days develop neovascularization originating from the deep capillary bed that grows through the photoreceptor layer into the subretinal space (Okamoto et al., 1997; Tobe et al., 1998a). Hemizygous transgene positive mice were given a periocular injection of 3 μl containing 100 μg of CGC-11144 ($n = 3$), 1 mg of CGC-11047 ($n = 3$), or vehicle ($n = 6$) at P7. At P21, mice were euthanized and the amount of subretinal neovascularization was quantified as previously described (Tobe et al., 1998a). Briefly, mice were anesthetized, perfused with fluorescein-labeled dextran, and the total area of