Experimental Eye Research 92 (2011) 464–472

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Optimization of laser-induced choroidal neovascularization in African green monkeys

Robin J. Goody [a], Wenzheng Hu [b], Afshin Shafiee [b], Michael Struharik [a], Stephen Bartels [b], Francisco J. López [b,1], Matthew S. Lawrence [a,*]

[a] RxGen, Inc., 100 Deepwood Drive, Hamden, CT 06517, USA
[b] Preclinical Pharmacology, Bausch & Lomb Inc., Rochester, NY 14603, USA

ARTICLE INFO

Article history:
Received 19 November 2010
Accepted in revised form 6 March 2011
Available online 15 March 2011

Keywords:
laser-induced choroidal neovascularization
age-related macular degeneration
nonhuman primate
African green monkey
Chlorocebus sabaeus
animal model
VEGF

ABSTRACT

We developed and validated a new nonhuman primate model of laser-induced choroidal neovascularization (CNV) that addresses study design limitations prevalent in laser-induced CNV-based efficacy studies. Laser-induced Bruch's membrane disruption triggers CNV and has been widely utilized in animals to model neovascular ("wet") age-related macular degeneration (AMD). Despite widespread use of the approach, detailed assessment of experimental parameters and their influence on pathophysiological endpoints critical for disease modeling has been extremely limited and largely based on anecdotal observations. We evaluated laser power parameters and endpoint measures to optimize methods for CNV formation and quantification to facilitate drug efficacy screening in African green monkeys. Six laser spots of 350, 550, 750, 950 or 1500 mW laser power were positioned bilaterally 1.5 disc diameters from the fovea, within the macula. Fluorescein angiograms were collected 3–5 weeks later and scored by trained masked investigators using graded (I–IV) and densitometric methods. Histopathology assessments were also performed, including determination of CNV area. Test system sensitivity to angiogenesis inhibition was subsequently assessed by evaluating the effect of intravitreal bevacizumab (Avastin) pretreatment (one day prior to laser photocoagulation) on incidence of CNV. Grade III and grade IV lesions were considered clinically relevant, demonstrating early hyperfluorescence and late leakage within or beyond the lesion borders. By 4 weeks post-laser all treatment groups demonstrated evidence of grade III lesions with greatest incidence observed in lesions induced by 750 and 950 mW laser power (72.9% and 69.4% respectively). Grade IV lesions were confined to eyes receiving 550 mW laser power or higher, with highest incidence of grade IV lesions observed in eyes receiving 950 (19.4%) and 1500 mW (31%) laser spots, incidence peaking 4 weeks post-laser photocoagulation. Densitometric analyses of angiograms corroborated visual scoring. Bevacizumab completely abolished grade IV lesion development and significantly lowered lesion fluorescein signal intensity ($P < 0.0001$) and CNV area ($P = 0.038$) compared to vehicle-treated controls. Our studies demonstrate that laser power of 950–1500 mW and angiography analysis 4 weeks post-laser are optimal parameters to evaluate treatment effects on CNV induction following laser photocoagulation. Bevacizumab significantly attenuated CNV development, as determined by fluorescein angiography and histopathology assessments in this model, supporting the application of African green monkeys in preclinical modeling of CNV. Laser parameters and time points for therapeutic dosing and angiography endpoints are critical factors to the laser-induced CNV model and must be validated for robust assessment of efficacy. The newly optimized nonhuman primate model described will facilitate preclinical efficacy assessments of novel therapeutics for CNV.

1. Introduction

Choroidal neovascularization (CNV) involves the invasion of new blood vessels from the choroid through breaks in Bruch's membrane. Choroidal neovascularization is a feature of several eye diseases, but is most commonly associated with age-related macular degeneration (AMD). In the advanced "wet" form of AMD, CNV develops in the choriocapillaries and subretinal space, disrupting the retinal pigment epithelium (RPE) and resulting in severe central vision loss (Green, 1999). Age-related macular degeneration remains a leading cause of blindness in older populations of developed countries (Resnikoff et al., 2004). Recent development of anti-vascular endothelial growth factor

* Corresponding author. Tel.: +1 203 498 9706; fax: +1 203 776 2893.
E-mail address: mlawrence@rx-gen.com (M.S. Lawrence).
[1] Current address: Integrative Pharmacology, Life Sciences, Corning Incorporated, One Science Center Drive, Corning, NY 14831.

0014-4835/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2011.03.006

(anti-VEGF) therapies has dramatically improved outcome for many patients with AMD (Ozkiris, 2010). The anti-VEGF agents pegaptanib sodium (Macugen™, Eyetech, Inc., New York, NY), ranibizumab (Lucentis™, Genentech, Inc., South San Francisco, CA) and bevacizumab (Avastin™, Genentech, Inc.) have all demonstrated efficacy at attenuating vision loss or improving visual acuity in the clinic following repeated intravitreal (IVT) injection (for review, see Ciulla and Rosenfeld, 2009). However, anti-VEGF based therapeutics are not effective in all patients. In the 2006 MARINA trial, substantial vision improvement was confined to only a third of patients; and in 1/6th of trial patients the disease still progressed to legal blindness over a 24 month period (Rosenfeld et al., 2006).

Early clinical observations of CNV 4–6 weeks after argon laser-mediated photocoagulation therapy for macular degeneration provided the basis for the development of animal models of the disease (Francois et al., 1975). Significantly, recent studies evaluating ranibizumab and bevacizumab in rat (Lu and Adelman, 2009) and mouse (Yu et al., 2008) models of CNV demonstrated that these humanized anti-VEGF antibody-based therapeutics offer no improvement in leakage or neovascularization in rodent models. The absence of efficacy in rodent models is presumed to be related to the structural differences between rodent and human VEGF. Due to the unique shared physiology and anatomy of human and nonhuman primate eyes, the primate laser-induced CNV model, which has been investigated in rhesus (Ryan, 1979; Zhang et al., 2008) and cynomolgus monkeys (Tolentino et al., 2000; Shen et al., 2004), has become the model of choice for preclinical evaluation of the efficacy of candidate wet AMD therapies, including the VEGF inhibitors, now in clinical use. This is exemplified by the efficacy of ranibizumab at attenuating CNV alone (Krzystolik et al., 2002) and in combination with verteporforin photodynamic therapy in cynomolgus monkeys (Husain et al., 2005; Kim et al., 2006) and the recent first demonstration of efficacy of bevacizumab in a cynomolgus monkey model (Lichtlen et al., 2010). The relative expense and scarcity of rhesus and cynomolgus monkeys, however, can be a prohibitive barrier in the evaluation of novel therapeutics. In contrast, the African green monkey (*Chlorocebus sabaeus*) is another well-validated old world primate model species (Carlsson et al., 2004) with greater abundance and available for research use at potentially more reasonable cost. Furthermore, use of this species in ophthalmic studies has been reported since the early 1960s (Barany, 1963, 1977; Merrill and Burge, 2007). Early evaluations of laser-induced CNV in nonhuman primates demonstrated low incidence of CNV (Ryan, 1982; Shen et al., 2002), however, more recent studies have shown increased CNV incidence after photocoagulation in the macular region (Shen et al., 2004). Laser photocoagulation has been performed with various laser parameters over recent years. A spot size of 50 μm and duration of 100 ms has been most widely used in nonhuman primates (Ryan, 1982; Shen et al., 2004; Husain et al., 2005). Laser power, however, has been more variable between research groups, ranging from 240 mW (Zhang et al., 2008) up to as high as 1500 mW (Shen et al., 2004). Significantly, while laser power settings of 300–700 mW are most commonly described (Miller et al., 1995; Husain et al., 2005; Koh et al., 2006; Zhang et al., 2008), many groups utilize a multiple hit approach, whereby laser photocoagulation is repeated at either the same spot or an immediately adjacent one until an audible "pop" is heard and a subretinal bubble visualized as Bruch's membrane is disrupted.

The objective of these studies was to validate a laser-induced CNV model of wet AMD in the African green monkey by demonstrating angiographically and histopathologically evident neovascularization following laser injury and by showing inhibition of neovascularization in this model by a well characterized existing therapeutic. This model represents a new nonhuman primate model and an optimal study design for assessment of efficacy of novel wet AMD therapeutic candidates.

2. Materials and methods

2.1. Animals

Twenty-four male African green monkeys (*C. sabaeus*), aged 4–8 years were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Permission allowing the use of bilateral treatments and all aspects of the animal studies was obtained from the primate facility (St. Kitts Biomedical Research Foundation, St. Kitts, West Indies) Animal Care and Use Committee. Monkeys were anesthetized for all procedures with intramuscular injection of 5:1 ketamine:xylazine mix (0.2 mL/kg of 100 mg/mL ketamine and 20 mg/mL xylazine). Supplemental anesthesia (ketamine:xylazine) was administered intramuscularly as needed. Proparacaine hydrochloride 0.5% was provided topically. Animals were euthanized using intravenous pentobarbital sodium and enucleation performed immediately thereafter.

2.2. Induction of experimental CNV

Baseline fundus photography and fluorescein angiograms were performed as part of animal and ocular health screening (day -1) prior to laser photocoagulation. On day 0, monkeys were randomly assigned to treatment groups. Six laser spots were concentrically spaced approximately 1.5 disc diameters from the fovea at the anatomic periphery of the macula, within the temporal vascular arcades. Laser spots were applied using an Iridex Oculight TX 532 nm laser at 350–1500 mW power. Laser pulse duration and spot size were fixed at 100 ms and 50 μm, respectively. A single laser photocoagulation treatment was provided per site, independently of the presence or absence of an audible "pop" sound and visualization of a subretinal bubble, which are routinely used as indications of successful disruption of Bruch's membrane. The laser was mounted on a slit lamp with a slit lamp adapter and the beam directed to the retina with a Volk Centralis Direct ANF+ 0.9× laser lens with a 10 mm contact diameter (Volk Optical, Mentor, OH). Saline was used as the coupling agent. In experiments assessing efficacy of bevacizumab a laser power of 1500 mW was used with all other parameters identical to that detailed here.

2.3. Ocular examinations

Eyes were examined by slit lamp and indirect ophthalmoscope immediately prior to IVT injections on day 0, immediately prior to laser treatment on day 1 and on days 15 and 22.

2.4. Laser power assessment

To determine optimal laser power for development of grade IV CNV lesions in African green monkeys 16 adult males were randomly assigned into treatment groups receiving 350, 550 (3 animals per group), 750 or 950 mW (4 animals per group) laser power. A separate cohort of four adult males received 1500 mW laser power, and was analyzed separately. No additional treatments were performed on these animals prior to or following laser treatment and all other parameters for lesion induction were as described above.

2.5. Bevacizumab treatment

To assess efficacy of an existing wet AMD therapeutic in this model, 8 adult male African green monkeys were randomly