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The non-human primate experimental glaucoma model☆

Claude F. Burgoyne*

Devers Eye Institute, Optic Nerve Head Research Laboratory, Discoveries in Sight Research Laboratories, Legacy Research Institute, Portland, OR, United States

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ABSTRACT

The purpose of this report is to summarize the current strengths and weaknesses of the non-human primate (NHP) experimental glaucoma (EG) model through sections devoted to its history, methods, important findings, alternative optic neuropathy models and future directions. NHP EG has become well established for studying human glaucoma in part because the NHP optic nerve head (ONH) shares a close anatomic association with the human ONH and because it provides the only means of systematically studying the very earliest visual system responses to chronic intraocular pressure (IOP) elevation, i.e. the conversion from ocular hypertension to glaucomatous damage. However, NHPs are impractical for studies that require large animal numbers, demonstrate spontaneous glaucoma only rarely, do not currently provide a model of the neuropathy at normal levels of IOP, and cannot easily be genetically manipulated, except through tissue-specific, viral vectors. The goal of this summary is to direct NHP EG and non-NHP EG investigators to the previous, current and future accomplishment of clinically relevant knowledge in this model.

1. Introduction

While varying forms of chronic experimental intraocular pressure (IOP) elevation were reported in the non-human primate (NHP) by a series of investigators beginning in the late 1950's and early 1960's, the first description of the current model of chronic, laser-induced, unilateral intraocular pressure (IOP) elevation is generally ascribed to a 1974 publication by Gasterland and Kupfer (Gaasterland and Kupfer, 1974). During the ensuing 40 years, the NHP experimental glaucoma (EG) model has become well established for studying human glaucoma in part because the NHP optic nerve head (ONH) shares a close anatomic association with the human ONH and in part because this association is consistent throughout each component of the visual system. The model is also important because it provides the only means of systematically studying the very earliest visual system responses to chronic IOP elevation, i.e. the conversion from ocular hypertension to glaucomatous damage. Human eyes at this stage of "damage" cannot be clinically recognized nor can they be expected to be found in subjects who are close to death (i.e close to post-mortem, tissue donation). However, NHPs are impractical for studies that require large animal numbers, demonstrate spontaneous glaucoma only rarely, do not currently provide a model of the neuropathy at normal levels of IOP, and cannot easily be genetically manipulated, except through tissue-specific viral vectors. NHP EG often occurs at levels of IOP that are higher than (Gardiner et al., 2012), and in eyes from animals that are "younger" than (Gardiner et al., 2012), most human primary open angle glaucoma (POAG) patients (Tielsch et al., 1991a, 1991b). The purpose of this report is to summarize the current strengths and weaknesses of the NHP EG model through sections devoted to its history, methods, important findings, alternative optic neuropathy models and future directions. To provide context, it starts with a review of what is known about spontaneous NHP glaucoma and other spontaneous forms of NHP optic neuropathy.

To clarify terminology within this article, "naive" normal eyes from bilateral normal animals will be referred to as "normal". The contralateral, "untreated" eye of an animal with unilateral EG will be referred to as a "control" eye and the treated eye will be referred to as the pre-EG eye during its period of baseline testing and the "EG" eye following the onset of trabecular meshwork lasering

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* Corresponding author. Devers Eye Institute, Optic Nerve Head Research Laboratory, Discoveries in Sight Research Laboratories, Legacy Research Institute, 1225 NE 2nd Ave, Portland, OR, 97232, United States.
E-mail address: cfburgoyne@deverseye.org.

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whether IOP elevation and/or the clinical onset of glaucoma has been detected or not. Glaucoma that has been recognized to be present in wild-caught NHPs will be referred to as "spontaneous" glaucoma.

1.1. Spontaneous NHP glaucoma and other spontaneous NHP optic neuropathies

A small colony of Rhesus Macaque monkeys from the closed Cayo Santiago colony of the University of Puerto Rico, was first reported to contain animals that demonstrated ocular hypertension, an optic neuropathy progressing in the setting of ocular hypertension and the presence of ONH changes suspicious for glaucoma in the absence of detected elevated pressure in 1993 (Dawson et al., 1993). A series of subsequent reports (Dawson et al., 1998, 2005; Komaromy et al., 1998; Toris et al., 2010) defined a variety of ocular and demographic characteristics of subsets of animals, but the genetics of the colony and the histologic features of the neuropathy have not yet been described. A second small group of ocular hypertensive animals has been identified within an NHP colony in Singapore. A formal description of these animals is in preparation *(verbal communication, Tina Wong)*. An idiopathic bilateral optic neuropathy that demonstrated non-glaucomatous pallor of the ONH accompanied by retinal nerve fiber layer (RNFL) thinning most predominant within the maculopapular bundle has been described in 9 NHPs of Chinese origin obtained from two different primate centers (Fortune et al., 2005). Evidence of a toxic or nutritional cause for that neuropathy could not be found.

1.2. History of the model

A variety of agents have been employed to achieve acute and short-term IOP elevation in NHPs including alpha chymotrypsin (Hamasaki and Ellerman, 1965; Hamasaki and Fujino, 1967; Kalvin et al., 1966; Lampert et al., 1968; Lessell and Kuwabara, 1969; Levy, 1974; Zimmerman et al., 1967) and red blood cells (Quigley and Addicks, 1980a). In general, these approaches were abandoned because IOP elevations were poorly controlled, short-term, and frequently led to a loss of posterior pole visualization (Quigley and Addicks, 1980a). Following the model's introduction in 1974 (Gaasterland and Kupfer, 1974), a series of investigators (Quigley, Anderson, Harwerth, Kaufman, Burgoyne and others) have employed the model in their laboratories, contributing to its development and extending its application to the study of human glaucoma.

2. Methods

2.1. Institutional animal care and use committee (IACUC) approval, animal costs, and tools to follow each animal

While there are no standardized protocols, the scientific details of all NHP experiments must be approved by the IACUC under whose supervision the work will be performed. For investigators contemplating NHP experiments, gaining experience with the model in a lab that practices it is both recommended and often IACUC required. Having access to an NHP lab's written IACUC proposals can be extremely helpful in crafting your own. Studying any animal species to advance human understanding is a privilege society grants to medical researchers uncomfortably. This discomfort is never more acutely felt than with NHP research. Because of the ethical issues involved in their care and the costs of purchase (currently 500 to 10,000 dollars per animal, averaging 5000 dollars, depending upon age, source and research protocol exposure (in our experience) and daily (per diem) care (currently 15 dollars per animal per day in our institution)), substantial infrastructure should be in place to monitor each animal prior to the start of their study. Such infrastructure is required to be certain that each animal is sacrificed for post-mortem study (if planned) at defensible endpoints. Close endpoint monitoring is a challenge when 4, 8, 12 or more animals are under study at a given point in time. To address these concerns we and others have constructed custom, online software which allows immediate access to animal demographic, running notes and IACUC approved protocols. IOP, clinical photo, longitudinal digital imaging and testing data, which updates and displays testing results relative to baseline mean and 95% confidence intervals at the end of each session, allows for same day onset and/or progression detection. Post-mortem tissue and data storage is also managed and accessed through these systems from whole globe to each visual system tissue type, from fixation through embedding, sectioning, staining, reconstructing, segmenting and quantification.

2.2. The implications of origin, age, gender and sub-species differences in study execution and design

While definitive studies have not yet been done for sub-species and gender, investigators need to be aware that they may need to control for these demographic variables in their work. Even though we have twice reported no detectible differences between cynomolgous and rhesus control eye ONH connective tissue architecture using 3D histomorphometry (Lockwood et al., 2015; Yang et al., 2011b), we have chosen to concentrate our work in the rhesus to remove any possible differences in species-related susceptibility from our experiments. Cynomolgus versus Rhesus differences in Multifocal Electroretinography (mfERG) responses during NHP EG have been reported (Nork et al., 2010). Less commonly used subspecies such as the squirrel and marmoset monkey will require similar evaluation to be used in combination with rhesus macaques. Age is an important risk factor for both the onset and progression of glaucomatous damage at all levels of IOP in human glaucoma (Burgoyne, 2011; Burgoyne and Downs, 2008; Downs, 2015). A series of NHP ocular age effects have been reported (see section to follow). Controlling for age should therefore be considered depending upon the hypotheses being tested. Gender differences in NHP EG phenotype and/or susceptibility have not been explicitly studied, but should also be considered.

2.3. Quarantine – baseline – intervention – post-intervention – pre-sacrifice – sacrifice and post-mortem stages of testing/study

Most institutions require a quarantine period after animal delivery (4 weeks is common) in which the new animals are housed together in an isolation room to protect the rest of the colony from infectious disease (most commonly tuberculosis).

Once quarantine is cleared, weekly testing sessions are allowed in our institution, assuming animal health is maintained. Anesthesia protocols are commonly test-type and institution-specific – depending upon the experience and preferences of the attending veterinarian. A period of baseline testing (commonly 3–5 sessions) to characterize eye-specific variability precedes the planned intervention. While unilateral interventions are most common, bilateral interventions can be proposed but the IACUC will require extensive justification and absolute avoidance of bilateral blindness. Post-intervention testing occurs at a frequency governed by the sensitivity and specificity of the endpoint determination. It is common for us to perform weekly or bi-weekly post-laser testing, which requires the confirmation of onset on 2 subsequent test days (1 and 2 weeks post onset detection) (He et al., 2014b). This criterion has achieved 95% specificity among control eyes in all of our studies to