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Research article

Differential visual system organization and susceptibility to experimental models of optic neuropathies in three commonly used mouse strains

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ABSTRACT

Mouse disease models have proven indispensable in glaucoma research, yet the complexity of the vast number of models and mouse strains has also led to confusing findings. In this study, we evaluated baseline intraocular pressure, retinal histology, and retinofugal projections in three mouse strains commonly used in glaucoma research, *i.e.* C57Bl/6, C57Bl/6-Tyr^{c}, and CD-1 mice. We found that the mouse strains under study do not only display moderate variations in their intraocular pressure, retinal architecture, and retinal ganglion cell density, also the retinofugal projections to the dorsal lateral geniculate nucleus and the superior colliculus revealed striking differences, potentially underlying diverging optokinetic tracking responses and visual acuity. Next, we reviewed the success rate of three models of (glaucomatous) optic neuropathies (intravitreal N-methyl-D-aspartic acid injection, optic nerve crush, and laser photocoagulation-induced ocular hypertension), looking for differences in disease susceptibility between these mouse strains. Different genetic backgrounds and albinism led to differential susceptibility to experimentally induced retinal ganglion cell death among these three mouse strains. Overall, CD-1 mice appeared to have the highest sensitivity to retinal ganglion cell damage, while the C57Bl/6 background was more resistant in the three models used.

1. Introduction

Glaucoma is a heterogeneous group of disorders that have in common the progressive death of retinal ganglion cells (RGCs) and degeneration of the optic nerve. Worldwide, over 60 million people are believed to be at risk to become irreversible blind, due to this neurodegenerative disease (Quigley and Broman, 2006; Tham et al., 2014). Although elevated intraocular pressure (IOP) is considered the major risk factor – and sole target for clinical treatment – glaucoma etiology is still not completely understood and thought to involve a dynamic interplay of genetic predisposition and age-related and environmental stressors (Calkins, 2012; Calkins and Horner, 2012; Leske et al., 2007; Nickells, 2007). This complexity and multifactorial nature of glaucoma is challenging scientists and clinicians to understand the underlying mechanisms leading to neurodegeneration and to find novel therapeutic approaches to fight this blinding disease. Mouse disease models have proven indispensable in this quest, yet the complexity of the vast number of models and mouse strains has also led to confusing findings.

Previous papers reported that CD-1 mice are more susceptible to ocular hypertension-induced glaucoma as compared to C57Bl/6 mice (Cone et al., 2010, 2012; Nguyen et al., 2013). This greater susceptibility could derive from structural differences in the eye/visual system of either mouse strain or from a differential response to elevated IOP. The assess whether albinism alone is the main factor predisposing CD-1 mice to more severe glaucomatous neurodegeneration, we tested CD-1, albino C57Bl/6 and pigmented C57Bl/6 mice in a model for ocular hypertension-induced glaucoma and two other optic neuropathy models (*i.e.* intravitreal N-methyl-D-aspartic acid (NMDA) injection and optic nerve crush (ONC)).

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Abbreviations

CTB	cholera toxin subunit β
dLGN	dorsal lateral geniculate nucleus
dpi	days post injury/injection
IOP	intraocular pressure
L-Dopa	L-3,4-dihydroxyphenylalanine
LP	laser photocoagulation
NMDA	N-methyl-D-aspartic acid
ONC	optic nerve crush
PBS	phosphate-buffered saline
PFA	paraformaldehyde
rd1	*retinal degeneration 1* mutation
RGC	retinal ganglion cell
ROI	region of interest
(SD-)OCT	(spectral domain-) optical coherence tomography
(s)SC	(superficial layers of the) superior colliculus
TBS	tris-buffered saline
Tyr	Tyrosinase gene
VGluT2	vesicular glutamate transporter 2

Whether or not related to albinism, many morphological and functional characteristic of the eye have been shown to vary among mouse strains, including IOP (Cone et al., 2012; John et al., 1997; Savinova et al., 2001), aqueous humor outflow resistance (Boussommier-Calleja and Overby, 2013), scleral biomechanics (Nguyen et al., 2013), RGC and cone density (Salinas-Navarro et al., 2009b; Whitney et al., 2011; Williams et al., 1996), congenital retinal degeneration (Chang et al., 2013; Clapcote et al., 2005; Mattapallil et al., 2012; Serfilippi et al., 2004; Wong and Brown, 2006), susceptibility to photoreceptor death (Matsumoto et al., 2014), visual projection patterns (Drager and Olsen, 1980; Rebsam et al., 2012; Rice et al., 1995), and performance in vision-guided behavior tasks (Balkema and Drager, 1991; Wong and Brown, 2006). We therefore first investigated the baseline phenotype of the retina and retinofugal projection in the three wild type mouse strains mentioned above, looking for traits that might relate to the differential glaucoma susceptibility.

2. Methodology

2.1. Experimental animals

Three mouse strains/stocks[2] were used in this study: C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice. The CD-1 and C57Bl/6-Tyr^c mouse strains both carry a homozygous Cys103Ser mutation (designated *Tyr^c*) in the tyrosinase (*Tyr*) gene, resulting in oculocutaneous albinism (Beermann et al., 2004; Lavado and Montoliu, 2006). Although C57Bl/6-Tyr^c and CD-1 are both albino mice, they have a very distinct genetic background: while C57Bl/6-Tyr^c inbred mice share the same genetic background as the C57Bl/6 strain, CD-1 mice are an outbred strain, implying high genetic heterogeneity (Chia et al., 2005).

All studies were conducted in compliance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and the Belgian legislation (KB of 29 May 2013), and were approved by the KU Leuven institutional ethical committee. Adult (2–4 months) C57Bl/6, C57Bl/6-Tyr^c, and CD-1 mice were obtained from the university breeding colony. Animals were kept under a 12/12 light–dark cycle and had *ad libitum* access to food and water.

2.2. Genotyping

Mice were genotyped for the *retinal degeneration 1 (rd1)* mutation of the *Pde6b* gene. Briefly, DNA was extracted from tail biopsies, and genotyping PCR was performed with the Fast Hotstart Genotyping PCR kit (Kapa Biosystems), in 30 cycles at the following temperatures: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and polymerization at 72 °C for 30 s. The amplified DNA products were analyzed by electrophoresis on a 2% agarose gel. The following primer sequences were used, producing 237 bp and 562 bp bands for wild type and *rd1* genotypes, respectively: 5′ ACCTGCATGTGAACCCAGTATTCTATC 3′ (*Pde6b1*); 5′ CTACAGCCCCTCTCCAAGGTTTATAG 3′ (*Pde6b2*); 5′ AAGCTAGCTGCAGTAACGCCATTT 3′ (*Pde6b3*).

2.3. Intra-ocular pressure measurement

The IOP was measured in awake animals with a calibrated rebound tonometer (Tono-Lab, iCare) (Haddadin et al., 2009, 2012). IOP was measured before LP (day 0) and on day 1 till 7 following LP. In short, the mouse was hold with one hand by loosely grabbing the fur in the neck and care was taken to avoid stress and pressure on the neck region. Next, 10 independent IOP measurements were taken per eye, from which the highest two and lowest two values are excluded in order to reduce variability. An average IOP for each eye was calculated from the remaining six values. IOP measurements were always performed in the morning, to avoid potential variability due to diurnal IOP variations. The untreated, contralateral eye was used as a control.

2.4. Spectral domain optical coherence tomography

Thickness of the retinal layers was evaluated using a spectral domain optical coherence tomography (SD-OCT) system (Envisu R2210, Bioptigen) (Buys et al., 2013). Upon general anesthesia (i.p. 75 mg/kg body weight ketamine, Anesketin, Eurovet; i.p. 1 mg/kg medetomidine, Domitor, Pfizer), pupils were dilated by topical application of 0.5% tropicamide (0.5% Tropicol, Thea). SD-OCT was performed using 100 consecutive B-scan lines composed of 1000 A-scans, in a 1.4 × 1.4 mm field. After the procedure, anesthesia was reversed by means of atipamezol (i.p. 1 mg/kg, Antisedan, Pfizer) and antibiotic ointment was applied to the eye (tobramycin 3 mg/g, Tobrex, Alcon). Total retinal thickness and thickness of different retinal layers were analyzed using InVivoVue Diver 2.2 software (Bioptigen).

2.5. Optokinetic tracking response

The optokinetic tracking response was measured under photopic conditions in a virtual-reality chamber (OptoMotry, Cerebral Mechanics), as described by Prusky et al. (Douglas et al., 2005; Prusky et al., 2004). Briefly, a virtual cylinder comprised of a vertical sine wave grating was projected on four computer screens facing into a box. The animal was placed on a platform in the center of the arena and a video camera, situated above the animal, provided real-time video feedback. Visual acuity was measured using a staircase procedure, in which different spatial frequencies (100% contrast, 12° per second speed) varied randomly and separate for each eye. The experimenter judged whether the mouse displayed optokinetic tracking, and the maximum spatial frequency at which optokinetic tracking seen, was determined.

[2] Outbred colonies are usually referred to as 'stocks', whereas inbred ones are referred to as 'strains' or 'lines'. However, to facilitate reading we will refer to all of them as 'strains'.