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In vivo imaging methods to assess glaucomatous optic neuropathy

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ABSTRACT

The goal of this review is to summarize the most common imaging methods currently applied for *in vivo* assessment of ocular structure in animal models of experimental glaucoma with an emphasis on translational relevance to clinical studies of the human disease. The most common techniques in current use include optical coherence tomography and scanning laser ophthalmoscopy. In reviewing the application of these and other imaging modalities to study glaucomatous optic neuropathy, this article is organized into three major sections: 1) imaging the optic nerve head, 2) imaging the retinal nerve fiber layer and 3) imaging retinal ganglion cell soma and dendrites. The article concludes with a brief section on possible future directions.

1. Introduction

Glaucoma is the most common optic neuropathy and the second leading cause of blindness worldwide (Quigley and Broman, 2006). Vision loss in glaucoma occurs primarily because retinal ganglion cells (RGCs) die and their axons degenerate, losing the capacity to convey visual information to the brain (Quigley, 1999, 2011; Quigley et al., 1981; Weinreb and Khaw, 2004). The initiating injury to RGCs and their axons is thought to occur within the optic nerve head (ONH) (Anderson and Hendrickson, 1974; Emery et al., 1974; Howell et al., 2013; Nickells et al., 2012; Quigley, 1999; Quigley et al., 1981; Vrabec, 1976). Indeed, chronic progressive deformation of the ONH tissues (traditionally referred to as "cupping" and/or "excavation") is considered the hallmark of glaucoma, a clinical sign used to distinguish it from other optic neuropathies (Danesh-Meyer et al., 2010; Quigley, 1993, 2011; Van Buskirk and Cioffi, 1992; Weinreb and Khaw, 2004). Thus clinical diagnosis and management of glaucoma depend not only on assessment of vision function, such as by measuring sensitivity across the visual field using automated perimetry, but also on assessment of structural integrity of the ONH and RGC axons within the retinal nerve fiber layer (RNFL).

Assessment of ONH and RNFL structural integrity can be achieved most easily by clinical examination using a direct ophthalmoscope, however, appreciation of finer detail is afforded by stereoscopic examination using binocular indirect ophthalmoscopy. Similarly, archival records of ONH and RNFL structure can be achieved in simplest form using a two-dimensional drawing or flash photograph, but simultaneous stereoscopic photographs offer superior capability for discerning changes in ONH surface topography due to their three-dimensionality.

More recent developments in ophthalmic imaging technology have enabled *quantification* of structural parameters, such as the width or volume of optic disc rim tissue, width and depth of the optic cup and cup-to-disc ratio, etc. One such technique known as confocal scanning laser tomography (CSLT) has become widely incorporated into both glaucoma research and clinical practice, and has even been used as an ancillary outcome measure for randomized clinical trials (Alencar et al., 2008; Fingeret et al., 2005; Sharma et al., 2008; Wollstein et al., 2000; Zangwill et al., 2013, 2005). Optical coherence tomography (OCT) enables cross-sectional images of ONH and retinal tissue to be acquired in the living eye and thus quantification of individual retinal layer thicknesses, as well as three-dimensional (3D) visualization and quantification of deeper ONH structures such as the lamina cribrosa (LC) (Inoue et al., 2009; Kagemann et al., 2008; Srinivasan et al., 2008; Strouthidis et al.,

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2010; Wollstein et al., 2005a). Modern OCT systems provide axial resolution of a few micrometers and the addition of adaptive-optics techniques are able to improve lateral (transverse) resolution to the same level (Hermann et al., 2004; Kocaoglu et al., 2011, 2014a, 2014b; Torti et al., 2009; Zawadzki et al., 2009, 2005; Zhang et al., 2005). These most recent developments hold great promise for helping to elucidate directly from studies performed in clinical settings a more precise understanding of glaucoma risk, the susceptibility of individual eyes and even the sequence of events in glaucoma pathogenesis.

Yet experimental models of glaucoma remain important for these purposes, for testing hypothesis about pathogenesis and for pre-clinical trials of novel therapeutic interventions (reviewed elsewhere this issue and others, e.g., (Burgoyne et al., 2005; Goldblum and Mittag, 2002; Morrison et al., 2008; Pang and Clark, 2007)). Common outcome measures for experimental glaucoma (EG) models include histological counts of RGC soma and/or orbital optic nerve axons, which require euthanasia and are thus only available at a single time point. Outcome measures based on *in vivo* imaging techniques, such as those used in the clinical setting and mentioned above, enable longitudinal evaluation within animals. Benefits of longitudinal imaging include reduction of the number of animals required to adequately power a scientific study (instead of sacrificing a different set of animals at each time point) and mitigation against errors that can arise when inferences about longitudinal time course and inter-relationships are drawn from cross-sectional data (Naskar et al., 2002; Prilloff et al., 2010; Sabel et al., 1997; Thanos et al., 2002). Moreover, application of imaging techniques that can be used across the spectrum of laboratory and pre-clinical studies in animals as well as clinical research and patient management in human glaucoma enables similar outcome measures to be assessed in order to better compare the pathophysiological sequence of an experimental model with the human disease. Such approaches may facilitate future translation of novel interventions and to ensure relevance and/or refine any given EG model accordingly. This review article summarizes the most common imaging methods currently applied for *in vivo* assessment of ocular structure in EG models with an emphasis on translation to clinical studies of the human disease. It is organized into three main sections based on target structure: ONH, RNFL, RGC soma and dendrites. Generally, since most ophthalmic imaging modalities were originally developed for clinical use in human eyes and then later adapted for use in animals, the discussion of each modality and target structure begins with some relevant background on development and application to basic and clinical studies of human eyes, then continues with laboratory studies in animals. It is hoped that this organizational structure will help emphasize the bidirectional translational nature of imaging in glaucoma research.

2. Imaging the ONH

Essentially any imaging modality used in a clinical setting for imaging human eyes can also be applied for imaging the eyes of larger laboratory animals – such as monkeys – with only minor modifications of technique to maintain optimal alignment, focus and corneal hydration since the latter are generally imaged under anesthesia. It is more challenging to image eyes of smaller laboratory animals such as rats and mice, primarily due to their smaller pupil size, higher optical power and aberrations. Thus, fundus photography in rats and mice benefits greatly from specialized equipment arrangements and procedures in order to obtain higher quality fundus images (Cohan et al., 2003; Hawes et al., 1999; Kocaoglu et al., 2007; Paques et al., 2007). High-resolution images of the mouse or rat fundus can also be obtained by confocal scanning laser ophthalmoscopy (CSLO) (Chauhan et al., 2002; Cordeiro et al., 2004; Paques et al., 2006; Seeliger et al., 2005). Advantages of CSLO for fundus imaging include improved image contrast due the confocal aperture and relative ease of imaging through the smaller pupil due to the narrow diameter of the scanning beam; disadvantages include greater expense of CSLO systems and lack of true color reflectance since the imaging source is monochromatic, though this aspect also contributes to greater image contrast and facilitates fluorescence applications (Cordeiro et al., 2004; Paques et al., 2006; Seeliger et al., 2005).

CSLO has been used to image the LC *in vivo* and perform quantitative morphometry in healthy and glaucomatous human eyes, measurements that were previously approachable only by histology. One of the earliest of such studies demonstrated that elongation of LC pores is related to the severity of glaucomatous visual field damage (Fontana et al., 1998). The addition of adaptive-optics (AO) to compensate for optical aberrations (Burns et al., 2007; Liang et al., 1997; Roorda et al., 2002) provides higher resolution in both transverse and axial dimensions for SLO imaging, which is particularly important for morphometric analysis of a complex three-dimensional structure such as the LC (Akagi et al., 2012; Ivers et al., 2011; Sredar et al., 2013; Vilupuru et al., 2007). Nevertheless, SLO imaging of the LC is ultimately limited to the visible portions of its anterior surface, which is masked across much of the ONH by major blood vessel branches and overlying rim tissue (consisting of axon bundles, astroglia and capillaries, all sources of scatter). Moreover, the LC of the rat ONH has only a few wispy vertical collagenous beams (Morrison et al., 1995) while the mouse ONH has none (May and Lutjen-Drecoll, 2002; Sun et al., 2009) and the major vessel branches also occupy a much greater proportion of the "optic disc" in rats and mice as compared with human and non-human primate (NHP) eyes (Chauhan et al., 2002; Fortune et al., 2011; Guo et al., 2005; Srinivasan et al., 2006; Zhi et al., 2011, 2012). Thus the applicability of two-dimensional *reflectance* imaging of the ONH in rat or mouse EG models is limited. However, Bosco and colleagues recently used *fluorescence* CSLO imaging *in vivo* to document early-stage ONH microgliosis in the DBA/2J mouse model of inherited glaucoma (Bosco et al., 2015). Ho et al., have also used *in vivo* longitudinal CSLO imaging to document gliotic responses of astrocytes within the ONH after RGC injury (Ho et al., 2009).

OCT imaging provides much higher axial resolution (~two orders of magnitude better) than CSLO, which has proven important to 3D visualization of the human LC (Inoue et al., 2009; Kagemann et al., 2008; Srinivasan et al., 2008). Use of the "enhanced depth imaging" (EDI) mode (Lee et al., 2012; Park et al., 2012a, 2012b; Yang et al., 2012a) and related strategies (Kim et al., 2012) in order to mitigate the signal roll-off inherent to spectral domain OCT improves measurement reliability and has revealed focal LC structural alterations that relate to glaucomatous disease severity (Faridi et al., 2014; Tatham et al., 2014; You et al., 2013). Similarly, post-processing techniques can be applied to recover contrast lost to signal attenuation (Girard et al., 2015; Mari et al., 2013) and further improve OCT imaging of deep ONH structures. The increased penetration of longer wavelength OCT sources (e.g. center wavelength of 1050 nm), such as those typically used in swept-source OCT may also improve imaging of the LC, peripapillary sclera and deep ONH structures (Srinivasan et al., 2008; Takayama et al., 2013a; Wang et al., 2013; Yoshikawa et al., 2014). Using polarization-sensitive swept-source OCT imaging at 1 μm further enhances visualization of these strongly birefringent connective tissues (Yamanari et al., 2009). However, one trade-off of the longer wavelength is a slight reduction of transverse resolution and the narrower frequency band available for most swept-source lasers results in reduced axial resolution (as compared with spectral domain OCT). These trade-offs can be overcome using adaptive