Published in final edited form as:

Jpn J Ophthalmol. 2015 May ; 59(3): 135–147. doi:10.1007/s10384-015-0373-5.

Ischemic optic neuropathies and their models: disease comparisons, model strengths and weaknesses

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Abstract

Ischemic optic neuropathies (IONs) describe a group of diseases that specifically target the optic nerve and result in sudden vision loss. These include nonarteritic and arteritic anterior ischemic optic neuropathy (NAION and AAION) and posterior ischemic optic neuropathy (NPION, APION). Until recently, little was known of the mechanisms involved in ION damage, due to a lack of information about the mechanisms associated with these diseases. This review discusses the new models that closely mimic these diseases (rodent NAION, primate NAION, rodent PION). These models have enabled closer dissection of the mechanisms involved with the pathophysiology of these disorders and enable identification of relevant mechanisms and potential pathways for effective therapeutic intervention. Descriptions of the different models are included, and comparisons between the models, their relative similarities with the clinical disease, as well as differences are discussed.

Keywords

Ischemic optic neuropathies; Nonarteritic anterior ischemic optic neuropathy; Optic nerve disease models; Arteritic anterior ischemic optic neuropathy model; Posterior ischemic optic neuropathy

Introduction

Ischemic optic neuropathy (ION) is a term describing a group of optic nerve (ON) disorders in which ischemia damages a part of the nerve. Most IONs occur acutely, which distinguishes them from chronic ON diseases such as open-angle glaucoma, in which ischemia likely plays an important part but develops over years. Nevertheless, both acute

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Conflicts of interest S. L. Bernstein, Patent (The use of Prostaglandin J2 for Optic nerve Disease); N. R. Miller, None.

and subacute or chronic ischemic ON disorders may have common pathophysiologic processes that result in a final pathway of ON damage and secondary retinal ganglion cell (RGC) loss. IONs usually are classified as anterior (AION) or posterior (PION) [1], and both are separated further into arteritic (AAION, APION), nonarteritic (NAION, NPION), and perioperative (PeAION, PePION) types [2].

Although the pathogenesis and pathophysiology of the arteritic forms of ION are well established based on both human and experimental studies, they are not as clear in either the non-arteritic or the perioperative forms. Fortunately, reasonable animal models now exist that can be used to understand these responses for all three forms. As no model is a perfect replica of a given human disease, it is important to understand how the human disorders differ from each other and how the animal models both simulate and diverge from the human condition.

Optic nerve anatomy and vascular supply

The optic nerve contains axons originating from retinal ganglion cells. These axons are unmyelinated within the retina and within the anterior intraocular portion of the ON. They become myelinated at the lamina cribrosa [3] and emerge from the eye as a distinct myelinated central nervous system (CNS) tract that passes through the orbit to the brain. The ON may be described as having four parts: (1) the intraocular portion (the optic nerve head or optic disk); (2) the intraorbital portion; (3) the intracanalicular (intraosseous) portion within the optic canal; and (4) the intracranial portion. The intraocular ON can be divided further, at least in humans and non-human primates, into three anatomically distinct zones: the prelaminar portion (anterior), the laminar portion (middle) and the retrolaminar portion (posterior) (schematic, Fig. 1a). Figure 1 is an integrated attempt to show an overall comparison among the different species. Vascular supply to the ON regions is complex, with much interaction between the laminar, prelaminar and retrolaminar regions (Fig. 1b). There is likely complex control of the blood flow through vessels in these three regions, as well as of the choroidal vessels derived from the Zinn-Haller circle, and the pial vessels. The rat ON (Fig. 1c) shares many structural similarities with the human nerve, differing primarily in overall complexity due to a reduction in the number of axons, capillaries, and size. In contrast, in the mouse, both the ON and its laminar vasculature (schematic, Fig. 1e) are markedly different from those of rat and human, due to a lack of communication between the laminar and the choroidal vasculature, the presence of retrograde retinal vasculature and reduced overall complexity. However, the mouse laminar capillary bed is more exuberant than the posterior ON, which is not apparent in the figure (data not shown). Schematic comparisons of human, mouse and rat ON circulation are shown in Fig. 1d–f.

A comparative analysis of the ON of different mammalian species is instructive [9]. In addition to the vast difference in axon numbers between mice (~50,000), rats (~100,000), and humans (~1,200,000), the lamina cribrosa structure is different in the primate and rodent, making direct model comparisons difficult. Although primates have dense connective laminar tissues made up of fibrous columns, rats have much less connective tissue [9, 10] and mice have only a glial lamina made up of glial plates rather than fibroblasts [8, 11]. These and vascular differences (see below) likely contribute to the

variations in difficulty in NAION model induction, and the speed of development and appearance of ON edema.

ON circulation is complex and is both species- and region- specific (Fig. 1). A simplified schematic illustration of primate lamina and ON supply is shown in Fig. 1d. For comparison, mouse is shown in Fig. 1e and rat in Fig. 1f. The anterior ON in mammals receives blood supply from three sources: the choroid, the pial vessels that perforate the ON surface, and capillaries derived from the retinal circulation [5, 11–13]. These interconnections form a plexus within the optic nerve lamina, normally enabling the anterior ON to sample the metabolic conditions of the retina, the choroid (in rats and primates) and the more posterior nerve. Primate and rodent laminar circulation primarily differ in that primates have a more complex and larger capillary bed. The mouse lamina circulation typically is not supplied by the choroid [8].

Behind the plexus region, the intraorbital and posterior ON receive blood supply from the pial circulation and perforating arteries derived from the pia. These vessels originate from the carotid artery and its branches [15]. Thus, there are numerous vascular sites for dysfunction, particularly in the anterior ON region. As the average axon in humans is 50 mm long, and as it takes only the loss of a single capillary anywhere along its route to cause dysfunction, it is surprising to the writers how stable and responsive the mechanisms of the average normal human ON and visual system are compared with the occasional unfortunate individual.

Clinical findings in the IONs

AAION is characterized clinically by sudden severe visual loss associated with pallid swelling of the optic disk, indicating a true infarction (Fig. 2c). Unlike the progressive edema and compartment syndrome associated with NAION (see below), AAION results from sudden vascular dysfunction due to inflammation-induced thrombosis of the medium-sized arteries supplying the choroidal circulation (the arterial circle of Zinn-Haller). The choroidal vasculature contributes a significant portion of the blood supply to the anterior ON circulation. Autoimmune vascular inflammatory disorders, most often giant cell arteritis (GCA), are largely responsible for AAION. Patients with this disorder typically are older than NAION patients.

In AAION, there is reduced choroidal blood flow from the compromised arteries identifiable by using intravenous fluorescein angiography (IVFA) as decreased filling of choroidal lobules near the ON [16, 17]. When there is sudden additional vascular compromise (vascular thrombosis), the previously compromised choroidal blood flow is insufficient to enable ON perfusion, and the remaining ON circulation likely cannot compensate for the sudden blood loss, resulting in severe regional ischemia and ON edema. This results in a severe vision loss and typical appearance of the swollen optic disk (Fig. 2c) [18].

NAION typically occurs in individuals over the age of 50 with small, crowded optic disks with little or no central cup (the disk at risk, Fig. 2a) and one or more underlying systemic vasculopathies such as hypertension, hyperlipidemia, or diabetes mellitus [19]. In such patients, it is assumed that slight damage to or occlusion of the capillaries will initiate a

cascade of events that will result in NAION in many cases. Additionally some NAION patients report first noticing vision loss upon waking up, leading a number of investigators to suggest that in such patients, the triggering event is nocturnal hypotension [20]. In all cases, however, NAION is characterized by hyperemic swelling of the optic nerve head (Fig. 2b). The swelling can worsen over days, during which the retinal vessels may dilate somewhat (Fig. 2b, arrow), suggesting a compressive restriction of central retinal venous return. Macular edema and submacular fluid accumulation can also occur [21], producing further visual dysfunction. Thus, there are at least three potential causes of visual loss in patients with NAION: an initial ischemic event, secondary ON damage from a compartment syndrome and macular edema and/or subretinal fluid collection. As if this were not enough, there is evidence that secondary inflammation at the site of the ischemia may contribute to or produce more visual loss (see below). Thus, further visual loss following the initial presentation (in the first days) is not uncommon. Nevertheless, visual improvement of three lines or better occurs in about 40 % of patients by 3 months post-onset, although a minority of individuals experience additional significant vision loss after this time [22], and even patients whose vision improves often experience a slight loss of a line of visual acuity by 2 years post-incident [22].

Following regression of ON edema, which typically takes 6–11 weeks the ON develops pallor/atrophy that may be sectoral or diffuse (Bellusci, 2008 3177/id). Clinical tissue is rare in early NAION: a single histological case has been published of a patient who still had OD edema when he died 21 days post-onset [68]. In that patient, ON swelling was not associated with any specific vascular bed, suggesting that the NAION lesion is similar to the compartment syndrome seen in other tissues in which there is compressive tissue edema in a restricted area. Progressive edema results in increased vascular resistance, ultimately resulting in additional compromise and loss of circulation in otherwise initially normal tissue.

As noted above, PION can be arteritic or nonarteritic [23, 24]. Like AION, the degree of visual loss in PION ranges from mild to complete, with patients who experience arteritic PION generally having much worse vision loss than patients with the nonarteritic type. In both types, however, the optic disk initially looks completely normal, only becoming pale after 6–8 weeks.

The diagnosis of PION, particularly the nonarteritic variety, should be one of exclusion [2] and should be made only after magnetic resonance imaging confirms the lack of a compressive or infiltrative lesion affecting the posterior orbital, canalicular, or intracranial regions of the optic nerve [24]. As is the case with NAION, there are few cases of pathologically studied NPION.

The pathophysiology behind perioperative ION (PeION) is unclear. PeAION typically occurs after cardiac bypass surgery, whereas PePION most commonly occurs after prone-position spine surgery. The factors associated with the development of PeION most often are hypotension and anemia, often associated with hemodilution, although relative fluid excess, head-down positioning, lengthy surgery, vasopressors and patient factors including atherosclerosis, diabetes mellitus, hypertension, obesity, obstructive sleep apnea, and

congenital or acquired hypercoagulability also have been suggested as risk factors. In a retrospective case-control study, the risk of PePION in patients undergoing prone-position spinal fusion surgery was increased by male sex, use of a Wilson frame, lengthy surgery, large blood loss, obesity, and relatively decreased colloid relative to crystalloid resuscitation, but not by intraoperative hypotension [25].

Models of AAION, NAION, and PeAION

AAION models

All models currently utilized for the arteritic, nonarteritic and perioperative forms of AION employ some acute or subacute loss of blood supply to the ON, rather than relying on the hypotensive dysfunction/disk-at-risk variables that are theorized to play a role in NAION and PeAION or the medium artery vascular inflammation that produces AAION. One reason these diseases have been so hard to model is that the vasculature of the ON is contiguous with but not identical to the retinal circulation. The short arterial vessels supplying the outer retinal vasculature through the circle of Zinn-Haller forms a plexus with the ON-laminar vasculature (Fig. 1d). This communication is utilized in the only published AAION animal model, which relies on partial destruction of the long or short posterior ciliary arteries (LPCAs/SPCAs) that, at least in primates, make up the arterial circle of Zinn-Haller [26]. This is shown schematically in Fig. 3a. The LPCAs and SPCAs supply the choroidal circulation, responsible for photoreceptor survival. Choroidal circulation forms an anatomic plexus under the retina (Fig. 3a); however, this anatomic plexus is probably functionally incomplete or only slowly responsive to changes in normal circulation [16, 27]. This is demonstrable by the lobular flow seen in choroidal circulation by IVFA, and the segmental loss of choroidal circulation demonstrable in patients with AAION [16, 28, 29]. Following sudden disruption of flow in the PCAs in these patients, there is segmental loss of the vascular supply to the ON from the anastomotic vessels connecting the choroid with the ON. When this occurs, sudden segmental disruption of axon transport results in ON head edema (Fig. 2c). Pathophysiology of the primate AAION model is based on sudden, segmental loss of the SPCA circulation (Fig. 3b). When this occurs, there can be both choroidal insufficiency and reduced flow in the cilioretinal arteries that are supplied by the choroidal circulation. IVFAs from both clinical AAION and early post-induction of the primate AAION model reveal segmental choroidal loss that extends to the optic disk. The SPCA-AAION model mechanism therefore is similar to the proposed mechanism of human AAION. Like clinical AAION, the primate AAION model generates pallid ON edema (Fig. 2c) [30].

Rodents have smaller eyes than primates, and thus have far fewer PCAs supplying the outer retina [5]. Accordingly, much larger areas of the posterior circulation are supplied by fewer vessels. This anatomic difference can result not only in ON ischemia and/or infarction after experimental PCA occlusion, but also in subtotal or total retinal infarction after PCA occlusion. These problems contribute to the failure of attempts to block only ON circulation by PCA ablation in rodents. Thus, there are no useful models of AAION in rodents.

Human AAION, as well as its models, is clinically associated with choroidal ischemia [31] and, as the photoreceptors are supplied from the choroid, outer retinal degeneration is often

present [32, 33]. Thus, it is not surprising that both human AAION-associated ischemic choroidopathy and model-associated choroidopathy result in electroretinographic changes consistent with photoreceptor loss or damage [26, 32] as indicated by decreased a- and b-wave responses.

The PCA ablation-AAION model has been produced only in nonhuman primates. This limits its usefulness, as it considerably reduces the opportunity for experimentation by increasing the expense of animals for each experiment. In addition, ethical concerns regarding primate experimentation also limit the use of the model in obtaining data not accessible by clinical methodology. Thus, no therapeutic studies are reported using the AAION-PCA ablation model. Additionally, PCA ablation is not directly equivalent to arteritic thrombosis because IVFA-based choroidal flow changes are clinically observable before thrombosis in GCA, when ON function is still relatively intact. As a result, few studies utilizing the AAION model are reported, and histological data resulting from this model are limited to specimens obtained from a few animals euthanized at relatively long (14 days) periods after induction, reducing the information obtainable for cellular pathophysiology. Nevertheless, data from these time periods reveal either total or regional loss of nerve fiber bundles in the lamina, as well as cellular inflammation [27].

Another model with similarities to human AAION has been generated in rabbits using sustained endothelin-1 release via minipump [34]. This model has been used to demonstrate glaucomatous changes; however, some findings could be related to mechanisms common in AAION. These include (at least in rabbits) a considerable reduction in ON blood flow and early RGC, ON axon and myelin loss [34]. Endothelin-1 injection results in damage beginning within 1 week of treatment. These changes are not evident in the primate model at early stages even though ON blood flow is significantly decreased within 7 days [35], suggesting that primate ON circulation is more robust than that of rodents. Indeed, nonhuman primates require endothelin-1 administration for 6 months or longer to show RGC loss [36], indicating that, at least as regards this model, lower mammals have different responses than nonhuman primates.

NAION models

Because the majority of NAION cases are believed to be due to a compartment syndrome initiated by ONH capillary dysregulation (rather than arterial occlusion), an appropriate NAION model requires selective capillary dysregulation acting at the ON head rather than SPCA occlusion. This has been accomplished in both rodents (rNAION) and non-human primates (pNAION) by intravenously injecting a photosensitive dye (e.g., rose bengal; RB) and using low-intensity laser light to generate dye-induced superoxide radicals that circulate within the ON capillaries [37] (Fig. 4). A simple, but specially designed 7-mm- (rat) or 5-mm- (mouse) diameter $\times$ 3-mm-thick plano-concave contact lens enables retinal visualization of rat or mouse retinæ [37, 38] and stabilizes the eye for laser induction in front of a slit lamp. A 532-nm laser light of either 500- μ m- (rat) or 300- μ m- (mouse) diameter is focused on the intraocular portion of the ON, sparing the rest of the retinal capillary bed (Fig. 4b). The laser power levels used are so low that this approach does not produce thermal damage, although higher fluency can cause direct damage and result in

burns or even central retinal vein or artery occlusion (CRVO/CRAO) (see Fig. 2d in [38]). The rNAION model is a simple approach that requires only an easily obtained (or generated) fundus contact lens, a widely available 532-nm clinical laser and a slit lamp.

The superoxide radicals generated by the photochemical reaction induce both photothrombosis [39] and regions of capillary endothelial damage without thrombosis [40]. This capillary dysregulation results in progressive accumulation of ON edema in the region of the anterior ON (histological cross section, Fig. 4d).

Typically, the normal rat optic nerve disk is flat against the retina (Fig. 4a), with definable borders (Fig. 4a, arrowheads), and retinal vessels of normal caliber. This suggests that venous vascular flow is unrestricted. On day 1 post-rNAION induction (Fig. 4c), optic disk edema is observable as disk swelling, obscuration of retinal vessels on the disk surface, blurred disk margins (arrowheads, Fig. 4c) and slight dilation of the retinal veins; all features similar to those seen clinically in humans with NAION. Histologically, edema is localized to the regions exposed to light: ON edema is localized to the region of the anterior ON and laminar region (Fig. 4d). Progressive edema results in an ON compartment syndrome with further compression and compromise of otherwise uninvolved ON capillaries. In addition, spectral domain-optical coherence tomography (SD-OCT) analyses utilizing the rNAION model have documented subretinal fluid in the posterior pole of the eye, similar to that which can be seen in human NAION soon after induction (data not shown), suggesting that the mechanisms of such fluid accumulation may be similar in both the NAION model and clinically, and that the model may be useful in identifying the pathophysiology of this clinical sign.

It has recently been shown that skillful use of an indirect ophthalmoscope laser can also be used to generate rNAION by enabling focal dye activation at the ON head without the need for a contact lens [41]. Other superoxide radical-generating dyes also have been used to induce AION in vivo [42, 43]. One of these, mesoporphyrin IX, has the advantage of being slowly eliminated, creating more stable blood levels and, thus, presumably allowing more time from injection to induction. This may result in better interanimal predictability with respect to the severity of damage.

A major problem with rodent models of CNS damage (and indeed, of all rodent models) is that many of the responses seen in rodents, including their responses to therapeutics, can differ markedly from those of primates [44–47]. Thus, confirmation of rodent:primate-conserved responses prior to clinical trials is crucial. Fortunately, a nonhuman primate NAION model has been developed [48] that utilizes intravenous RB with a larger spot size (1.2 mm) and shorter exposure times (7–10 s) directed to the intraocular portion of the ON. This model generates a lesion with many of the findings consistent with those seen in both human NAION and rNAION (Fig. 5), including progressive anterior ON edema and regional BBB breakdown, clinical (pupillary response to light) and electrophysiological evidence of partial vision loss, subretinal fluid, intraretinal edema, early cytokine-driven inflammation followed by cellular inflammation, and subsequent regional RGC and ON axon loss [40, 48–50].

This ‘vertical model integration’ enables direct confirmation of rodent-obtained responses in an old-world primate species, which may have a greater likelihood of clinical relevance. Both the rNAION and pNAION models have already been used to evaluate and confirm effectiveness of a potential therapeutic agent (prostaglandin J2) for clinical NAION [40, 50, 51] and will allow testing of other agents in the future.

NPION model

Because of the location of the ON lesion in human NPION, until recently, this condition has been difficult to model for study; however, Wang et al. have developed a rodent model that appears similar to the human condition. This model requires careful orbital dissection to expose the anterior orbital portion of the optic nerve. The animal is then injected with erythrosin B dye and the exposed area of the optic nerve is subjected to 532-nm-wavelength light (Fig. 6a, b; modified from Fig. 2 in [43]). In this model, like the rNAION model, capillary leakage occurs soon after induction, but unlike the rNAION model, which produces optic disc swelling shortly after induction, the optic discs of these animals show no obvious swelling at any time examined (Fig. 6f), for up to 2 weeks post-induction, following which the discs become pale (Fig. 6g). Also, unlike the rNAION models, early hemorrhages can be observed on the portion of the treated nerve itself (Fig. 6d, arrow). Histologically, this model shows axon loss, glial scarring and cellular inflammation weeks after the initial insult [43]. These findings again are similar to those seen in the rNAION and pNAION models. Although the method requires a somewhat complex set-up, complex surgery and a complex focusing procedure, all of which require significant practice, this model is an exciting new approach to evaluating PION-induced visual loss and may be useful in evaluating PION-specific features and potential treatments for this poorly understood disorder.

PeAION model

Roth et al. have attempted to create a rodent model of PeAION (personal communication). These investigators placed adult rats dorsally in a 70-degree head-down position for 5 h and hemodiluted them over 90 min to about 40 % of their normal hematocrit. This resulted in clinical, electrophysiological, and histological evidence of optic nerve damage. Interestingly, these investigators found that the ONs of the animals showed inflammatory changes similar to those seen in the rodent and non-human primate NAION models [49, 52, 53]. Having said this, there are some confusing features of this model. In particular, the authors indicate that in this model, the optic disc initially appears normal with optic disc edema apparently developing over a period of 28 days. This would seem at odds with most cases of human ION of any type. We are unaware of any other models of PePION.

Histological comparisons: human disease vs animal models

AAION: clinical histology

The histological findings in AAION result from inflammation of the posterior ciliary arteries [54–56]. Inflammation tends to affect these arteries in either a segmental or patchy fashion, but long portions of arteries may be affected [57, 58]. In mild cases, collections of T-lymphocytes may be confined to the region of the internal or external elastic lamina or to the

adventitia [59, 60]. Intimal thickening without prominent cellular infiltration is usually present. In areas of more marked inflammation, all layers are affected [59]. Necrosis of portions of the arterial wall, including the elastic laminae, and granulomas containing multinucleated histiocytic and foreign-body giant cells, histiocytes, lymphocytes (both T-helper and T-suppressor cells) and some plasma cells and fibroblasts are found [60, 61]. The inflammatory process is usually most marked in the inner portion of the media adjacent to the internal elastic lamina. Fragmentation and disintegration of elastic fibers occur, closely associated with an accumulation of giant cells.

ON histological findings in AAION-affected individuals are well documented [62–66]. In cases autopsied during the acute phase of AAION, ONs show ischemic necrosis, with the area of greatest damage at or just posterior to the lamina cribrosa. Histologic ON specimens obtained days to weeks after infarction show liquefactive necrosis of the retrolaminar axons and phagocytosis of debris by microglia. In some specimens, hyaluronic acid may be present in the area of necrosis. Presumably, the hyaluronic acid arises from the optic nerve head through a defect in the prelaminar glial membrane. The resulting histologic picture thus is identical with that seen in cavernous degeneration.

Animal model

As opposed to the optic nerve findings in human AAION, the few reported histologic studies of enucleated eyes in AAION animal models reveal well-circumscribed infarctions of the optic disc and retrolaminar portion of the optic nerve [33]. These lesions can involve either the entire diameter of the ON, or a subregion. Both cellular inflammation and liquefactive necrosis were apparent in the late specimens: no early (<30 days) specimens were reported.

NAION: clinical histology

Humans

Due to the nonfatal nature of NAION, few histopathological studies of human tissue are available [67], and it is even less common to have tissue obtained near the time of onset, with only one case of clinically confirmed NAION having been reported [68, 69]. In this case (20 days post-onset), the optic nerve lesion was consistent with that seen in compartment syndromes, where vascular compression is responsible for the majority of damage without respect for vascular territories, and subsequent RGC death occurred by apoptosis [69, 70]. Although inflammation was not studied initially in this case, a later immunocytochemical analysis of the same tissue revealed macrophage-based inflammation similar to that seen in the rNAION and pNAION models [49] (see below). In an extensive histological study of the pathology of presumed NAION (there was no clinical correlation), there were ‘acellular’ early lesions with later macrophage infiltration [67]. OCT in NAION has revealed loss of both the retinal nerve fiber layer and the RGC layer that correlates with visual field loss [21]. ON specimens obtained by the authors from a patient with well-documented bilateral NAION that occurred 21.5 years prior to death revealed both severe axonal loss, and continued bilateral cellular inflammation that was greater in the more recently affected nerve.

Animal model

In contrast to AAION and clinical NAION specimens, the availability of tissue from the highly reproducible rNAION and pNAION models has enabled numerous studies to be performed on the responses of the retina and ON to conditions that closely resemble human NAION [37, 38, 48]. This also has enabled correlation with the few human specimens available [49]. Early phases of rNAION demonstrate retinal stress gene induction, notably the proto-oncogene c-Fos, within 12 h post-induction [37]. Markers of retinal ischemia also revealed transient RGC layer and ON ischemia that normalized within 24 h [71]. Retinal c-Fos expression is histologically demonstrable in the RGC layer 3d post-induction (Fig. 7). Similar to most cases of clinical NAION, the rNAION lesion affects different retinal regions to a greater or lesser extent; this is seen by retinal flat-mount immunostaining for cFos activity in a c-Fos/LacZ reporter mouse, which reveals high expression levels in some retinal regions (Fig. 7b, indicated in RGC cell body, arrow), and much lower expression levels in relatively unaffected retinal areas (Fig. 7a, arrow). Initiation of c-Fos expression begins much earlier than 3 days [37]. RGC-specific transcription factors such as Brn3b decline within 18 h post-induction [37]. RGCs apparently die by apoptosis, demonstrable by TUNEL staining, with peak RGC death occurring by 10.5 days in the rat model [72]. Other models of ON damage exhibit different RGC death patterns. For example, RGC death peaks earlier in models of ON transection and later in ON crush, with RGC death beginning even later if the lesion is intracranial [73]. As the lesions in the NAION models are close to the eye, this suggests that there are distinct physiological differences in RGC response after ON ischemia compared with ON trauma (as well as with the type of direct trauma).

A significant caveat in both the rodent and primate laser-induced models of NAION is that the extent of ON capillary damage directly relates to both the time of exposure and the intensity of the light used. Thus, animals with prolonged induction times exhibit considerably more RGC loss than animals with shorter induction time. This relationship is linear only within certain limits. Prolonged times of exposure or higher laser intensities can result not only in ON ischemia but also in retina infarction. It is important to evaluate laser power with a power meter prior to each experiment and to maintain identical amounts of exposure time. In addition, both rodents and nonhuman primates exhibit individual differences in their response to NAION model induction. Each animal should be evaluated at baseline and shortly after induction (typically 1–2 days post-induction).

Recently, it was found in the rNAION model that, in addition to simple photothrombosis, dye-induced photoactivation and transient ON ischemia also cause endothelial dysfunction and active fluid transport [40]. This is similar to the mechanism seen in CNS infarcts [75, 76] and supports the hypothesis that ON responses to ischemia are likely similar to those seen in other parts of the CNS.

Although previously unsuspected, inflammation appears to be a key factor in early ON damage in both rodent and animal models of NAION. The earliest inflammatory responses are cytokine based and occur within hours of induction [40], whereas later responses include sequential involvement of neutrophils and macrophages (both microglia- intrinsic and extrinsic invading macrophages) [49, 53]. There is observable breakdown of the blood–brain barrier at the region of the primary lesion (see Fig. 4d), followed by extrinsic macrophage

invasion at that site [53]. Thereafter, microglial-based inflammation proceeds along the ON from the primary site in an anterograde fashion, associated with myelin breakdown, demyelination and remodeling [52]. Both cytokine and cellular inflammation can be pathologic to retina and the optic nerves [77–79]. Recently, macrophage-based inflammation has been characterized as M1 (classical/neurodegenerative) and M2 (alternative inflammatory pathway-neuroprotective) [80]. Alteration of the M1 and M2 responses can be neuroprotective [81] and effect both ON repair and remyelination [82]. These reports suggest that selective inflammatory immunomodulation may be a viable approach to the treatment of human NAION.

Comparative differences between the rodent and primate NAION models

Humans, nonhuman primates and rodents have different responses to disease and disease models that can extend even to individual strains. Prime examples of these are the lack of correlation in spinal damage and infection models [45, 83] and the rodent species-specific differences in laminar structure [10]. In addition, there can be species' selective reliance on different mechanisms of vascular regulation, such as calcium channel blockers in rodents, that have less impact in humans [47]. While cellular inflammation is consistently found in both clinical NAION and the nonhuman primate and rodent models of NAION, there are differences in the type of early cellular infiltrate in primates (neutrophilic invasion in the first 3 days, followed by macrophage infiltration) vs rodents (macrophage invasion alone).

ON edema is an important sign in early clinical NAION; however, mice do not necessarily exhibit edema, but rather disk pallor and capillary leakage, as well as peripapillary retinal displacement [38, 84]. In contrast, rats nearly always express ON edema, as well as peripapillary displacement, similar to that seen in humans. This is also seen in the nonhuman primate model [37, 48]. These differences may be due either to the difference in ON wall resistance or to the laminar structure [3, 10]. NAION model-induced ON edema also resolves much faster in the rat (resolution is complete by 5 days), than in nonhuman primates, as pNAION-induced monkeys show ON edema even 2 weeks post-induction [48]. Human NAION-related edema can last for more than 40 days [84]. Thus, differences in the speed of different pathophysiological processes must be taken into account when considering both treatment and disease responses. Experimental results must be interpreted with caution, and it may not be possible to directly correlate results found in one species to another. Nevertheless, the strongly parallel nature(s) of the NAION model lesion in the mouse, rat and nonhuman primate models can enable greater understanding of the general mechanisms occurring after clinical NAION. The newly developed NAION and PION models may prove to be excellent platforms for evaluation of various reparative therapies, and greatly increase the speed of development of effective drugs for these disorders.

Acknowledgments

The authors would like to thank all of the individuals who have worked on the rodent and nonhuman NAION models in our laboratory. These include Drs. M.A. Johnson, N. Goldenberg-Cohen, C. Chen, C. Zhang, C. Salgado, V. Touitou, Y. Guo, Z. Mehrabyan, and J. Nicholson. The invaluable assistance of our many students, including B.J. Slater, S.L. Vilson, D.L. Bernstein, A.M. Bernstein, and S. Hwang are also gratefully acknowledged. This work was funded by an unrestricted grant from Research to Prevent Blindness (SLB), The Hirschhorn Foundation

(NRM), The Donegan Fund for Optic Nerve Research (NRM), and NIH grants EYRO1-019529 (SLB and NRM) and EYRO1-015304 (SLB).

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Fig. 1.

Structure and vasculature of the human ON, and vascular schematic comparisons of the human, mouse and rat lamina and optic nerve. **a** Human ON structure. The ON is divided into prelamina (intraocular), lamina and retrolamina (postlamina) regions. The prelamina region (P-lam) is contiguous with the retina (Ret), and the RGC axons are unmyelinated and derive from the nerve fiber layer, with little structural delineation or restriction. Unmyelinated axon bundles are restricted by the scleral walls (Sc) in the lamina region, and separated by collagen columns. Myelination occurs in the retrolamina region of the ON, resulting in marked enlargement of the ON diameter. Modified from [3]. **b** Vascular corrosion cast of the human ON regions. The lamina region (Lam) is supplied by both prelamina (PrL-intraocular) capillaries, as well as indirectly from the choroid, and directly from branches of the posterior ciliary arteries. The lamina vasculature is also continuous with the vascular supply of the retrolamina (RL) ON. Capillary density of the lamina is more exuberant than the prelamina or retrolamina ON. Lamina blood supply is therefore more complex than any other single region. Modified from [5]. **c** Vascular corrosion cast of the rat ON regions. Similar to humans, the lamina capillary bed is more complex than either the pre- or retro-lamina ON regions and receives blood from retina, choroid, posterior ciliary arteries and posterior ON. Central retinal artery (A) and vein (V) are indicated. **d** Schematic of the human ON. CRA, central retinal artery; ZHC, zonal hypodense capillary; Pv, posterior vein; OA, optic artery; ON, optic nerve. **e** Schematic of the mouse ON. CRA, central retinal artery; Ch, choroid; Sc, sclera; OA, optic artery; ON, optic nerve. **f** Schematic of the rat ON. CRA, central retinal artery; SC, sclera; PCA, posterior ciliary artery; ZHC, zonal hypodense capillary; OA, optic artery; ON, optic nerve.

Modified from [5]. **d** Schematic of human ON blood supply. Vessels derived from the posterior ciliary arteries forms a plexus derived from the circle of Zinn- Haller (ZHC). The central retinal artery (CRA) derives from the ophthalmic artery and penetrates the retrolaminar ON, emerging to supply the inner retina from the center of the prelaminar (intraocular) portion of the ON. Posterior optic nerve capillaries extend from pial vessels (Pv), which themselves derive from the ophthalmic artery (OA) and vein (OV). Modified from [7]. **e** Schematic diagram of the mouse ON circulation. There is less complexity of the posterior ON capillaries compared with rat, but these communicate with the lamina. Retinal retrograde vasculature communicates with the lamina past the sclera (Sc), but there is reduced direct communication with the choroidal (Ch) circulation. Modified from [8]. **f** Schematic diagram of the rat ON circulation. Rat lamina is similar in overall structure to the primate but with reduced vascular complexity and much less supporting collagen, present mainly as plates. The posterior ciliary artery (PCA) derives from the ophthalmic artery (OA) in all species. Modified from [5]

Fig. 2.

Optic disk appearance in a patient with unilateral NAION (**a**, **b**) and in a patient with AAION (**c**). **a** In a patient with NAION in the opposite eye, the unaffected optic disk (ON) is small, with little or no cup, and the disk margin is sharply delineated (*arrowheads*). **b** NAION-affected disk. The disk is swollen and hyperemic, with blurred disk margins (*arrowheads*) and obscuration of vessels (*white arrow*). A disk hemorrhage is present (*black arrow*). The veins are mildly dilated, suggesting compression at the disk. **c** AAION-affected disk. The ON is both pale and swollen. A cotton-wool spot is visible (*arrow*)

Fig. 3.

A schematic diagram of the primate AAION model and effect on the nonhuman primate (NHP) retina and choroid. **a** Schematic diagram of the normal posterior of the eye. The posterior ciliary arteries (PCAs) include the short posterior ciliary arteries (SPCAs) surrounding the optic nerve (ON) in the primate. **b** Primate AAION model schematic diagram. Ligation of multiple SPCAs at the entrance to the sclera results in regional choroidal ischemia, which also affects a portion of the circle of Zinn-Haller (choroidal vascular circle, in red) supplying a portion of the anterior ON, resulting in regional anterior ON ischemia. **c** Fluorescein retinal angiogram of a NHP immediately following regional ligation of SPCAs. The regional loss of choroidal blood flow results in compromise of the flow in the cilioretinal artery, leading to retinal capillary nonperfusion as well as nonperfusion of the underlying choroid. Regional choroidal nonperfusion has also caused loss of capillary perfusion to a portion of the ON (*arrow*). Photo taken from [27]

Fig. 4. Rodent NAION (rNAION) model. **a** Control rat ON. Retinal veins are of normal caliber. The optic disk has defined margins (*arrowheads*) and the disk is flat against the retina. A scale bar (500 μm) indicates the diameter of the disk. **b** Schematic diagram of the rNAION model. The photoactive dye rose Bengal (RB) is injected, and a 500- μm -diameter laser light spot/532 nm wavelength/50 mW power is focused on the optic disc using a fundus contact lens. The laser light activates the RB, causing capillary damage. This capillary damage results in axonal ischemia. **c** Photo of the rat ON 1 day post-induction. The optic disk is swollen (*arrowheads*), with blurred margins. There is mild retinal venous dilation. **d** Confocal micrograph of an ON near the lamina, and overlying retina. One day post-rNAION induction, the animal was injected intravenously with a mixture of rhodamine-dye-linked 3-kDa dextran and fluorescein-linked bovine serum (66 kDa) albumen (FITC-BSA), and euthanized 1 h later. There is accumulation of both molecules in the anterior ON with edema (*asterisk*) and retina overlying the nerve, suggesting localized breakdown of the blood-brain barrier (BBB) in the anterior ON and retina

Fig. 5.

Characteristics of the pNAION model. **a** Normal NHP optic disk. The ON is flat, with well defined margins. Arteries (A) and veins (V) are normal in caliber. **b** pNAION, 1day post-induction. The optic disk is edematous and swollen, with disk hemorrhage (*arrowhead*) and obscuration of vessels due to edema (*arrow*). **c** pNAION, 30days post-induction. There is temporal pallor of the affected nerve (indicated by *arrows*). **d** Fluorescein angiogram (late phase) of the pNAION-induced (1 day) ON. There is disk leakage from the ischemic regions (*arrowheads*). **e** SD-OCT of an pNAION-affected eye, 1day post-induction. The ON is swollen, and subretinal fluid is apparent in the macula (indicated by *arrows*). Mac: macula. **f** SDOCT ring scan of retina shown in **e**. The RNFL is regionally swollen, and there is subretinal fluid (SRF) apparent in the region of the macula. **g** Histological (H&E stained) section of a pNAION lamina and ON, 70 days post-induction. There is regional axonal loss (indicated by *arrows*) and increased cellularity in the loss region, consistent with inflammatory cell infiltrate

Fig. 6. Rodent PION model. **a** photograph of the laser set-up. Light from a continuous wave laser is segmented by a 'chopper', further processed and filtered, then directed inferiorly using a mirror. **b** The ON is exposed posteriorly to the globe. **c** The photoreactive dye (erythrosin B), is activated by a laser spot placed on the surface of the ON. **d** Post-illumination, the treated nerve exhibits vascular damage and surface hemorrhage (*arrow*). **e**. Disk photo of the untreated ON. **f** Disk photo of the PIONinduced ON 4 days posttreatment. There is no

observable disk edema. **g** Disk photo of a PION-induced eye 28 days post-induction. The optic disk is slightly shrunken in appearance and exhibits slight pallor. Modified from [43]

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Fig. 7. ON ischemic stress results in regional RGC stress in the mouse after rNAION. A transgenic mouse line was generated with a c-Fos-driven promotor-reporter gene construct (cfos-lacZ) [74]. Three days following induction, the whole retina was immunostained for lacZ expression. **a** Region 1 reveals low levels of lacZ protein (*arrow*) in the RGC layer. **b** Region 2 reveals that nearly all RGCs express high levels of cfos-driven lacZ (*arrow*). *Scale bar in b* 50 microns