

Potential Neuroprotective Therapy for Glaucoma

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SUMMARY

Glaucoma is often associated with high intraocular pressure (IOP), but continues to progress even after normalization of IOP. We have suggested that such progression is at least partly due to delayed degeneration of spared neurons by their exposure to the degenerative milieu created by degenerating neurons, the primary victims of high IOP. The extent of delayed (secondary) degeneration is apparently a function of the severity of the primary insult, which affects the level of toxicity mediators and the spared neurons' susceptibility to them. We developed a model of adult rat partial optic nerve lesion to quantify the extent of primary and secondary damage, respectively, and find out why patients with severe pre-existing damage are more prone to deterioration than patients without pre-existing visual loss. The model also screens compounds for neuroprotective efficacy. Our findings support our suggestion that glaucoma therapy should consist of a combination of neuroprotection and ocular hypotensive therapy.

Keywords: Glaucoma; Neuroprotection; Optic nerve injury.

Glaucoma is a leading cause of blindness in the USA, where it accounts for 10% of all reported cases ¹. The loss of vision in glaucoma patients is a consequence of damage to optic nerve axons, with resulting death of their retinal ganglion cells ^{2,3}. The pathogenesis of optic neuropathy in glaucoma is still a matter of debate. The fact that increased intraocular pressure (IOP) is probably the most important risk factor in primary open-angle glaucoma ^{4,5} has led to the widely held view that IOP plays a central role in the initiation and development of glaucomatous neuropathy. Increased IOP may act directly on optic nerve axons at the level of the lamina cribrosa ² or cause a change in the posterior microcirculation of the eye that could influence the optic nerve head ⁶. Alleviation of IOP is currently the treatment of choice in attempting to attenuate the propagation of optic neuropathy and ganglion cell loss in glaucoma patients. However, many patients with glaucoma continue to experience visual field loss long after therapeutic normalization of their IOP ⁷. Moreover, as many as one-sixth of all patients with glaucomatous damage show no evidence of elevated IOP, even on repeated testing ^{8,9}. These findings suggest that, at least in some cases, high IOP alone cannot explain the propagation of glaucomatous optic neuropathy ^{6,10,11} and that additional primary risk factors and/or secondary factors are involved ^{7,11}. These additional factors might be a reflection of the toxic environment created by mediators of toxicity produced by or associated with the degenerating neurons, as well as of changes in the susceptibility of remaining neurons to such toxicity. If this is the case, progression of neuropathy might be related to what was recently defined in the field of central nervous system (CNS) trauma as secondary degeneration.

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The last decade has seen substantial progress in understanding of the processes leading to secondary degeneration of neurons in the CNS following acute or chronic trauma. It was shown that regardless of the primary cause of neuronal cell death, damage spreads beyond the directly injured neurons to adjacent neurons that escaped the primary lesion. The excessive loss of neurons has been attributed to delayed biochemical processes, which lead to neuronal death over and above that caused directly by the primary injury¹²⁻¹⁴. It appears that regardless of the nature of the primary lesion (ischemia/hypoxia, stroke, mechanical trauma or degenerative neuronal disease), the resulting damage to cells and fibers leads to similar changes in the extracellular milieu including alterations in ion concentrations, increased amounts of free radicals, release of neurotransmitters, depletion of growth factors and involvement of the immune system^{12,13,15,16}. These changes trigger secondary degeneration in neighboring neurons that escaped the primary injury^{13,14,16,17}. Functional damage continues to progress even after the primary cause of neuronal death has been removed. This knowledge is the basis for the therapeutic approach of "neuroprotection", which aims to protect these initially spared neurons from secondary degeneration, thereby improving functional outcome following CNS injury¹⁸.

The objective of neuroprotective therapy is to employ pharmacological or other means to attenuate the hostility of the environment and/or to supply the cells with tools to deal with these changes. According to this approach, any chronic degenerative disease of the neurons may be viewed as a process in which at any given time, regardless of the primary cause, some neurons are undergoing an active process of degeneration, which contributes to the hostility of environment toward other neurons that are still intact. The similarity of the mediators of secondary degeneration found in various types of acute CNS trauma suggests that the mediators are the outcome rather than the cause of neuronal death. This would support the idea that in designing neuroprotective therapy the critical factor may not be the nature of the primary injury but rather the cellular milieu in which the insult occurs. Because neurons from various CNS regions share many common properties, some of the mediators involved in propagation of secondary damage of the optic nerve in glaucoma may be similar to those causing secondary degeneration in any part of the CNS. Support for this theory comes from the observed presence of the excitatory amino acid glutamate in the vitreous body of glaucomatous eyes at concentrations that are potentially toxic to retinal ganglion cells¹⁹. According to this view, in the selection of an animal model to screen for neuroprotection, its characteristics should include not only the ability to distinguish unequivocally between primary and secondary events but also the capacity to induce secondary degeneration in a cellular milieu similar to that in which the disease is localized (for example, the optic nerve in the case of glaucoma). The availability of a model that meets these two criteria would make it possible to screen candidate compounds for their ability to protect the affected

nerve or other neuronal elements from the mediators of secondary degeneration.

We have developed an animal model of a partial lesion of the optic nerve of adult rat²⁰⁻²⁶. This model provides a system in which the initial mechanical neuronal loss can be controlled and the secondary loss can easily be quantified. It can therefore be used (i) to simulate the continuing progression of optic nerve degeneration following a primary insult even after the primary risk factors (e.g. increased IOP) have been removed or attenuated, (ii) to show that appropriate neuroprotective therapy may be effective in halting progressive degeneration of the optic nerve, (iii) to screen potential neuroprotective drugs, and (iv) to examine whether continued progression is not only a function of the mediators but also of changes in susceptibility of remaining neurons to them. This model can shorten the time-consuming studies prior to the use of experimental glaucoma models in primates and clinical trials aimed at halting the propagation of optic neuropathy in glaucoma patients with normalized IOP.

A model of secondary degeneration of the optic nerve

The model is a well-controlled, reproducible, calibrated partial lesion of the optic nerve of adult rat, in which some of the fibers are left undamaged²⁰⁻²⁶. The directly damaged axons undergo both anterograde and retrograde degeneration, the latter culminating in apoptotic death of cell bodies²⁷ like that seen in glaucoma³. The propagation of degeneration is self-perpetuating, as neurons that escaped the primary injury are exposed to injury-induced mediators of toxicity and will therefore also eventually degenerate. Such degeneration mediators, including excitatory amino acids, free radicals and potassium ions^{12,13}, are present in the vicinity of the cell bodies and are likely to be present also in the vicinity of the site of injury. Thus they can presumably trigger the process of secondary degeneration in the axons as well as in the cell bodies of initially spared neurons. The extent of such degeneration is a function of the severity of the primary insult, which governs the levels of released toxicity or of secondary degeneration mediators. Using this model, we recently showed that in addition to elevation of levels of secondary degeneration mediators, the remaining neurons embedded in such hostile milieu show an increase of susceptibility to this milieu, which fact may further account for degenerative progression. This might explain, for example, why propagation of glaucomatous neuropathy, when IOP is normalized, does not occur in all cases, and also varies from one case to another. Its occurrence and extent might depend on the stage at which the patient was diagnosed and on the severity of the disease when pressure-relieving treatment was initiated.

Screening for neuroprotective compounds

Glutamate is a major cause of cytotoxicity in brain injury and brain disorders leading to secondary degeneration²⁸. Therefore, one of the most promising approaches to treatment

of brain lesions, when the aim is to attenuate propagation of secondary damage, is to block the glutamate receptor subtype, N-methyl-D-aspartate (NMDA), with suitable antagonists²⁹. These receptor-operator channels are known to be widely distributed on cell bodies of CNS neurons but are apparently absent in white matter³⁰. Using our optic nerve model, we recently showed that the NMDA-receptor antagonist MK-801, when administered systemically, does protect optic neurons from secondary degeneration³¹. This finding, taken together with the wide distribution of NMDA receptors on retinal ganglion cells, suggests that the cell bodies are the primary site of NMDA antagonist-induced protection of optic neurons from secondary degeneration.

Our results with MK-801 provide an insight into the nature of secondary damage mediators in the optic nerve. They also indicate that it is worth pursuing the development of neuroprotective therapy for glaucoma, possibly using NMDA antagonists as a starting point. In seeking potential drugs for neuroprotective treatment, however, it is preferable to avoid compounds that block receptors mediating important functions, or at least to use antagonists whose affinity toward such receptors is lower than that of MK-801. This is because the powerful receptor blockers, although beneficial in neutralizing the excitatory amino acids that secondary degeneration, may have harmful side effects.

Several compounds shown to be effective in reducing injury-induced deficit in brain lesions have been found effective in optic nerve lesions as well. These include GM1²² and the analog of the cannabinoid HU-211²³. Recently, α -2 adrenoreceptor agonists, known to have an anti-hypertensive effect, were found in this model to have a neuroprotective effect as well²⁴.

An alternative choice might be drugs which neutralize extracellular toxic agents, or anti-apoptotic drugs which act intracellularly by inducing cell cycle regulatory genes that enable the nerve to cope with the intracellular consequences of toxicity leading to death. In glaucoma, death of the cell bodies appears to be apoptotic³. Among the apoptotic cells are victims of the primary insult (e.g., increased IOP) and of other hostile factors such as the degenerative environment. It is important to note in this connection that apoptotic retinal ganglion cells were observed in the rat optic nerve following axotomy²⁴. Therefore, in seeking suitable neuroprotective drugs one should also consider anti-apoptotic compounds, not only compounds designed to neutralize mediators of secondary degeneration or their receptors. Anti-apoptotic compounds might protect the initially spared neurons from secondary degeneration by increasing their "resistance" to intracellular signals for cell death. Compounds likely to promote increased resistance are those that promote survival at the gene level, for example, by activating survival-related cell-cycle regulatory genes, such as bcl-2³². In the case of neurons that have fallen victim to the primary axotomy, at most their rate of degeneration may be slowed down by anti-apoptotic treatment or any other form of

neuroprotection; their eventual degeneration is inevitable, however, unless regeneration can be induced to occur.

CONCLUSIONS

It is now commonly accepted that in many cases of glaucomatous neuropathy, propagation of the disease continues even after the primary cause, such as elevated IOP, has been removed or attenuated. We suggest that such progression is probably mediated by substances originating from those optic nerve axons and cell bodies (retinal ganglion cells) that were directly injured as a result of the primary disease process and are actively undergoing degeneration and cell death. Spreading of damage to apparently healthy neurons or marginally damaged neurons follows any type of CNS injury, and some of the mediators are likely to be common to different insults as they originate from the dead and dying neurons regardless of the primary cause of death. Therefore, in studying the nature and dynamics of secondary neuronal death in the case of optic nerve damage like that occurring in glaucoma, we considered it appropriate to employ a partial injury of the rat optic nerve as a model. Our model causes limited and controlled primary death and enables us to quantify and characterize the secondary neuronal loss.

We suggest that progression of optic nerve degeneration after removal of its primary cause in glaucoma might occur because of the hostile environment created by neurons degenerating as a result of the primary cause, e.g. increased IOP. Accordingly, at any given time during the course of the disease there should be some neurons that are victims of the primary cause, others that are victims of the hostile environment, and yet others that are still intact or only marginally and transiently damaged. The last group of neurons is the potential target of neuroprotective therapy. Studies of human glaucoma have suffered from the lack of an animal model that fully simulates the etiology and pathogenesis of the disease. This is mainly because of the heterogeneity of the disease syndrome and the lack of consensus with regard to its risk factors. As the targets of neuroprotective therapy are the still-healthy neurons and the mediators of secondary degeneration, it seems reasonable to assume that the requirement of a model for studying neuroprotection, at least as a first step, is not simulation of the primary cause but of the resulting damage. This is especially valid when studying neuroprotection in glaucoma because of the number and diversity of possible risk factors, all of which may lead to the same disease manifestation. The possibility of adapting neuroprotective therapy for glaucoma has only recently been attempted. Our model seems to be suitable for testing the working hypothesis regarding potential use of neuroprotective compounds for optic neurons immersed in a pathological environment. The model does not mimic the disease itself, but sustains damage that resembles the damage incurred in the disease, including the death of cell bodies (apoptosis), and the progression of degeneration to neurons that were still apparently

undamaged at the time of pressure attenuation (in the case of the disease) or withdrawal of the external insult (in the case of our model). It also applies to the possible site of pressure-induced damage initiation (axons). The model can therefore be employed as an efficient screening device in the search for drugs capable of protecting optic axons from secondary degeneration. The most promising compounds, once identified, should be tested in primate models of glaucoma. At the stage of clinical trials in patients with glaucoma, the candidate compound should be applied in conjunction with a drug that alleviates the primary cause of the disease.

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