Developments in Neuroprotection

Novel neuroprotective agents and their mechanisms.

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In a multifactorial disease such as glaucoma, the concept of neuroprotection is predicated on enhancing the survival of retinal ganglion cells independent of the beneficial effects of lowering IOP (Figure 1). It is well established that retinal ganglion cells die by a programmed death process called apoptosis (and not by necrosis). The genetic regulation of apoptosis is being investigated in experimental animals, with the hope that this regulation can be modified as a treatment for glaucoma in the future. Researchers are also investigating the injection of neurotrophic factors in experimental animals. This article reviews our current understanding of promising new agents in neuroprotection.

BRIMONIDINE

In a model of optic nerve crush injury in rats, researchers have increased the survival of retinal ganglion cells with a single injection of brimonidine, an $\alpha_2$-adrenergic agonist. Although the exact mechanism of this neuroprotective action is unknown, it may involve (1) the reduction of oxidative stress; (2) the induction of a basic fibroblast growth factor; (3) the up-regulation of various neuroprotective factors such as bcl-2 and bcl-xL; or (4) any combination of the previous three possibilities. These mechanisms could ultimately prevent apoptosis. Brimonidine also reduces IOP, but it may be difficult to isolate the IOP-independent neuroprotective action of the drug. Recently, a clinical study showed that treatment with brimonidine led to the attenuation of visual field loss in patients with normal-tension glaucoma, an effect presumed to be somewhat independent of its IOP-lowering effect.

N-METHYL-D-ASPARTIC ACID–RECEPTOR ANTAGONISTS

Glutamate, a major excitatory neurotransmitter in the central nervous system, is one of the biological mediators that have received a lot of attention for causing secondary neuronal damage. Researchers believe the potentially toxic effects of excessive levels of glutamate occur due to overstimulation of the $N$-methyl-$D$-aspartic acid (NMDA) receptor, a situation that leads to increased intracellular calcium levels and apoptosis. Based on this knowledge, scientists have studied NMDA-receptor antagonists as potential neuroprotective agents.

In the early development of NMDA-receptor antagonists, side effects upon the central nervous system occurred, because NMDA plays a crucial role in the learning and memory processes. For example, low doses of one NMDA-receptor antagonist, Selfotel, led to sedation, dizziness, and...
disorientation transiently. Higher doses led to agitation, hallucination, and confusion. A phase 3 clinical trial of the drug was stopped due to an increase in the mortality rate.

Memantine is approved for use in the US to treat patients with Alzheimer’s disease. By virtue of its selective targeting of only overly stimulated NMDA receptors, researchers have shown memantine to have fewer side effects than previously studied NMDA-receptor antagonists. In primates with experimental glaucoma, researchers have demonstrated that the drug enhanced the functional and structural integrity of retinal ganglion cells. Memantine is currently undergoing phase 3 clinical trials for the treatment of glaucoma.

**INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITORS**

Among other tissue-specific functions, nitric oxide (NO) is able to induce apoptosis. Although there are many proposed ways in which this occurs, one recent work has suggested that it may involve S-nitrosylation of a protein known to participate in apoptosis and its subsequent translocation into the nucleus, thus making the cells susceptible to stress-induced apoptosis. In tissues, NO can be synthesized by one of the isoforms of NO synthase (NOS), which are expressed ubiquitously. One of the isoforms of NOS, NOS-2 or iNOS, is not ubiquitously expressed but is expressed only when induced by various stimuli, however. In postmortem glaucomatous human eyes, Liu and Neufeld have shown that NOS-2 was present in reactive astrocytes, mostly in the damaged areas of the nerve head. Also, in vitro, these cells were induced to express NOS-2 in the setting of increased IOP. The inhibition of NOS-2 was considered a good target for neuroprotection because the number of cells entering apoptosis could be decreased by lowering the amount of NO produced in the environment.

Using episcleral venous cautery in a rat glaucoma model, researchers tested aminoguanidine, an inhibitor of NOS-2, for neuroprotective effects in glaucoma. Aminoguanidine was added to the drinking water of rats for 6 months postprocedure, and resulted in an increased survival rate of retinal ganglion cells. This result was reproduced using another prodrug that had better specificity than aminoguanidine. Even when the treatment was delayed by 3 months, the survival rate of retinal ganglion cells was shown to be higher in that group than in the untreated group. Contradictory results were observed using a different rat glaucoma model, however. The study showed no induction of NOS-2 and, subsequently, no effect of the treatment with aminoguanidine on damage caused by increases in IOP.

Questions remain unanswered regarding the protective effects of NOS-2 inhibition in glaucoma. It is interesting to note that aminoguanidine is in clinical trials for patients with diabetes mellitus based upon its inhibition of the end-formation of advanced glycation end products.

**PROTECTIVE AUTOIMMUNITY**

Schwartz and Kipnis concluded that the immune system may be involved in neuroprotection. In one study of rats thymectomized at birth—a procedure that causes a loss of T-cell function—the researchers showed decreased survival rates of retinal ganglion cells in response to increased IOP levels. For example, in the setting of IOP-induced loss of retinal ganglion cells in mouse models, a vaccination with myelin-derived proteins from axons failed to protect retinal ganglion cells. In contrast, a vaccination with retinal photoreceptor-derived peptides led to significant increases in the survival rates of retinal ganglion cells. Thus, T cells localized to the site of injury appeared to be capable of decreasing the extent of secondary neurodegeneration. In another study, a vaccination with synthetic Cop-1, a weak agonist of self-antigens, provided protection against an IOP-induced loss of retinal ganglion cells.

These findings support the potential benefits of a therapeutic vaccination in the future treatment of glaucoma. Given our lack of understanding of the role of autoimmunity in diseases of the central nervous system, this line of therapy should be studied with extra caution, however.

**ERYTHROPOIETIN**

Erythropoietin (EPO), a glycoprotein primarily known for its role in hemopoiesis, was recently shown to participate in neuroprotection. Researchers theorized that the prevention of neuronal apoptosis occurs by binding EPO to a modified tissue-protective EPO receptor, which is a complex that is structurally different from the EPO receptor associated with hemopoiesis. This new potential receptor complex contains βcR, which is involved in other cytokine-receptor complexes. In the βcR family of receptors, a stereotypical stoichiometry of receptor complexes exists; one ligand has the potential to evoke many different responses through various combinations of receptor subunits. Moreover, βcR knockout mice, in which the gene encoding of the βcR receptor is erased from the genome, do not appear to benefit from the neuroprotective effects conferred by EPO. In light of this novel neuroprotective function, recent studies have investigated the use of exogenous EPO in animal models of retinal degeneration such as glaucoma.

In our laboratory, we have utilized the model of episcleral vessel cautery to elevate IOP and cause subsequent glaucomatous optic neuropathy in rats. In initial pilot studies involving rats, we found that a single intravitreal injection of EPO significantly increased the survival of retinal ganglion cells during a 2-day period of increased IOP levels compared with animal subjects that received no injection or a placebo. In another animal model of retinal degeneration (optic nerve axotomy-induced apoptosis), intravitreal EPO injections increased the survival of retinal ganglion cells,
mainly by preventing caspase-3 activation, which leads to apoptosis.23 Furthermore, EPO is able to cross the blood-brain barrier,13,34 a favorable property for any potential neuroprotective agent. Systemic injections of EPO have protected retinal neurons histologically and functionally in models of acute ischemia injury.35

Safety concerns regarding the use of EPO include the potential risk of stimulating angiogenesis and the potential for polycythemia vera26 and increased hematocrit levels.53 A recent study implicated the possible role of EPO as an angiogenic factor in patients with proliferative diabetic retinopathy.38

CONCLUSION

No therapeutic agents are currently FDA approved for neuroprotection in the treatment of glaucoma. Moreover, the intravitreal route of delivery may circumvent the problem of having systemically delivered agents cross the blood-brain barrier. As research proceeds, we anticipate a better understanding of the effects of the aforementioned agents, including the dose-response extent of neuroprotection, side effects related to the agents’ long-term use, and their possible synergies with other medications.

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