Animal and culture models of glaucoma for studying neuroprotection

L.A. LEVIN
Dept. of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI - USA

PURPOSE. Neuroprotection aims to treat nervous system disease by maintaining the health and function of neurons. The final proof of the neuroprotective strategy relies on randomized, controlled clinical trials, but the choice of which agents to study for these trials depends on studies in the laboratory using culture and animal models. Most culture models for studying ocular neuroprotection use retinal cells, and a range of mechanisms can be studied in culture, e.g. axotomy and serum or growth factor deprivation.

METHODS. A variety of animal models are available for studying neuroprotection as possible therapy for glaucomatous optic neuropathy. Those most closely related to glaucoma are probably associated with moderate elevation of the intraocular pressure to levels similar to those seen in patients with untreated glaucomatous optic neuropathy.

CONCLUSIONS. Care should be taken when applying the results of these models to humans, and there is no single criterion for deciding which culture or animal model is most relevant to the clinical situation. The most important feature is whether the model's results correlate with clinical results, and this information will only become available over time, as randomized clinical trials are completed. Eur J Ophthalmol 2001; 11 (Suppl 2): S23-S29

KEY WORDS. Neuroprotection, Glaucoma, Optic neuropathy, Animal models, Retinal ganglion cell

INTRODUCTION

Neuroprotection is an example of a cytoprotective strategy for preventing neuronal loss seen with diseases of the central and peripheral nervous system. Neuroprotection is of particular interest with respect to glaucoma, a progressive optic neuropathy associated with stereotyped morphological features of the optic disk (cupping without accompanying pallor of the neuroretinal rim), loss of retinal ganglion cell (RGC) axons within the nerve fibre layer, and loss of RGC bodies of the retina. Glaucomatous optic neuropathy is also associated with functional changes, particularly visual field defects, which mirror the loss of nerve fibre bundles within the retina.

Neuroprotection may be useful as a therapy for glaucoma (1). Glaucoma is a disease previously thought to be due to increased intraocular pressure (IOP), but is now recognized to have IOP as the primary risk factor. For example, some patients do not respond well or at all to lowering of the IOP. These patients may presumably benefit from other therapies that address the optic neuropathy inherent in glaucoma, reflecting the loss of RGCs. Therefore, neuroprotection, a therapeutic paradigm for prophylaxis of death of neurons from injury so as to maintain physiological function, may eventually be proven as a valid strategy for treatment of patients with glaucoma.

One of the main problems in assessing whether neuroprotection will be effective as a glaucoma therapy...
Neuroprotection models

is that assessment of this strategy is extremely difficult, requiring clinical trials that last years and cost millions of dollars. Therefore, before investing in such trials, it is important that the pre-clinical studies supporting the neuroprotective strategy be valid. This article discusses and analyses culture and animal models for studying neuroprotection in the laboratory, and provides a framework for evaluating these models.

Differences between pre-clinical studies and clinical trials

There is a long history of failure in neuroprotection clinical trials, despite multiple pre-clinical studies using animal models (2). For example, several studies described the use of N-methyl-D-aspartate (NMDA) antagonists showing improved outcome in experimental cerebral ischaemia. However, Phase III trials of NMDA antagonists failed (3). The same has been true for several studies of free radical scavengers and other neuroprotective agents.

There are many reasons why animal models might not predict the results of clinical trials.

1. There may be no good model for a specific type of disease. For example, there is no agreed upon animal model for normal tension glaucoma.
2. Sometimes animal models used in pre-clinical studies are not correctly chosen for the disease. For example, studies of photic injury may not be good models for looking at the possible neuroprotective effects of a pharmacological agent on RGCs.
3. There are many differences between animals and humans in the anatomy of the optic nerve and lamina cribrosa, types of RGCs and pathophysiology of disease.
4. An animal model may be somagenic, i.e. involve damage to the neuronal cell body, while the human disease may be axogenenic, i.e. mediated by axonal damage (4). Since the rate of cell death after an axonal injury is usually much slower than when the cell body is injured, an agent which might work for axonally mediated disease might not work for a neuronally mediated disease.

Other differences that may explain why animal models do not reflect the course of clinical trials relate to the timing of the cell death cascade:

1. In human disease, the treatment is usually given after the onset of the disease, while in animal models, the treatment is often given before or at the time of injury.
2. Human disease can be chronic, while animal models are sometimes acute. When human diseases are acute, they often ‘stutter’, meaning that the disease can intermittently worsen over the short term.
3. In animal models, the onset is usually all or none.
4. Human patients do not often realize that the disease has started until the symptoms begin, and therefore do not enter a trial early enough. With an animal model, the experimenter knows exactly when the disease was induced, and thus the treatment is always given at the same time with respect to the onset of pathology.

Types of neuroprotective models

Neuroprotection was initially studied in diseases of the central nervous system (CNS) that affect the brain and spinal cord, e.g. stroke, traumatic injury and degenerative diseases. Early models reflected this emphasis, and many are still applicable to pre-clinical work in glaucoma (Tab. I). The culture models of neurological diseases are usually based on the culturing of cerebral cortical neurons, cerebellar granule cells or other CNS cells. A variety of mechanisms can be used in culture to induce cell death, e.g. hypoxia, chemical hypoxia with metabolic inhibitors, hypoglycaemia, induction of apoptosis (e.g. with the agent staurosporine or similar agents), and serum or growth factor deprivation. Similarly, there are many animal models of neurological disease. Cerebral ischaemia can be produced by transient or permanent occlusion of the carotid and/or vertebral arteries (5), and simi-

<table>
<thead>
<tr>
<th>TABLE I - CULTURE MODELS RELATED TO GLAUCOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Retinal ganglion cells (RGCs)</td>
</tr>
<tr>
<td>A. Purified RGCs</td>
</tr>
<tr>
<td>B. Identified RGCs in mixed or explant cultures</td>
</tr>
<tr>
<td>II. Mixed retinal cells</td>
</tr>
<tr>
<td>III. Transformed retinal cells</td>
</tr>
<tr>
<td>IV. Neurons from nervous system tissue other than retina</td>
</tr>
<tr>
<td>V. Neuronal-like cell lines</td>
</tr>
</tbody>
</table>
lar techniques can be used with the spinal cord. Trauma to the brain (6) or spinal cord (7) can be produced mechanically. Chemical agents can induce specific disease states, e.g. MPTP (8) or rotenone (9) toxicity for Parkinson’s disease. Transgenic animals that simulate disease states, e.g. the Cu, Zn-superoxide dismutase mutations (10) that mimic familial amyotrophic lateral sclerosis, are also amenable to neuroprotective strategies.

Culture models

Other models are more applicable to the study of glaucomatous optic neuropathy from the standpoint of the visual scientist. Some of these are listed in Table II. The best cell culture models for studying neuroprotection are derived from the retina. Mixed retinal cultures, in which the retina is dissociated and cell death of individual retinal neurons studied, are used in many laboratories. The disadvantage of studying mixed retinal cultures without identification of individual neurons as to their type is that it is not clear whether a neuroprotective strategy is helping photoreceptors, bipolar cells, Müller cells, RGCs or any other specific cell type. Since RGCs are predominantly the only cells that die in glaucoma, a mixed retinal cell culture is not always representative of the cell death that occurs in the disease itself. Similar attributes exist for retinal explant cultures, in which entire pieces of retina are cultured.

Instead, RGCs can be studied in vitro, by either identifying them in the culture or by making pure RGC cultures. One technique for identifying RGCs is to label the RGCs by injecting a tracer, e.g. the fluorescent dyes, DiI or FluoroGold (11), into target areas of the RGC axons (usually the superior colliculus and/or dorsal lateral geniculate nucleus). A second technique for identifying RGCs in culture is to stain them with an antibody to Thy-1, a cell-surface molecule, which is predominantly seen on RGCs within the retina (12).

A pure RGC culture can be produced by using a panning technique (13), in which dissociated retinal cells are passed over Petri dishes coated with antibodies to surface Thy-1. Only the RGCs will adhere to the Petri dish, and they then can be dissociated from the plastic and studied further. A similar technique using antibodies linked to magnetic beads has been described (14).

Some researchers use neuronal-like cell lines derived from the retina (15). These are not completely physiological, since post-natal neurons are usually non-mitotic. However, cell lines do have the advantage of providing a simple method for propagating and maintaining the cultures over time. Alternatively, primary cultures or cell lines derived from neurons not of retinal origin can be used, but may not necessarily display the biological behaviour of retinal neurons.

Once a culture model is established, multiple mechanisms can be used to simulate injury and study the effectiveness of neuroprotective therapies. As with neuronal cultures, ischaemia can be simulated by either hypoxia, chemical hypoxia or hypoglycaemia (16). Apoptosis and growth factor deprivation can also be induced in culture. The procedure of making a dissociated culture injures the axon of the RGC, since taking the retina out of the eye transects those axons from the rest of the optic nerve, and thus is also an axotomy model.

<table>
<thead>
<tr>
<th>TABLE II - ANIMAL MODELS RELATED TO GLAUCOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Elevated intraocular pressure (IOP)</td>
</tr>
<tr>
<td>A. Monkey trabecular meshwork injury</td>
</tr>
<tr>
<td>B. Rat outflow blockade</td>
</tr>
<tr>
<td>1. Hypertonic saline sclerosis</td>
</tr>
<tr>
<td>2. Heat cauterization</td>
</tr>
<tr>
<td>3. Laser cauterization</td>
</tr>
<tr>
<td>C. Inherited glaucoma</td>
</tr>
<tr>
<td>II. Optic nerve injury</td>
</tr>
<tr>
<td>A. Optic nerve crush</td>
</tr>
<tr>
<td>B. Optic nerve transection</td>
</tr>
<tr>
<td>III. Excitotoxicity</td>
</tr>
<tr>
<td>A. N-methyl-D aspartate injection</td>
</tr>
<tr>
<td>B. Glutamate injection</td>
</tr>
<tr>
<td>IV. Retinal ischaemia</td>
</tr>
<tr>
<td>A. Acute IOP elevation</td>
</tr>
<tr>
<td>B. Central retinal and/or ophthalmic artery occlusion</td>
</tr>
<tr>
<td>V. Outer retinal damage</td>
</tr>
<tr>
<td>A. Experimental retinal detachment</td>
</tr>
<tr>
<td>B. Phototoxicity</td>
</tr>
<tr>
<td>C. Retinal degeneration</td>
</tr>
</tbody>
</table>
Neuroprotection models

Animal models

A variety of models using animals for retinal and optic nerve disease have been described. Exposure to constant light produces retinal phototoxicity (17). Retinal degeneration can be studied in transgenic animals, most notably in Drosophila (18,19) and rodents, e.g. the retinal degenerative mouse (20). Experimental retinal detachment can be produced by injecting fluid between the neural retina and retinal pigment epithelium (21). All of these models suffer from the drawback that the injury primarily affects photoreceptors, with relatively little contribution to RGC death. Therefore, they do not possess the predominant pathological finding seen in glaucoma, namely RGC loss over time.

A more suitable group of animal models are those associated with optic nerve injury or inner retinal injury. Any injury to the optic nerve will cause RGC death. Therefore, transecting or crushing (either completely (22,23) or partially (24)) the optic nerve accomplishes this, as does chronic ischaemia to the optic nerve head, achieved by infusing endothelin-1 into the subarachnoid space around the nerve itself (25,26). By causing axonal injury, these models all result in RGC death.

Another group of models depends on injuring the RGCs directly. Acutely raising the IOP higher than the systolic blood pressure will decrease the perfusion pressure sufficient to cause inner retinal ischaemia (27). Since the RGC layer is the innermost cellular layer of the retina, this retinal ischaemia will affect RGCs, and therefore will indirectly cause an optic neuropathy. Injection of excitotoxic agents, such as glutamate or NMDA, will predominantly injure RGCs (28–31), and thus also simulate the pathological findings seen in glaucoma (32).

Spontaneous elevation of IOP in inbred animals shares features with human glaucoma, and has the advantage that no experimental manipulation is necessary. A wide variety of animals have been used, including the DBA/2 mouse (33,34), the buphthalmic rabbit (35) and the beagle (36). The animal models most closely related to glaucoma are those associated with moderate elevation of the IOP to levels similar to that seen in patients with untreated glaucomatous optic neuropathy. Most commonly, these models are performed by raising the IOP by decreasing the aqueous outflow. In rats, the episcleral veins can be occluded with cautery (37), the aqueous veins sclerosed with hypertonic saline (38) or the limbal and episcleral veins occluded with laser (39). These all increase the IOP, and result in an optic neuropathy. In the non-human primate, the trabecular meshwork can be injured with argon laser (40,41), also resulting in raised IOP. This latter method is the most commonly used primate technique for studying experimental glaucoma.

Applicability of animal models

Given the variety of cell culture and animal models available to the researcher, what is the relative value of each for studying glaucomatous optic neuropathy in patients? To answer this, several measures can be used. If one looks at how closely the technique of the model simulates the disease itself, then it is easy to be misled. For example, raising the IOP on a chronic basis in the rat or primate causes the same morphological changes as seen in humans, namely loss of RGCs and their axons. One might therefore think that elevation of the IOP is a good model system for pre-clinical studies. However, studies where the IOP is raised acutely to very high levels, so as to exceed or approach the systolic blood pressure and make the retina ischaemic, cause a very different pattern of disease. Although RGCs are also lost in this disease, there are two major differences between this and the effects of moderate IOP elevation on the optic nerve. First, other parts of the inner retina are affected when the retina becomes ischaemic. Second, the time course over which RGC injury occurs is quite different from that seen in glaucoma. The injury initially occurs at the cell body in models of retinal ischaemia, while in glaucoma it appears that the injury initially occurs at the axon (42). Therefore, the very fact that the IOP is raised is not enough to make this model directly applicable to studying neuroprotection in glaucoma.

One could consider whether the results of the model reflect that seen in glaucoma. For example, we know that glaucoma causes death of neurons within the retina. One could therefore make the assumption that a model where neuronal retinal loss occurs, e.g. a phototoxicity or retinal degeneration model, are good models for studying neuroprotection in glaucoma. The same is true for models in which retinal neurons are cultured without specific identification of RGCs. However, it is the loss of the RGCs in particular that is specific to glaucoma, and not the loss of retinal neurons in general.
Finally, one could assume that the pathological mechanism of the model is most important. However, one of the difficulties is that even now the exact pathogenesis of the glaucomas has not been completely clarified, and might differ depending upon the specific type of glaucoma an individual has. For example, models in which RGC death is induced by excitotoxicity, which takes place at the cell body, might reflect what occurs in glaucoma if a patient has an excitotoxic mechanism. However, it is possible that this might not be true for all patients. Other patients might have primarily axonal disease and relatively little contribution from excitotoxicity, and thus a model produced by axonal injury, e.g. optic nerve crush, might be more applicable.

CONCLUSIONS

There is thus no specific single criterion for deciding which culture or animal model is best for studying neuroprotection. Neither the technique, the pathological results or the pathogenesis of the disease within the model are by themselves sufficient to make the model applicable to human disease. In the end, the most important feature is whether the results of the model correlate with the results of clinical trials of a neuroprotective agent in human disease. In other words, whether a model is a good model for studying a neuroprotective agent can only be shown if the model’s results with the neuroprotective agent are similar to the clinical results. Unfortunately, this operational definition is not one that can be predicted in advance. Only over time will we be able to determine which models are optimal for studying neuroprotection.

In summary, a wide variety of animal models are possible for studying neuroprotection as possible therapy for glaucomatous optic neuropathy. These animal models differ widely in their applicability to the human disease. The likely probability that a neuroprotective strategy will actually be useful for clinical glaucoma will rely heavily on the careful choice of which animal model(s) are chosen for pre-clinical studies of these neuroprotective agents.

ACKNOWLEDGEMENTS

Supported by the Retina Research Foundation, the Glaucoma Foundation, NIH EY12492 and an unrestricted departmental grant from Research to Prevent Blindness, Inc. LAL is a Research to Prevent Blindness Dolly Green scholar.

REFERENCES

Neuroprotection models

39. Schori H, Kipnis J, Yoles E, et al. Vaccination for protection of retinal ganglion cells against death from...

