Research reveals potential new strategies for neuroprotection in glaucoma

Rolbeard O'Neill in Berlin

AN increasingly detailed understanding of the mechanisms by which retinal ganglion cells die in eyes with glaucoma is revealing several potential new neuroprotective strategies for the treatment of the disease, according to a series of presentations at the 7th International Symposium of Ocular Pharmacology and Therapeutics (ISOPT).

Retinal ganglion cell death is the main pathological finding of glaucoma and is the cause of visual loss in eyes with the disease. Most current therapies aim to protect the retinal ganglion cells and the optic nerve indirectly through reduction of IOP. However, while in three major trials (AGIS, EMGT and OHTS) this strategy has been shown to significantly reduce the rate of glaucoma progression, in a proportion of patients the disease will still progress, despite IOP reduction.

Recent years have seen a growth in the research into neuroprotection in glaucoma and other neurodegenerative diseases. In particular, a large clinical trial due to be completed in 2007 is investigating the use of memantine (Namenda, Allergan) in patients with primary open-angle glaucoma. The agent is currently used in the treatment of Parkinson's disease and Alzheimer's disease. It appears to achieve its neuroprotective effect by preventing glutamate from binding to neuronal NMDA-receptors, which in turn reduces the influx of calcium and thereby prevents apoptosis, or cellular suicide.

Meanwhile, several new alternative neuroprotective strategies for glaucoma are also under investigation. They include approaches designed to improve the function of mitochondria, interfere directly with apoptosis, or prevent axonal degeneration. Some of the agents being studied for these purposes occur naturally within the body and have a reasonably high safety profile, the ISO PT Congress heard.

Improving mitochondrial function

The mechanical and ischaemic insults induced by raised IOP can result in energy dysregulation and oxidative stress in retinal ganglion cells, which can ultimately lead to their death because of the inability of their mitochondria to maintain their normal function. Agents that enhance mitochondrial function may therefore confer a neuroprotective effect in glaucoma, said Professor Neville Osborne PhD DSc, Nuffield Laboratory of Ophthalmology, Oxford, UK.

“In the initiation of glaucoma, an alteration in the blood flow dynamics in the optic nerve head causes a compromise in the retinal ganglion cell axon energy requirement rendering the ganglion cells susceptible to additional insults. Thus, agents targeted specifically at enhancing ganglion cell mitochondrial energy production should be beneficial in a disease like glaucoma,” he added.

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Neville Osborne PhD DSc

Dr Osborne noted that retinal ganglion cell axons are functionally specialised and contain many mitochondria to meet the high-energy requirements for nerve conduction. Following ischaemic injury, secondary damage can result from exposure to light and to substances released from damaged astrocytes, such as glutamate and TNF-alpha, further undermining mitochondrial function and viability, he explained.

However, retinal ganglion cells appear to die at different rates, depending on factors that have yet to be fully elucidated. It may therefore be possible to intervene pharmacologically at an early stage to halt or slow down the neurodegenerative process and thereby preserve vision in the glaucomatous eye, he pointed out.

Several agents showing promise in animal studies

Studies on animal models and cell cultures have demonstrated that substances which enhance mitochondrial energy function can also blunt the apoptotic effect of ischaemic injuries in retinal ganglion cells, Prof Osborne noted. The agents include compounds found naturally within the human body such as alpha-lipoic acid, creatine monohydrate and nicotinamide.

“These substances weren't selected at random; they were selected because they can be tolerated by man and can be taken over a long period of time. All can be taken orally and have a high safety profile with minimal side effects,” Prof Osborne said.

The three agents each play important roles in the ADP/ATP system of mitochondria. In animal experiments, they appear to reduce the death rate of retinal ganglion cells when given about four weeks prior to ischaemic injury.

“Ganglion cell death in glaucoma ultimately occurs because of a collapse of the mitochondrial function, which initiates apoptosis. Facilitated mitochondrial function could be used to attenuate apoptosis. Therefore we suggest that a prophylactic treatment with the substances that can facilitate mitochondrial function might slow down the apoptosis of still functioning ganglion cells.”

Anti-apoptotic approaches

Neuroprotection of retinal ganglion cells may also be achieved through direct interference with the cascade of chemical reactions that results in apoptosis, said Matthias Bähr MD PhD, Department of Neurology, University Hospital, Göttingen, Germany.

Some of the approaches Dr Bähr and his associates are investigating include the administration of agents that block specific parts of the apoptotic cascade and gene therapy with genes for anti-apoptotic proteins, Dr Bähr told the ISO PT meeting.

To study apoptosis in retinal ganglion cells, Dr Bähr and his associates used an axonal lesion model rather than an ischaemic model of neurodegeneration in their in vivo studies. It involves pre-labeling the ganglion cells through injection of a dye and then crushing or transecting the optic nerve. The retina may then be flattened or sectioned for morphological or immunohistochemical analysis.

“This is a very nice and straightforward in vivo apoptosis model. We don’t study glaucoma, which also has ischaemic aspects of retinal ganglion cell death, but just concentrate on the apoptosis aspects.”

Apoptosis in this model will normally occur in about 80% of retinal ganglion cells. During that time the cells undergo specific changes in their gene expression pattern of transcription factors, pro- and anti-apoptotic proteins and growth-associated genes, leading finally to the activation of neuron-intrinsic enzymes, called caspases (Caspases 3, 8 and 9) which irreversibly initiate apoptosis.

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Matthias Bähr MD PhD

“As a critical step, mitochondrial dysfunction occurs, which seems to be the point of no return for functional rescue of affected cells. Thus, long-term survival and initiation of regeneration programmes of adult CNS neurons critically depends on inhibition of mitochondrial dysfunction upstream of caspase activation,” he added.

Erythropoietin

Dr Bähr and his associates have been able to demonstrate in animal studies that neurotrophic factors such as IG F can prevent cell death following axonal injury by inhibition of Caspase 3 and preservation of mitochondrial function. However, side effects
of this agent make it unacceptable for use in humans. Fortunately, erythropoietin (EPO) can achieve the same effect following virtually the same pathway and is already in wide use in humans for a number of indications.

The rationale behind the use of EPO in glaucoma lies in the fact that it increases red blood cell counts by inhibiting apoptosis in erythrocyte progenitors. Furthermore, research has shown that receptors for EPO are present in retinal ganglion cells.

More recently, investigators have investigated for this purpose using both adeno-associated virus and HIV-Tat-derived fusion proteins with transduction properties as vectors. They found that animals transfected with the gene in this way had resistance to axotomy-induced retinal ganglion cell death that lasted for many months, Dr Bähr said.

“This shows that over-expression of anti-apoptotic proteins upstream of mitochondrial dysfunction protects the cells from secondary degeneration. In the future we hope that by combining these strategies we can have a very efficient block of cell death, not only by blocking Caspase 3 pathways but by preserving mitochondrial function upstream,” he added.

Preventing Wallerian degeneration

Another approach to preventing retinal ganglion cell death may be by preventing the Wallerian degeneration of the cells’ axons, said Keith R Martin MD FRCOpht, Centre for Brain Repair, Cambridge University, Cambridge, UK.

Wallerian degeneration occurs in many neurodegenerative diseases including Alzheimer’s disease and Parkinson’s disease, and as a consequence of stroke, he noted. It is an active, orderly process that usually follows severe focal axon damage and involves the breakdown of the endoplasmic reticulum, microtubules, neurofilaments and mitochondria, Dr Martin explained.

In addition, the Wallerian process occurs independently of apoptosis and is not prevented by inhibitors of apoptosis. However, the loss of axons inevitably results in neuronal death.

Researchers have identified a mutant gene in mice that appears to inhibit the Wallerian process. It is called the slow Wallerian degeneration gene (WldS) and it delays axonal degeneration 30-fold in several mouse models of neurological disease and postpones secondary cell body death in a mouse model of motor neuron disease, Dr Martin noted.

The mutation occurred spontaneously at the Harlan-Olac laboratory in Bicester, UK and is not harmful. In fact, mice with the gene are identical to those without the gene in terms of appearance and behaviour and can be distinguished only by the remarkable ability of their axons to survive without a nucleus after separation from the cell body.

The WldS gene is a chimera of two normal genes, whose protein products control ubiquination and NAD synthesis, respectively. The WldS gene product appears to achieve its neuroprotective effect by interfering with the ubiquitation process of neural proteins, and in this way prevents the targeting of axonal proteins for degradation.

Gene protects retinal ganglion cells

With regard to glaucoma, Dr Martin noted that in a study he and his associates recently conducted the WldS gene conferred a significant protective effect on retinal ganglion cell axons in transgenic rats following unilateral transection and crush injury.

Dr Martin noted that their research findings indicate that inhibition of the protein ubiquitation system may be a promising avenue for preventing axonally mediated cell death. Other potential therapeutic approaches to protect retinal ganglion cell axons include the inhibition of autophagy (a process in which lysosomes consume cellular organelles), neurotrophin supplementation by gene therapy; and calcium antagonists, he said.

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