Summary of the First Meeting of the EGS Neuroprotection SIG

3 - 4 May 2008
Convent van Chièvres Begijnhof, Leuven, Belgium
Dear Colleagues & Friends

Last weekend, the EGS Neuroprotection SIG held its first roundtable meeting. There were 30 attendees, and it proved a memorable event, thanks not only to a great venue but wonderful, thought-provoking & poignant discussions.

As the date of the meeting was so near ARVO, there were many who were disappointed they could not attend, but requested details of what was discussed, and a willingness to participate in the future.

Consequently, here is a summary of the roundtable meeting, with contributions from each speaker covering lectures and core messages from the day, which I am sure you will find interesting.

If you would like to participate in future meetings, I would invite you to attend our next event which takes place during the **8th EGS CONGRESS BERLIN 1-6 JUNE, 2008**:

The Neuroprotection SIG  
EGS CONGRESS BERLIN  
Room London 4  
11:00 – 12:00, Friday 6th June 2008

In addition, at the end of this summary, there is a form which I would urge you to complete to register your interest in receiving updates and information on this new SIG.

I hope to see many of you in Berlin, but most of all look forward to working with you in promoting research and collaborations on neuroprotection in Europe.

Best wishes

Dr M Francesca Cordeiro  
Chairman, EGS Neuroprotection SIG
Introduction

In summer 2007, Clive Migdal (President-Elect of the EGS) took the initiative to set up an EGS Neuroprotection SIG, and this was announced during the Closed EGS Meeting in Palma, following which a meeting was held in London in January 2008 to discuss its formation and development. At the meeting I was appointed Chairman, and a mission statement was agreed, with the aims as itemized below. It was also suggested that a roundtable pre-EGS Berlin conference was needed and we are extremely grateful to Ingeborg Stalmans who at short notice organized a tremendous meeting in her home town in Leuven, the proceedings of which can be found in the following pages.

Broad Aims

- To provide a forum to facilitate the introduction of neuroprotective treatment in the clinical practice of glaucoma
- To understand the science of neurodegeneration in glaucoma
- To develop strategies directed at future neuroprotective treatments

Specific Aims

- To promote understanding of science behind neuroprotective strategies by encouraging dialogue between glaucoma, allied clinicians & basic scientists
- To maintain a comprehensive and updated database of clinical trials & basic research in area of neuroprotection
- To disseminate the results of translational research and facilitate appropriate clinical trials
- To provide a forum for the discussion of new, interesting & controversial findings in the area
- To encourage high standards of evidence-based medicine and research in the application of neuroprotection in glaucoma leading to a consensus on effective therapies
Attendees

BELL Katharina
CORDEIRO Francesca
DEBAERE Lieven
DEFLORENNE Carine
FARRELL Stephen
FEDRIGO Alessandra
GANDOLFI Stefano
GOETHALS Marc
GRAU Anna
GRUS Franz
HITCHINGS Roger
HOSTE Ann
KESSING Svend Vedel
LARSSON Lil-Inger
MARTIN Keith
MIGDAL Clive
MORGAN James
NISSEN Ole
NORRGREN Gunilla
NUCCI Carlo
O'BRIEN Colm
OSBORNE Neville
PAQUES Michel
ROSSETI Luca
SAHEL Jose
STALMANS Ingeborg
STEVENS Anne-Marie
SWIFT Anne-Marie
VIDAL-SANZ Manuel
ZEYEN Thierry
Neuroprotection – The Science behind the Theory
James Morgan
Cardiff University

Background

Interest in neuroprotection in ophthalmology was raised by work published by Dreyer et al. 1996 reporting elevated levels of glutamate in the vitreous of patients with glaucoma. This finding led to the hypothesis that glutamate mediated excitotoxic damage was likely to play a key role in initiating retinal ganglion cell death in glaucoma. Subsequent work has cast doubt on these findings as others have not be able to replicate Dreyer’s findings. While marked glutamate elevation seems unlikely the possibility remains that variation in glutamate levels within the retinal ganglion cell layer may be important. The study remains important in that it highlighted the possibility that neuroprotective strategies could be developed to prevent retinal damage in glaucoma.

Since that time many studies have focused on factors that, apart from elevated IOP influence the rate of retinal ganglion cell death. It is now clear that a number of distinct cellular processes may be involved ranging from elevated nitric oxide at the level of the optic nerve head to complement activation in the retinal ganglion cell layer. During this time important descriptions of the process of retinal ganglion cell death have emerged which indicate that RGCs are likely to undergo a prolonged period of atrophy prior to death. These changes are manifest as shrinkage and remodelling of the dendritic tree with associated changes in RGC function. These ‘sick cells’ represent an excellent opportunity for neuroprotective strategies and raise the prospect that the early changes in glaucoma might be reversed. Considerable work is still required to elucidate the processes that result in these changes so that appropriate therapeutic agents can be used.

Key points:

• Neuroprotection is directed at the direct rescue or regeneration of retinal ganglion cells in glaucoma using agents that compliment treatments directed at the reduction of IOP.
• Retinal ganglion cells undergo a prolonged period of remodelling and are likely to be dysfunctional prior to cell loss.
• Sick retinal ganglion cells represent an excellent target for the prevention of RGC death and, possibly the restoration of function.
• Elevated glutamate levels in the vitreous are unlikely to occur in glaucoma but the possibility remains that excitotoxic damage may occur at a local level within the retinal ganglion cell layer.
Take Home Messages

➢ *We are at an early stage in our understanding of the processes that cause retinal ganglion cell death. Evidence supports the idea that the entire retinal ganglion cell population shows signs of damage prior to the onset of cell death – this presents a great opportunity for the early detection of retinal damage and for the restoration of function.*

➢ *It is also important that we develop clinical and research tools for the detection of these early changes.*

Discussion

1. We should consider carefully the models that we use in the laboratory setting. The generation of high intraocular pressures may not necessarily provide the best model of glaucoma

2. Data should be considered from a range of models: changes in different species following IOP increase are more likely to be a common feature of disease.
Traditional End points for Neuroprotection

F. Grus PhD MD, Experimental Ophthalmology, University of Mainz, Germany

Background:

Glaucoma is one of the leading causes of blindness worldwide. The primary injury is still unknown and at time of detection 50% of retinal ganglion cells are already lost. Furthermore, the elevated IOP can only be considered as a main risk factor. Thus, neuroprotective treatments could be very promising in glaucoma. Neuroprotection can be considered as any therapy which prevents, retards, or reverses apoptosis-associated cell death targetin “only” the loss of cells and not the cause of the disease process.

Key points

• Very unsuccessful transition from lab to human clinical trials
• Suitability of animal models in glaucoma?
• Did the clinical trials fail because the neuroprotection did not work or due to unsuitable clinical endpoints?
• How to measure efficacy and progression in clinical trials, how to define clinical endpoints?
• In most of the studies: only visual fields and disc photographs
• Both are variable and mostly insensitive and subjective to detect progression
• Better parameters needed to define better endpoints
• Structural and functional parameters e.g. HRT, OCT, GDx could improve study design
• In vivo counting of retinal ganglion cells? (Annexin V)
• Other protoemics, genomics, or autoimmune biomarkers
Take Home Message

➢ *Improvement of endpoints needed to define better study designs*

Discussion

1. Always consider: neuroprotective trials might work better with improvement of endpoints, but

2. What about the clinical relevance of the results?

3. What if traditional endpoints were suitable but the drugs not effective at all?
Novel End Points for Neuroprotection
Keith Martin, Cambridge University

Background
A major problem with clinical trials of neuroprotection in glaucoma is our current reliance on conventional visual field tests. Visual fields are variable and therefore verification of visual field progression is slow, leading to long and expensive clinical trials. The use of newer technologies such as HRT and GDx as surrogate biomarkers of progression is currently limited by a lack validation against visual fields and a lack of acceptance by regulatory authorities. Thus, there is an urgent need to develop and validate new endpoints for use in clinical trials of neuroprotection.

Key points
• Rapidly improving imaging technologies mean it is now possible to visualise surviving and apoptosing retinal ganglion cells (RGC) in animal models of glaucoma.
• In vivo imaging of apoptosing RGC is an attractive strategy for assessment of glaucoma progression, assuming the techniques can be shown to be safe and reproducible in humans.
• Detection of apoptosing cells in human glaucoma is likely to be technically challenging particularly when the rate of progression is slow. It is estimated that around 20 cells enter apoptosis each day in the normally ageing human retina. If a dying cell is only detectably in apoptosis for 3-6 hours, a typical normal retina is only likely to contain a maximum of 5 detectably apoptosing RGC at any one time, assuming a uniform rate of RGC death. A glaucoma eye progressing from normal to blindness over 10 years would be expected to have around 30 – 60 detectably apoptosing cells using similar assumptions, and slower rates of progression will be even more challenging to detect.

Take home message
➢ Current clinical trial endpoints far from ideal, making clinical trials of neuroprotection too long and expensive.
➢ Better validation of existing optic nerve head and nerve fibre layer imaging technologies as markers of progression would be very useful.
➢ Imaging of surviving and dying RGC in the retina is technically feasible now, and may be useful as an endpoint for neuroprotection trials in the future. However, the technical challenges for safe detection of slow progression in humans are formidable.

Discussion
• Should our priority for neuroprotective trials in the short term be to optimise the markers we already have or to delay further trials until we have better novel endpoints?
Moving Results of Translational Research into Clinical Trials
Jose Sahel, Quinze Vingts Paris.

Background
The frequent failure of clinical trials following successful experimental studies raises questions about preclinical development processes. Improvement in the preclinical determination of the therapeutic interest of drugs is the main objective to reach in the next few years. The most important bottlenecks of translational research are relevance of animal models, definition of adequate biomarkers, and the degree of understanding of pathogenic processes. For the definition of adequate biomarkers in the specific area of retina, it is likely that in vivo, non-invasive imaging will have growing importance, in parallel to the evolution observed in the clinical practice. The understanding of pathogenic process may benefit from the experience of our team which has a large experience in neuroprotection of photoreceptors; thus, the tools and concepts that we developed may be relevant for retinal ganglion cell neuroprotection as well.

Key points
Recent failures in neuroprotection trials (e.g. Memantine) raises many questions:
• Ineffective drugs or ineffective trials ?
• Are preclinical models predictive ?
• Need for novel or better biomarkers
• Need to target the right phase of progressing disease

The course of retinal ganglion cell death (if death is assumed to be the sole cause of dysfunction, which may of course not be the case) may follow a course similar to what is observed during degeneration of photoreceptors, in which two populations degenerate at different rates with different functional consequences (i.e. rods before cones). This duality accounts for the complex clinical pattern, and if the genetic heterogeneity (and probably also epigenetic/environmental factors) is taken into account, this helps to understand the high variability of the disease. Thus, understanding basics mechanisms such as trophic interactions may play a role, as was shown by our discovery of trophic factors for cones derived from rods, may help to define a time-window in which neuroprotective agents may be efficient. State-of-the-art imaging techniques, which are growing at a tremendous pace, will without doubt considerably increase our knowledge on retinal cell death during glaucoma. We have been developing techniques for labelling retinal cells and observing them non-invasively. Currently, SLO allows imaging of retrogradely labelled retinal ganglion cells, of microglial cells, of axons. Other laboratories have developed metabolic imaging such as for apoptotic retinal ganglion cells. Thus, it is conceivable that a better understanding of the course of retinal ganglion cell death during glaucoma will be obtained, in animals models as well as in humans.
Take Home Message

- Clinical trials should rely on solid proofs of concept of preclinical studies with clinically relevant endpoints.

- In vivo, non-invasive imaging will probably be a key factor to understand retinal ganglion cell death.
Natural Neuroprotection
Carlo Nucci, University of Rome Tor Vergata, Rome Italy

Background
Antioxidant activity and/or neuroprotective effects based on other mechanisms have been reported in a number of natural substances, including plant compounds and elements of the human body. Many of these compounds have been on the market for over 30 years. They are sold as nutritional supplements, and as such they can be purchased without a doctor’s prescription. The problem is that patients’ use of these substances is often based on information obtained from unreliable sources, above all the Internet, and while the risk of serious adverse effects is low, patients may be led to use these compounds in ways that are absolutely ineffective.

Key Points:
- The list of natural compounds believed to be beneficial for the retina is long and varied.
- One of the most thoroughly studied is Ginkgo biloba extract (GBE). In the eye, GBE has been reported to protect retinal ganglion cells in a rat model of chronic glaucoma. In a crossover study, low dose, short-term treatment with GBE increased ophthalmic artery blood flow by a mean of 24%. In another recent study, visual field defects in patients with normal tension glaucoma improved after 4 weeks of treatment. The improvement was no longer detectable after 8 weeks of washout, so GBE would probably have to be used continuously to provide lasting effects.
- Another extensively studied neuroprotective agent is citicoline, an endogenous compound involved in the synthesis of the neuronal membrane. It has been showed that 60 days of oral or intramuscular citicoline improved electrophysiological parameters in patients with glaucoma. However partial regression of this improvement was detected after 120 days of wash out. But in 12 patients who continued the treatment for periods as long as 8 years, the improvement remained stable, suggesting a potential neuroprotective effects of citicoline.
- Green tea is particularly rich in flavonoids, the most abundant being epigallocatechin gallate (EGCG). In several laboratory studies, EGCG has displayed specific properties capable of attenuating several events implicated in the pathogenesis of glaucomatous damage. Neville Osborne has presented electrophysiological, structural, and biochemical evidence indicating that intravitreal, systemic, or oral administration of EGCG reduces the retinal damage induced by ischemia and reperfusion.
- The next compound on our list is coenzyme Q10, a fundamental player in the mitochondrial respiration pathway. Recent studies have shown that CoQ10 is not only a powerful antioxidant: it also inhibits the opening of the mitochondrial permeability pore and thereby exerts a specific anti-apoptotic effect.
application of CoQ10 reduces retinal ganglion cell loss in rats with retinal ischemia induced by increased IOP. The neuroprotective effects of topical CoQ10 were also demonstrated in vivo by Cordeiro et al. in a model of chronically elevated IOP. Based on our experimental studies, our hypothesis is that CoQ10 reduces the negative effects of high IOP-induced ischemia on glutamate transport by protecting mitochondrial energy metabolism. This limits the accumulation of extracellular glutamate induced by the insult and prevents apoptotic death in the RGCs.

• I would like to briefly mention the retinal endocannabinoids system. Our studies suggest that this system provides endogenous neuroprotection that can be weakened under certain conditions, such as acute raise of IOP. Restoration of physiological anandamide levels with agents that inhibit its enzymatic degradation or act as agonists of retinal cannabinoid receptors appears to be a promising strategy for strengthening this endogenous protective system and preventing retinal cell loss.

Take Home Messages

- *Gingko biloba extract, citicoline, catechins and coenzyme Q10 are natural products that seem to provide neuroprotection.*
- Their efficacy in glaucoma has not been confirmed in large clinical trials and it is unlikely that testing of this sort will be undertaken in the near future.
- *At the present time, however, in light of their relative safety, and the study data I have summarized, use of these compounds might be considered when conventional therapy for glaucoma is inadequate.*
Review of alpha-2 agonists
Manuel Vidal Sanz  Universidad de Murcia  Spain

Background

These studies were aimed at studying the effects of transient ischemia of the retina on the inner and outer retina as well as on axonal transport and the retino-tectal projection. In addition we investigated whether topical administration of an alpha-2 agonist, (brimonidine; BMD) had protective effects against ischemia-induced retinal damage.

Key points

- **Inner retina.** The thickness of the inner nuclear and inner plexiform layers of the saline-treated groups of retinas had decreased to approximately 71% of the thickness in the BMD-treated groups.
- **Retrograde transport.** In the groups of animals that had been treated with saline, approximately 15% of the RGCs that survived retinal ischemia had their retrograde transport impaired.
- In the BMD-treated groups of animals, RGC densities amounted to 90% of the RGC population 7 or 14 days after ischemia and were comparable to those obtained in their contralateral non-ischemic retinas.
- **Orthograde transport** The density of CTB-labeled profiles in the contralateral SC of the vehicle-treated rats represented less than one half the area occupied by CTB-labeled profiles in unlesioned control rats.
- In the BMD-treated rats CTB-immunoreactivity occupied an area of approximately 83% of that observed in the unlesioned group.

Take Home Messages

- Retinal ischemia induces degeneration of the inner retinal layers, loss of RGCs, alters retrograde axonal transport in a proportion of surviving RGCs and results in the loss of retinal afferents to the superior colliculus.
- BMD rescues RGCs from ischemia-induced cell death, preserves retrograde axonal transport in surviving RGCs and protects against ischemia-induced degeneration of the retinotectal projection.

Discussion

- Transient ischemia of the retina does not mimic the situation found in the adult human retina of glaucomatous patients.
- Nevertheless, these models provide a valuable tool to investigate injury – induced cell death and possible rescue in the retina.
Review of NMDA antagonists
Neville Osborne, Oxford University

Background
Glutamate elicits excitatory responses on neurones by influencing a number of receptor-types. The glutamate NMDA receptor appears to be particularly important because when overactivated a large influx of calcium occurs to cause cell death. Importantly, the NMDA receptor is only activated when two sites on the receptor are occupied, one site by glutamate and the other site by glycine. Moreover, when the glycine and glutamate sites are occupied their combine influence can be non-competitively modulated by various extracellular substances that include magnesium, zinc and nitric oxide.

Key points:
- Overactivation of NMDA receptors are implicated in the pathogenesis of many neurodegenerating diseases.
- Some evidence exists to suggest that glutamate is involved in the pathogenesis of glaucoma.
- Possible rise in extracellular glutamate in glaucoma may come from activated astrocytes caused by an insult at the optic nerve head.
- Whether a rise in extracellular glutamate is sufficient to overactivate NMDA receptors located to ganglion cells is debatable because the receptor functions by a combined action of both glutamate and glycine.
- No evidence exists for extracellular glycine levels to be elevated in experimental models of glaucoma or in the vitreous humour of advanced glaucoma patients.
- It is difficult to be persuaded by present evidence that overactivation of NMDA receptors located to ganglion cells is the main cause for ganglion cells dying in glaucoma.
- The pathogenesis for ganglion cell death in glaucoma probably involves an ischemic insult to the optic nerve head causing the ganglion cells to be energetically compromised. Moreover, an affect on optic nerve head glial cells might result in an elevation of various extracellular substances (e.g. glutamate, nitric oxide, TNFα, endothelin) that can differentially be toxic to the energetically compromised ganglion cells to result in apoptosis.
Take Home Messages

- Glutamate probably plays a minor part in the pathogenesis of glaucoma.
- Blocking unnecessary stimulation of all types of glutamate receptors associated with ganglion cells may slow-down ganglion cell death in glaucoma.
- All information available does not support the idea that blocking solely the NMDA receptor from any negative effect of a rise in extracellular glutamate is the best way forward to help glaucoma patients.
- Effective neuroprotection in glaucoma is unlikely to be realised by use of compounds that have a single mode of action.

Discussion

- Sustained activation of optic nerve head astrocytes may cause a definitive rise in extracellular glutamate in glaucoma.
- Preventing the actions of possible excessive extracellular glutamate on retinal ganglions would reduce neuronal injury. Experimental studies show that excessive depolarisation of neurones caused by high levels of glutamate causes cell death.
- Two agonists are necessary to stimulate a NMDA receptor, glutamate and glycine. The receptor function is also modulated by other chemicals. Weak evidence exists only for an elevation of extracellular glutamate in glaucoma patients.
- Present knowledge argues that NMDA receptor antagonists alone are unlikely to cause significant neuroprotection for glaucoma patients.
NMDA antagonists – A Critical Analysis
Roger Hitchings   Moorfields Eye Hospital London

Background

• RGC survival is dependent on a maintained balance between ‘life and death’ signals. Amongst these ‘death signals’ is Calcium ion induced excitotoxicity.
• Memantine is a selective ‘open channel’ blocker that restricts the entry of calcium ions into the cell when there is excess glutamate or cell disease. It has been shown to offer ‘neuroprotection’ to the retinal ganglion cell in the experimental primate model of ocular hypertension, and is in clinical use as an NMDA antagonist in mild/moderate Alzheimer’s disease.

Key points

1) The two multicentre clinical trials testing oral memantine in 10 or 20mg doses failed to reach the primary End Points (which differed in trial 1 and trial 2)
2) Memantine is not likely to be retested in a clinical trial for glaucoma because of patent issues
3) Neuroprotection other than IOP reduction or untested complimentary medicine is not a therapeutic option at the present time
What is the Evidence for Treatment
Luca Rosseti  Milan University

Background
Despite the bulk of evidence coming from experimental studies on animals, there is no convincing demonstration of a neuroprotective effect supporting treatment in human glaucoma. Concerning the question whether neuroprotection is effective in treating glaucoma, we should probably consider 2 different issues:

• Are neuroprotective therapies effective in protecting human retinal ganglion cells (RGCs)?
• Is there evidence supporting neuroprotection to cure glaucoma.

Key Points
• 2 different types of studies could answer these question:
  o Small clinical studies adopting surrogate outcomes might be adequate to demonstrate an effect of neuroprotection on human RGCs
  o Large trials using visual function as an outcome should be chosen in order to prove a clinically relevant effect on glaucoma (e.g. memantine trial).
• There are only 4 proper clinical studies on neuroprotection in glaucoma, despite the hundreds of citations about neuroprotective drugs in glaucoma.
• Majority of the published clinical trials on neuroprotection in glaucoma are pilot studies, no mention of a sample size calculation is provided, surrogate outcomes are adopted and usually treatment seems surprisingly effective. Are results of these studies reliable? Are these findings applicable to our clinical practice?
• Examples of small clinical trials are the Parisis’s study on citicoline (Ophthalmology 1999) and the Quaranta’s study on gingko biloba (Ophthalmology 2003), both appeared in one of our “top” clinical journals. Both these trials reported a significant (and surprising) effect of those compounds in either improving electrophysiology parameters or improving the patients’ visual fields.
• A recent systematic review (Gunasekera, Ophthalmology 2008), criticized the low quality of information and the lack of evidence about complementary and alternative medicine for glaucoma and mentioned the potential risk for the patients when such therapies are advertised on internet.
• Concerning brimonidine, there are 2 studies that, unfortunately have not been published yet, and therefore cannot provide any useful evidence.
• The only properly designed large clinical trial was the memantine trial, but unfortunately this was not able to demonstrate a significant effect on visual function in about 2,000 glaucoma patients.
Take Home Message

- Like for all “translational medicine”, there is a lot of evidence from neuroprotection in experimental models which has had difficulty in being applied to human studies.

- At present, there is no evidence suggesting that we should cure glaucoma with neuroprotection. Of course, science is running fast and evidence from a few studies could change this conclusion completely.

- Up to now there is strong evidence indicating that lowering IOP is by far the best neuroprotective option in our hands.
Lessons We Have Learnt
Stefano Gandolfi  University of Parma
Francesca Cordeiro University College London

Background
Until now a classical approach has been applied to testing neuroprotection in glaucoma, which appears to have inherent problems, as described below:

Key Points:
• Choosing a molecule with good experimental evidence in animal models does not necessarily translate to successful application in humans
• We should when at all possible endeavor to assess a drug with a “clear” mechanism of action, and using different and multiple animal models, when possible, primates,
• In setting up prospective, randomized, clinical trials, it is important to consider a proper sample size, adequate study duration, individual variation, accurate and consistent phenotyping, with matched risk factor across groups
• Ideally, it would be nice to establish biomarkers before the start of a trial
• Adequate attention should be given to the placebo-control groups
• Problems occur with having to establish good IOP control across groups, due to ethical considerations (eg Memantine)
• Being constrained by the only possible hard end point: visual fields, proved difficult for Memantine
• May be outcome evaluation should include “trend” and not “event” criteria
• Perhaps more “dose-response” and “cross-over” designs should be advocated to test efficacy
• New methodologies for establishing objective end points should be encouraged

Take Home Messages
➢ We must re-consider the design(s) of clinical trials in glaucoma (in general) and neuroprotection (in particular)

➢ We must start applying biomarkers to design the best study for the individual compound. In case of positive outcome, biomarkers will help the clinician to dissect out the best candidate for a selected therapy.

➢ We still have a “long-way-to-go”.... But not that long !!
Expression of Interest

EGS Neuroprotection SIG

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Please complete the form and fax or email to:

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