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REVIEW

Investigational neuroprotective compounds in clinical trials for retinal disease

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ABSTRACT

Introduction: Retinal neurodegeneration causes irreversible vision loss, impairing quality of life. By targeting neurotoxic conditions, such as oxidative stress and ischemia, neuroprotectants can slow or stop sight loss resulting from eye disease. Despite limimted clinical use of neuroprotectants, there are several promising compounds in early clinical trials (pre-phase III) which may fulfil new therapeutic roles. Search terms relating to neuroprotection and eye disease were used on ClinicalTrials.gov to identify neuroprotective candidates.

Areas covered: Research supporting neuroprotection in eye diseases is focused on, ranging from preclinical to phase II, according to the ClinicalTrials.gov database. The compounds discussed are explored in terms of future clinical applications.

Expert opinion: The major challenge in neuroprotection research is translation from basic research to the clinic. A number of potential neuroprotectants have progressed to ophthalmology clinical trials in recent years, with defined mechanisms of action – saffron and CoQ10 – targeting mitochondria, and both CNTF and NGF showing anti-apoptotic effects. Enhancements in trial design and patient cohorts in proof-of-concept trials with enriched patient populations and surrogate endpoints should accelerate drug development. A further important consideration is optimising drug delivery to improve individualised management and patient compliance. Progress in these areas means that neuroprotective strategies have a much improved chance of translational success.

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Neuroprotectants; clinical trials; NGF; saffron; CoQ10; ubiquinone; coenzymeq; CNTF; eye disease

1. Introduction

The complex and intricate structure of the retina predisposes its physiology to disruption from a wide range of pathological factors. Retinal disease may arise from a myriad of causes including age, diabetes, high myopia, hypertension, genetic predispositions, and external environmental factors. Diseases such as glaucoma, diabetic retinopathy, and age-related macular degeneration (AMD), all impair vision and severely quality of life by very different mechanisms; however, the neurodegeneration of retinal cells is common to all pathologies.

With very few exceptions, once neurons are lost, they are lost forever [1], and cannot be replaced. Neuroregeneration – restoration of lost neurons – is extremely difficult, and such research is in its infancy. For the foreseeable future, slowing or avoiding neuronal death (neuroprotection) is the most effective way to treat retinal disease, with the identification and application of neuroprotective agents having become a focal point of modern medicine. As the understanding of the cellular intricacies of neurons expands, the identification of compounds able to support the healthy function of neurons is accelerated. This presents an ever-changing clinical landscape, as novel compounds are identified and tested as treatment for various disorders.

The present review highlights four promising neuroprotective agents which have yet to reach clinical phase III trials, discusses their mechanisms of action, and details their use in clinical trials. In order to identify appropriate neuroprotective agents for this review, the database on www.clinicaltrials.gov was searched, with 'eye disease' and 'retina disease' being entered into the 'condition or diseases' field and 'neuroprotection' and 'neuroprotectant' being entered into the 'other terms' field. Once a compound was identified, it was then searched for within the clinical trials database to determine whether or not it had reached phase III for a given disease. Only compounds which were yet to reach clinical phase III were included in the present review. As such, some highly promising compounds, such as nicotinamide, which has demonstrated efficacy in retinal vein occlusion and thyroid eye disease (and entered phase 3 trials) are beyond the scope of this review.

2. Nerve growth factor (NGF)

Nerve growth factor (NGF) was discovered by Rita Levi-Montalcini in the 1950s and is the best-characterized member of the neurotrophin family, which also includes brain derived neurotrophic factor (BDNF), neurotrophins 3 (NT-3), 4/5 (NT-4/5) and 6 (NT-6) [2]. NGF plays a key role in the maturation and development of the central nervous and peripheral systems. It acts directly on peripheral sensory and sympathetic neurons promoting survival, differentiation, functional activity and maintaining their phenotypes.

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Article highlights

- NGF treatment appears to offer beneficial neuroprotection in a range of diseases including glaucoma, ocular ischaemia, and retinitis pigmentosa, via mechanisms of promotion of cell survival
- Saffron seems to behave as a potent antioxidant in human trials, making it an interesting candidate for reducing conditions of oxidative stress in AMD
- CoQ10 has demonstrated a broad range of efficacy in neuroprotective applications, yet its applications in clinical trials have been limited, it is therefore hoped that it will be further investigated in the near future
- CNTF treatment may dramatically reduce patient discomfort associated with treatment of AMD by utilizing Encapsulated Cell Technology
- Encapsulated Cell Technology (namely NT-501) could improve treatment for a range of eye diseases and will hopefully become an established method of drug administration within a few years

This box summarizes key points contained in the article.

NGF exerts its action through two classes of transmembrane receptors, the high-affinity tropomyosin tyrosine kinase receptor (TrkA) and the low-affinity $p75^{NTR}$ [3].

NGF and its receptors are expressed in the anterior and posterior segments of the eye, in both physiological and pathological states, where they exercise different specific tissue functions. Changes in NGF expression and its receptors in these segments of the eye are correlated with the onset or severity of ocular pathologies in both animal models and patients.

The neuroprotective mechanisms of NGF modifying events occurring in retinal diseases, such as apoptosis, oxidative stress, synaptic plasticity, etc. remain unclear, though it is possible that this ability is associated with NGF's inhibition of oxidative stress, achieved via the phosphatidtlinositol-3-kinase (PI3K)/AKT survival pathway [4]. NGF also plays a key role in controlling intracellular calcium levels as it mediates the presynaptic uptake of calcium that stimulates the presynaptic release of the neurotransmitter, fundamental for synaptic plasticity [5].

The potential neuroprotective role of NGF has been shown in several studies, which have shown that the local administration of NGF on the eye affects not only the ocular surface but is also able to reach the optic nerve, retina, and brain [6,7]. NGF improved optical nerve survival and prevented retinal ganglion cell (RGC) functions loss, which are both present in glaucoma. The effects of topically administered NGF were studied in both animal models and patients with glaucoma. The neuroprotective action of NGF has been seen to be able to inhibit apoptosis of RGCs and degeneration of the optic nerve, as well as improve visual functionality. NGF promoted RGC recovery, reduced retinal cell damage, as well as protected from axon loss of the optic nerve resulting from damage caused by elevated intraocular pressure (EIOP) although, there is currently no evidence of NGF's action on the change of the IOP, suggesting other mechanisms of action [8,9]. In addition, long-term NGF treatment improved visual field up to 90 days after discontinuation of treatment, with the neuroprotective effects being noted to extend to promotion of axonal regeneration, and neuronal growth and plasticity [10]. Furthermore, other findings have shown that NGF acts on the mechanisms of RGC degeneration following ocular ischemia and retinitis pigmentosa [11,12]. The topical application of NGF twice daily for 3 weeks in a partial optic nerve transection (pONT) rat model showed neuroprotective effects on retinal neurons as it protected RGCs from secondary degenerative processes, which are among the major concerns in optic neuropathies. NGF administration reduced apoptosis of RGC *in vivo* and thus increased the RGC population in the lower retina, which is primarily affected by this disease. In addition, NGF inhibits astrocytic activity in the optic nerve and promotes axonal survival after pONT [13]. Visual acuity and electrofunctional parameter improvements were detected after topical treatment with NGF in an AMD patient after 3 months [14], and in five children with optic glioma and optic nerve atrophy [15]. NGF has been approved for treatment of patients with retinitis pigmentosa and stage II clinical trial has been completed in order to study the safety and tolerability of NGF to administer [16].

3. Saffron

Saffron is a spice commonly used in many cuisines, consisting of red dried stigmas of *Crocus sativus* L., a flowering plant that belongs to the family of Iridaceae [17]. Numerous reported therapeutic properties of saffron have encouraged its use in traditional medicine across a variety of clinical applications for a long time [18]. Among the 150 metabolites that saffron contains, two of particular therapeutic relevance are crocin and safranal [19]. Crocin is glycolysed to crocetin, a carotenoid dicarboxylic acid which possesses antioxidant activity through hydroxyl radical scavenging, being reported to result in antiaging effects [20,21].

Saffron also has a protective role against free radicals as it modulates enzymes such as superoxide dismutase, glutathione-*S*-transferase, and catalase, involved in oxidative stress [22]. The neuroprotective effects of saffron, studied in various pathologies, especially of the central nervous system, are attributable to crocin due to its antioxidant effect [23,24]. Also, the presence, among its metabolites, of many antioxidants (such as vitamin B12 and lycopene) and carotenoids [20], plays a key role in its neuroprotective action against oxidative stress, which is the cause of many neuronal disorders [25,26]. It is reported to have anti-depressive, and anti-hypertensive properties [27,28]. The therapeutic role of saffron is further enhanced by its antidiabetic abilities, anti-cancer, and anti-inflammatory properties afforded by its ability to regulate genes that release pro-inflammatory cytokines, the ability to modulate the NF-κB pathway, and to suppress cell death by blocking TNF-α (tumor necrosis factor) released by microglial cells [29–32]. Saffron is also able to inhibit the increased activity of caspase, Bcl-2 and Bax resulting from retinal damage [33]. It has been noted that saffron can protect damaged retinal photoreceptors following their exposure to continuous bright light, preventing their death and maintaining their functions and morphology [34].

The therapeutic potential of saffron has been demonstrated in patients with AMD, an age-related multifactorial disease of central retina, and the primary cause of blindness in developed countries. Falsini's Phase II study [35] showed

Table 1. Neuroprotective potential of saffron in retinal diseases.

Disease investigated	Mechanisms of action	Participants	Saffron concentration	References
Bilateral early AMD	Retinal flicker sensitivity improvements Macular function and vision improvements Reducing neurodegenerative processes	25	20 mg/day	[40]
Early/moderate AMD	Stabilize the function of the photoreceptors Prevent cell loss Improvement in amplitude and response in analysis with the fERG	100	20 mg/day	[37]

that short-term oral saffron administration (20 mg/day) for 90 days in patients with bilateral early AMD led to a significant improvement in flicker generated by photoreceptors and bipolar cells in focal electroretinogram (fERG), and in retinal function and vision, compared to patients treated with placebo. These results were then affirmed by a subsequent study in which it was observed that saffron administration induced an improvement in macular function from baseline extended to a long-term follow up (over a 15 month) [36]. This finding suggests that saffron may stabilize the function of the photoreceptors and prevent cell loss even in patients with early/moderate AMD, having also been shown to improve amplitude and response in fERG analysis (results summarized in Table 1) [37]. The antioxidant and neuroprotective potential of saffron has also been demonstrated by its ability to act directly on gene expression [38], showing efficacy in reducing neurodegenerative processes both in animal models and in humans. In a comparative study between two groups of patients with AMD, treated with two different protocols, it was shown that treatment with saffron appeared to be more effective than classical antioxidant protocol as it had reduced the activation of metalloproteins which lead to disorganization of the extracellular matrix in retinal damage. In addition, visual function remained stable in patients treated with saffron compared to those treated with AREDS (a mixture of antioxidants), implicating that the neuroprotective properties of saffron extend beyond that of an antioxidant [39].

4. CoenzymeQ

Coenzyme Q (also known as ubiquinone) is a quinone that performs an essential function within the inner mitochondrial membrane, forming a critical link in the electron transport chain in the process of oxidative phosphorylation [41]. Its role is so vital that it is conserved across all aerobic organisms, with only the length of the isoprene tail varying, which in humans is 10 units long (CoQ10) [42]. However, CoQ10 possesses utility beyond facilitation of oxidative phosphorylation, as it can transition between its fully oxidized state (ubiquinone), partially reduced free radical state (ubisemiquinone), and fully reduced state (ubiquinol). CoQ10 is thus an excellent antioxidant and free radical scavenger, thereby performing crucial functions to support not only mitochondrial health but the health of the cell as a whole [43].

Genetic CoQ10 deficiency manifests heterogeneously as a diverse range of symptoms, reflecting the universal importance of the molecule throughout the body [44], though many of these symptoms can be effectively treated with oral CoQ10 supplements [45]. Beyond cases of clinical CoQ10 deficiency, there is evidence to suggest that administration of CoQ10 may afford beneficial health effects. The potential health benefits of CoQ10 have been widely explored as a dietary supplement in areas of physical fitness [46], enhancing fertility [47,48], antiaging [49,50], diabetes [51,52], and heart failure [53]. The mechanisms of these therapeutic effects are understood to center around an enhancement of oxidative phosphorylation, and an increased capacity to deal with oxidative stress. Based on these promising applications of CoQ10, it is not surprising that attention has turned to utilizing it as a neuroprotectant [54], in which the enhancement of mitochondrial function, and antioxidant activity are particularly beneficial.

Diabetic retinopathy is the most common cause of blindness in adults of working-age worldwide [55]. Diabetic hyperglycemia damages pericytes, cells that regulate and protect capillaries. The subsequent retinal microaneurisms generate ischemic conditions and oxidative stress [56]. As this pathology develops, VEGF-driven neovascularization attempts, and fails, to reduce the ischemia. This proliferative neovascularization process can eventually result in sight-threatening complications, such as retinal detachment [57]. Furthermore, hyperglycemia causes glucose to be processed via the polyol pathway, consequently decreasing antioxidants, disturbing macrophage activity, and generating reactive oxygen species, which can result in the buildup of pathological conditions within the retina [58]. Based on the mitochondrial enhancing and antioxidant properties of CoQ10, there is a strong foundation to suggest its potential therapeutic efficacy within diabetic retinopathy.

Phase II double-blind, randomized, placebo-controlled clinical trials have been conducted investigating the potential therapeutic effects of ubiquinone when applied in non-proliferative diabetic retinopathy (NPDR) [58,59]. Patients were orally administered 400 mg of CoQ10 daily for 12 weeks to 6 months (in different studies; Table 2). Significant improvements to the fluidity of submitochondrial particles of platelets, membrane fluidity of erythrocytes, and ATP hydrolysis, were noted in patients that received CoQ10 compared to patients that received placebo treatment. These promising results may indicate that CoQ10 could delay the progression of NPDR to proliferative diabetic retinopathy, by improving blood supply and metabolism within the retina, thereby potentially alleviating ischemic conditions. Further clinical studies are warranted investigating the utility of CoQ10 in delaying the onset of proliferative diabetic retinopathy in NPDR patients.

Table 2. Dose regimen for CoQ10 trials.

Number of CoQ10-treated patients (controls)	Dose of CoQ10 (daily)	Duration of treatment	Reference
24 (25)	400 mg	12 weeks	[58]
40 (20)	400 mg	6 months	[59]

5. CNTF

Ciliary neurotrophic factor (CNTF) is a 22.9-kDa polypeptide, expressed in glial cells of the peripheral and central nervous systems [60]. It has been observed to promote survival in the broad range of neuronal cells which express the CNTF receptor [61], and encourage neurite outgrowth [62]. Notably, the action of exogenous CNTF binding to its receptor has been found to exert neuroprotective effects on photoreceptors [63,64] and RGCs [65–-65–68]. In fact, research suggests that following injury to the eye, the release of CNTF from retinal astrocytes is upregulated (indicated by western blot and immunohistochemistry) to facilitate neuronal repair [69–71]. It is thus unsurprising that CNTF has been one of the most popular neuroprotective candidates for the development of novel eye therapies.

CNTF has a promising track record for demonstrating neuroprotection in various *in vivo* models of retinal disease. In Li et al. (2010) [72], CNTF was intravitreally administered to a transgenic rat model of cone photoreceptor death (mimicking late-stage retinitis pigmentosa and AMD). This treatment reversed and prevented the loss of cone outer segments (an indication of the early stages of cone cell degeneration). Protection was seen to persist with the long-term release of CNTF from implanted microdevices, lending credence to potential human applications. Pease et al. (2009) [66], showed that in a rat model of laser-induced glaucoma, intravitreal injection of a viral vector for CNTF reduced RGC loss by 15%.

A total of nine clinical trials involving CNTF in eye disease are listed on the ClinicalTrials.gov database, predominantly having taken place in the 2010s; these are listed in Table 3. Rather than administer discrete doses, clinical trials have opted for implants which release CNTF over the course of months to years, with all of these utilizing Neurotech Pharmaceuticals' NT-501, a CNTF Encapsulated Cell Technology [73]. Many trials have terminated in phase I or phase II, which in a large proportion of cases likely reflects ongoing development and application of the NT-501 drug delivery technology.

Of particular note within the CNTF trials are the significant improvements found in a phase II double-blind randomized sham-controlled trial for dry AMD [74]. Zhang (2011) et al. demonstrated that patients who received a high dose of CNTF outperformed a lower dose group, which outperformed the sham group in tests of visual acuity after receiving treatment for 1 year. The authors noted that the slowing of visual acuity decline was most effective in patients that began the trial with better vision.

Following a successful phase I trial, a randomized sham-controlled patient-blinded study is currently underway, investigating the efficacy of CNTF in glaucoma. The endpoints of this trial are visual field assessment at 6 months, with visual acuity and contrast sensitivity, and optic nerve structure measured at 6, 12, and 24 months (NCT02862938). The study is estimated to complete in December 2020. In addition, a series of trials sponsored by Neuortech Pharmaceuticals (NCT00447993, NCT00447980, NCT01530659) have investigated the efficacy of using CNTF implants within retinitis pigmentosa patients. Most notably, a trial that was expected to end in 2019 (though results have yet to be published) investigated whether cone photoreceptor cells in the implant-treated eye were preserved by CNTF compared to the sham-treated-eye, which was assessed at 6-monthly intervals up to 36 months post-implant.

6. Conclusions

In this review, we described potential neuroprotective compounds, their mechanisms of action and protective effects on retinal neurons, to shed light on neuroprotective strategies which may one day be used in the clinic. All compounds discussed have of course already demonstrated neuroprotection *in vivo* in animals and have shown promise in early clinical trials. However, there is a clear hurdle in progressing neuroprotectants toward the clinic, with the typical pattern being that excellent *in vivo* efficacy cannot be replicated in human trials. While the research surrounding all four of the potential neuroprotective compounds discussed is promising, it does not guarantee that they will prove successful when applied to phase III trials and beyond.

7. Expert opinion

A number of potential neuroprotective molecules have progressed to ophthalmology clinical trials in the last few years,

Table 3. CNTF clinical trials: a summary of clinical trials involving CNTF.

Condition(s)	Phase	Participants	Status	NCT
Achromatopsia	I/II	5	Completed 2015	NCT01648452
AMD (dry)	II	51	Completed 2009	NCT00447954
Glaucoma	I	11	Completed 2014	NCT01408472
Glaucoma	II	54	Estimated completion: 2020	NCT02862938
Ischemic optic neuropathy	I	11	Completed 2014	NCT01411657
Retinitis pigmentosa	I	10	Completed 2006	NCT00063765
Retinitis pigmentosa (early stage)	II	68	Completed 2010	NCT00447980
Retinitis pigmentosa (early stage)	II	30	Completed 2019	NCT01530659
Retinitis pigmentosa (late stage)	II	65	Completed 2009	NCT00447993

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* [59] Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, et al. The antioxidant effect of ubiquinone and combined therapy on mitochondrial function in blood cells in non-proliferative diabetic retinopathy: A randomized, double-blind, phase IIa, placebo-controlled study. Redox Rep. 2016;21:190–195.
This study revealed a significant improvement in mitochondrial health following application of CoQ10 to human patients

* [72] Emerich DF, Thanos CG. NT-501: An ophthalmic implant of polymer-encapsulated ciliary neurotrophic factor-producing cells [Internet]. Curr. Opin. Mol. Ther. 2008. p. 506–515.
This paper details the development of the NT-501 implant, which hopefully will play a pivotal role in the application of neuroprotectants in the near future

** [73] Zhang K, Hopkins JJ, Heier JS, et al. Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. Proc Natl Acad Sci U S A. 2011;108:6241–6245.
CNTF administered by NT-501 implant presents a viable and effective method of treating dry AMD, for which there is currently no effective treatment

including the growth factors NGF and CNTF and natural neuroprotectants CoQ10 and Saffron. Until recently, the biggest hurdle in this area of research was the translation of results from basic research to patients. However, there has been increasing recognition of the need to use multiple experimental models and paradigms to test neuroprotective preclinical efficacy, resulting in more potential molecules reaching clinical phase trials. The mechanism of action of saffron and CoQ10 is targeting the mitochondria, with both CNTF and NGF showing anti-apoptotic effects.

The action of saffron allows it to play an important antioxidant-neuroprotective role against oxidative stress, which has been seen to play a key role in the progression of primary open-angle glaucoma (POAG) [75,76]. NGF has been observed to reliably promote not only survival but also the recovery of damaged RGCs. The clinical application of CoQ10 in a broad variety of pathologies, many of which being shown as therapeutic [77], should help to encourage its progression to a phase III clinical trial in NPDR. CNTF has shown excellent *in vivo* neuroprotective efficacy and has demonstrated successful therapeutic efficacy in phase II. Neurotech began two parallel phase III trials in 2017 using their NT-501 CNTF ECT (the same technology in their dry AMD trial) for macular telangiectasia (NCT03316300 & NCT03319849), with results expected in 2022. If these trials prove successful, they may pave the way for other NT-501-based treatments to progress to later clinical trials.

A major challenge now, however, is optimizing the delivery of adequate therapeutic levels of neuroprotective drugs to the target site, with poor solubility and unpredictable systemic absorption often associated with variable uptake. Furthermore, sustained delivery systems should improve individual management and issues surrounding compliance. Another key factor is trial design and choice of patient cohorts in these chronic diseases with a wide spectrum of manifestations. Proof-of-concept trials with enriched populations could aid development of therapies using surrogate endpoints to provide rapid results and build confidence in drug efficacy before embarking on expensive, large-scale multicenter trials. Progress in all these areas means that neuroprotective strategies have a much-improved chance nowadays of translational success.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Brann JH, Firestein SJ. A lifetime of neurogenesis in the olfactory system. Front Neurosci Front Res Foundation. 2014;8. DOI:10.3389/fnins.2014.00182
2. Levi-Montalcini R. The nerve growth factor: thirty-five years later. Biosci Rep. 1987;7(9):681–699.
3. Wang H, Wang R, Thrimawithana T, et al. The Nerve Growth Factor Signaling and Its Potential as Therapeutic Target for Glaucoma. Biomed Res. Int. Hindawi Publishing Corporation; 2014:759473.
4. Salinas M, Diaz R, Abraham NG, et al. Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. J Biol Chem. 2003;278(16):13898–13904. .
5. Jiang H, Takeda K, Lazarovici P, et al. Nerve growth factor (NGF)-induced calcium influx and intracellular calcium mobilization in 3T3 cells expressing NGF receptors. J Biol Chem. 1999;274(37):26209–26216. .
6. Lambiase A, Manteli F, Sacheti M, et al. Clinical applications of NGF in ocular diseases. Arch Ital Biol. 2011;149(2):283–292. .
7. Aloe L, Rocco ML, Bianchi P, et al. Nerve growth factor: from the early discoveries to the potential clinical use. J Transl Med. 2012;10:239;p. 1–15.
8. Ferrari MP, Mantelli F, Sacchetti M, et al. Safety and pharmacokinetics of escalating doses of human recombinant nerve growth factor eye drops in a double-masked, randomized clinical trial. BioDrugs 2014;28(3):275–283. .
9. Colafrancesco V, Parisi V, Sposato V, et al. Ocular application of nerve growth factor protects degenerating retinal ganglion cells in a rat model of glaucoma. J Glaucoma. 2011;20(2):100–108. .
10. Lambiase A, Aloe L, Centofanti M, et al. Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: implications for glaucoma. Proc Natl Acad Sci USA. 2009;106(32):13469–13474. .
11. Siliprandi R, Canella R, Carmignoto G. Nerve growth factor promotes functional recovery of retinal ganglion cells after ischemia. Invest Ophthalmol. 1993;34:3232–3245.
12. Lenzi L, Coassin M, Lambiase A, et al. Effect of exogenous administration of nerve growth factor in the retina of rats with inherited retinitis pigmentosa. Vision Res. 2005;45(12):1491–1500. .
13. Guo L, Davis BM, Ravindran N, et al. Topical recombinant human nerve growth factor (rh-NGF) is neuroprotective to retinal ganglion cells by targeting secondary degeneration. Sci Rep. 2020;10(1):3375. .
14. Lambiase A, Coassin M, Tirassa P, et al. Nerve growth factor eye drops improve visual acuity and electrofunctional activity in Age-related macular degeneration: a case report. Ann Ist Super Sanita.. 2009;45(4):439–442.
15. Falsini B, Chiaretti A, Barone G, et al. Topical nerve growth factor as a visual rescue strategy in pediatric optic gliomas: a pilot study including electrophysiology. Neurorehabil Neural Repair. 2011;25(6):512–520.

•• **This paper demonstrated the safe application of NGF, accompanied by a successful neuroprotective effect in pediatric optic glioma patients over a treatment course of 180 days, resulting in measured improvments to treated patients.**

[16] From: C gov. IDFS p. A 2014V A. Available from: https://clinicaltrials.gov/ct2/show/NCT02110225

17. José Bagur M, Alonso Salinas GL, Jiménez-Monreal AM, et al. Saffron: an old medicinal plant and a potential novel functional food. Molecules 2017;23(1):1–21.
18. Giaccio M. Crocetin from saffron: an active component of an ancient spice. Crit Rev Food Sci Nutr. 2004;44(3):155–172.
19. Rameshrad M, Razavi BM, Hosseinzadeh H. Saffron and its derivatives, crocin, crocetin and safranal: a patent review. Expert Opin Ther Pat. 2018;28(2):p. 147–165.
20. Assimopoulou AN, Sinakos Z, Papageorgiou VP. Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phyther Res. 2005;19:997–1000.

21. Heidari S, Mehri S, Shariaty V, et al. Preventive effects of crocin on neuronal damages induced by D-galactose through AGEs and oxidative stress in human neuroblastoma cells (SH-SY5Y). J Pharmacopuncture. 2018;21(1):18–25. .
22. Bukhari SI, Manzoor M, Dhar MK. A comprehensive review of the pharmacological potential of Crocus sativus and its bioactive apocarotenoids. Biomed Pharmacother. 2018;98:733–745.
23. Ahmad AS, Ansari MA, Ahmad M, et al. Neuroprotection by crocetin in a hemi-parkinsonian rat model. Pharmacology, Biochemistry, and Behavior. 2005;81(4):805–813. .
24. Nassiri-asl M, Hosseinzadeh H. Chapter 3 - Neuropharmacology Effects of Saffron (Crocus sativus) and Its Active Constituents. Bioact. Nutraceuticals Diet. Suppl. Neurol. Brain Dis. Elsevier Inc.. 2015. Academic Press
25. Teleanu, Chircov, Grumezescu, et al. Antioxidant Therapies for Neuroprotection-A Review. J Clin Med. 2019;8:1659.
26. Skladnev NV, Ganeshan V, Kim JY, et al. Widespread brain transcriptome alterations underlie the neuroprotective actions of dietary saffron. J Neurochem. 2016;139(5):858–871.
27. Christodoulou E, Kadoglou NP, Kostomitsopoulos N, et al. Saffron: a natural product with potential pharmaceutical applications. J Pharm Pharmacol. 2015;67(12):1634–1649. .
28. Ghaffari S, Saffron RN. An updated review on biological properties with special focus on cardiovascular effects. Biomed Pharmacother. 2019;109:p. 21–27.
29. Kianbakht S, Hajiaghaee R. Anti-hyperglycemic effects of saffron and its active constituents, crocin and safranal, in alloxan-induced diabetic rats. J Med Plants. 2011;10:82–89.
30. Poma A, Fontecchio G, Carlucci G, et al. Anti-inflammatory properties of drugs from saffron crocus. Antiinflamm Antiallergy Agents Med Chem. 2012;11(1):37–51. .
31. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of Crocus sativus L stigma and petal extracts in mice. BMC Pharmacol. 2002;2(2):7.
32. Soeda S, Ochiai T, Paopong L, et al. Crocin suppresses tumor necrosis factor-α-induced cell death of neuronally differentiated PC-12 cells. Life Sci. 2001;69(24):2887–2898. .
33. Yamauchi M, Tsuruma K, Imai S, et al. Crocetin prevents retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity. Eur J Pharmacol. 2011;650(1):110–119. .
34. Maccarone R, Di Marco S, Bisti S. Saffron supplement maintains morphology and function after exposure to damaging light in mammalian retina. Investig Ophthalmol Vis Sci. 2008;49 (3):1254–1261.
35. Falsini B, Piccardi M, Minnella A, et al. Influence of saffron supplementation on retinal flicker sensitivity in early age-related macular degeneration. Invest Ophthalmol Vis Sci. 2010;51(12):6118.
• Successful application of Saffrn in AMD patients, demonstrating improvements in electrophysiology
36. Piccardi M, Marangoni D, Minnella AM, et al. A longitudinal follow-up study of saffron supplementation in early age-related macular degeneration: sustained benefits to central retinal function. Evid Based Complement Altern Med. 2012;2012:429124.
37. Broadhead GK, Grigg JR, McCluskey P, et al. Saffron therapy for the treatment of mild/moderate age-related macular degeneration: a randomised clinical trial. Graefe's Archive for Clinical and Experimental Ophthalmology. 2001;69(1):31–40. .
38. Natoli R, Zhu Y, Valter K, et al. Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina. Mol Vis. 2010;16:1801–1822.
• Identification of gene regulation effects of orally administered saffron in rats, integral to its neuroprotective activity.
39. Di Marco S, Carnicelli V, Franceschini N, et al. Saffron: a multitask neuroprotective agent for retinal degenerative diseases. Antioxidants 2019;8(7):224. .
40. Falsini B, Piccardi M, Minnella A, et al. Influence of saffron supplementation on retinal flicker sensitivity in early age-related macular degeneration. Investig Ophthalmol Vis Sci. 2010;51(12):6118–6124.
41. Crane FL. Biochemical functions of coenzyme Q10. J Am Coll Nutr. 2001;20(6):591–598.
42. Ayer A, Macdonald P, CoQ SR. CoQ10 Function and role in heart failure and ischemic heart disease. Ann Rev Nutr. 2015;35 (1):175–213.
43. Sohal RS, Kamzalov S, Sumien N, et al. Effect of coenzyme Q10 intake on endogenous coenzyme Q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice. Free Radic Biol Med. 2006;40(3):480–487. .
44. Hernández-Camacho JD, Bernier M, López-Lluch G, et al. Coenzyme Q10 supplementation in aging and disease. Front Physiol Frontiers Media S A. 2018;9:p. 44.
45. Montini G, Malaventura C, Salviati L. Early coenzyme Q10 supplementation in primary coenzyme Q10 deficiency [Internet]. N Engl J Med - Massachussetts Medical Society. 2008;358(26): p. 2849–2850.
[46] Cooke M, Iosia M, Buford T, et al. Effects of acute and 14-day coenzyme Q10 supplementation on exercise performance in both trained and untrained individuals. 2008
47. Balercia G, Mancini A, Paggi F, et al. Coenzyme Q10 and male infertility. J Endocrinol Invest. 2009;32(7):p. 626–632. .
48. Ben-Meir A, Burstein E, Borrego-Alvarez A, et al. Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell. 2015;14(5):887–895. .
49. Elena Díaz-Casado M, Quiles JL, Barriocanal-Casado E, et al. The paradox of coenzyme Q10 in aging. Nutrients 2019;11(9):2221.
50. Janson M. Orthomolecular medicine: the therapeutic use of dietary supplements for anti-aging. [Internet] Clin Interv Aging Dove Press. 2006;1(3):p. 261–265.
51. Zahedi H, Eghtesadi S, Seifirad S, et al. Effects of CoQ10 supplementation on lipid profiles and glycemic control in patients with type 2 diabetes: a randomized, double blind, placebo-controlled trial. J Diabetes Metab Disord. 2014;13(1):1–8. .
52. Moradi M, Haghighatdoost F, Feizi A, et al. Effect of coenzyme Q10 supplementation on diabetes biomarkers: a systematic review and meta-analysis of randomized controlled clinical trials. Arch Iran Med. 2016;19:588–596.
53. Oleck S, Ventura HO. Coenzyme Q10 and utility in heart failure: just another supplement? Curr. Heart Fail Rep. 2016;13(4):p. 190–195.
54. Garrido-Maraver J, Cordero MD, Oropesa-Ávila M, et al. Coenzyme Q_{10} Therapy. Mol Syndromol. 2014;5(3–4):187–197. .
[55] Yau JWY, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. 2012
[56] Hammes H-P, Lin J, Renner O, et al. Pericytes and the pathogenesis of diabetic retinopathy.
57. Porta M, Bandello F. Diabetic retinopathy: a clinical update. Diabetologia 2002;p. 1617–1634.
58. Hernández-Ojeda J, Cardona-Muñoz EG, Román-Pintos LM, et al. The effect of ubiquinone in diabetic polyneuropathy: a randomized double-blind placebo-controlled study. J Diabetes Complications. 2012;26(4):352–358.
59. Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, et al. The antioxidant effect of ubiquinone and combined therapy on mitochondrial function in blood cells in non-proliferative diabetic retinopathy: a randomized, double-blind, phase IIa, placebo-controlled study. Redox Rep. 2016;21(4):190–195.
•This study revealed a significant improvement in mitochondrial health following application of CoQ10 to human patients.
60. Sleeman MW, Anderson KD, Lambert PD, et al. The ciliary neurotrophic factor and its receptor, CNTFRα. Pharm Acta Helv Pharm Acta Helv. 2000;74(2–3):p. 265–272. .
61. Wen R, Tao W, Li Y, et al. CNTF and retina. Prog Retin Eye Res Pergamon. 2012;31(2):p. 136–151.
62. Richardson PM. Ciliary neurotrophic factor: a review. Pharmacol Ther. 1994;63(2):187–198.
63. Tao W, Wen R, Goddard MB, et al. Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. Investig Ophthalmol Vis Sci. 2002;43:3292–3298.
64. Kassen SC, Thummel R, Campochiaro LA, et al. CNTF induces photoreceptor neuroprotection and Müller glial cell proliferation

through two different signaling pathways in the adult zebrafish retina. Exp Eye Res. 2009;88(6):1051–1064.
65. Ji JZ, Elyaman W, Yip HK, et al. CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. Eur J Neurosci. 2004;19 (2):265–272. .
66. Pease ME, Zack DJ, Berlinicke C, et al. Effect of cntf on retinal ganglion cell survival in experimental glaucoma. Investig Ophthalmol Vis Sci. 2009;50(5):2194–2200.
67. Müller A, Hauk TG, Leibinger M, et al. Exogenous CNTF stimulates axon regeneration of retinal ganglion cells partially via endogenous CNTF. Mol Cell Neurosci. 2009;41(2):233–246.
68. Parrilla-Reverter G, Agudo M, Sobrado-Calvo P, et al. Effects of different neurotrophic factors on the survival of retinal ganglion cells after a complete intraorbital nerve crush injury: a quantitative in vivo study. Exp Eye Res. 2009;89(1):32–41 .
69. Müller A, Hauk TG, Astrocyte-derived FD. CNTF switches mature RGCs to a regenerative state following inflammatory stimulation. Brain 2007;130(12):3308–3320.
70. Wen R, Song Y, Cheng T, et al. Injury-induced upregulation of bFGF and CNTF mRNAs in the rat retina. J Neurosci. 1995;15 (11):7377–7385.
71. Cao W, Wen R, Li F, et al. Mechanical injury increases bFGF and CNTF mRNA expression in the mouse retina. Exp Eye Res. 1997;65 (2):241–248.
72. Li Y, Tao W, Luo L, et al. CNTF induces regeneration of cone outer segments in a rat model of retinal degeneration. Li X-J, editor. PLoS One. 2010;5:e9495
•This paper details the development of the NT-501 implant, which hopefully will play a pivotal role in the application of neuroprotectants in the near future.
73. Emerich DF, Thanos CG. NT-501: An ophthalmic implant of polymer-encapsulated ciliary neurotrophic factor-producing cells [Internet]. Curr. Opin. Mol. Ther. 2008. p. 506–515
••CNTF administered by NT-501 implant presents a viable and effective method of treating dry AMD, for which there is currently no effective treatment.
74. Zhang K, Hopkins JJ, Heier JS, et al. Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. Proc Natl Acad Sci USA. 2011;108(15):6241–6245.
75. Majsterek I, Malinowska K, Stanczyk M, et al. Evaluation of oxidative stress markers in pathogenesis of primary open-angle glaucoma. Exp Mol Pathol. 2011;90(2):231–237.
76. Bagnis ASSIA. The role of oxidative stress in pre-eclampsia. Pre-Eclampsia Etiol Clin Pract. 2007;612:121–137.
77. Akbari A, Mobini GR, Agah S, et al. Coenzyme Q10 supplementation and oxidative stress parameters: a systematic review and meta-analysis of clinical trials [Internet]. Eur J Clin Pharmacol Springer. 2020;76(11):1483-1499.