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REVIEW

Topical administration of adrenergic receptor pharmaceutics and nerve growth factor

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Abstract: Topical application of nerve growth factor (NGF) and adrenergic receptor pharmaceutics are currently in use for corneal ulcers and glaucoma. A recent interest in the neuroprotective abilities of NGF has led to a renewed interest in NGF as a therapeutic for retinal and choroidal diseases. NGF can promote cell proliferation through actions of the TrkA receptor or promote apoptosis through receptor $p75^{NTR}$. This understanding has led to novel interest in the role of NGF for diseases of the posterior eye. The role of β-adrenergic receptor agonists and antagonists for treatments of glaucoma, diabetic retinopathy, and their potential mechanisms of action, are still under investigation. This review discusses the current knowledge and applications of topical NGF and adrenergic receptor drugs for ocular disease.

Keywords: NGF, β-adrenergic receptor agents, α-adrenergic receptor agents, retina, cornea, glaucoma

Introduction

Nerve growth factor

Nerve growth factor (NGF) was the first discovered member of a family of factors noted to prevent neuronal death called the neurotrophins.[1] Levi-Montalcini found that NGF was produced in significant levels in mouse salivary glands, and it could prevent apoptosis of neurons in culture.[2] NGF and one of its receptors, TrkA, are predominantly found on sympathetic neurons.[3] During development, NGF is the factor that regulates neuronal remodeling to establish the precise connections of neuron to target. The target produces NGF that is taken up by the nerve terminal and transported retrogradely to the cell body. The neuron becomes anti-apoptotic after NGF has reached the cell body.[4] In the establishment of target-neuron connections, sensory and sympathetic nerves often will compete for available NGF.[5]

NGF actions are mediated by two receptors, $p75^{NTR}$ and neurotrophic tyrosine kinase receptor type 1 (TrkA). NGF is produced as a proneurotrophin (pro-NGF), which preferentially binds to $p75^{NTR}$. Activation of $p75^{NTR}$ can activate three major signaling pathways, including NFκB, c-Jun, and RhoA.[6] $p75^{NTR}$ is a low-affinity receptor for NGF and is a member of the tumor necrosis factor superfamily. Due to the activation of the c-Jun and RhoA, NGF binding to $p75^{NTR}$ is considered to be a predominantly pro-apoptotic event. In contrast to the binding of NGF to $p75^{NTR}$, NGF binds to TrkA with high affinity. Phosphorylation of tyrosine residues on TrkA creates binding sites for a variety of proteins. The major pathways activated by Trk receptors are Ras, Rac, PI3K, PLC-γ1.[7] We have previously shown that NGF promotes choroidal endothelial

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cell migration through PI3K signaling.[8] Activation of Ras and downstream effectors promotes choroidal cell proliferation.[8] In addition, work on CD34+ cells has demonstrated that NGF treatment will increase both migration and tube formation (key factors needed for wound healing) via phosphorylation of TrkA and activation of PI3K and Ras (Jadhao et al unpublished results). Thus, increased NGF binding to TrkA will promote a neuroprotective effect and should promote effective wound healing.

Sympathetic neurotransmitters

While NGF is required for the health of sympathetic neurons, norepinephrine signaling may also play a role in neuroprotection and wound healing. Norepinephrine signals through activation of either β-adrenergic receptors or α-adrenergic receptors. Within the eye, the presence of norepinephrine and noradrenergic receptors has been noted around the blood vessels of the choroid and iris.[9] More recently, work has shown that β2-adrenergic receptors are present in the corneal endothelial cells.[10,11] The presence and role of adrenergic receptors in the retina is a bit more controversial. Laties and Jacobowitz did not observe sympathetic innervation in the retina by immunohistochemical analysis.[12] However, Furukawa, using immunohistochemical methods as well as transmission and scanning electron microscopy, demonstrated that the retina receives sympathetic innervation.[13] Since then, others have demonstrated the presence of dopamine-β-hydroxylase, the enzyme that converts dopamine to norepinephrine, in specific layers of the retina.[14,15] Activation of β-adrenergic receptors in the retina stimulates the accumulation of cyclic adenosine monophosphate (cAMP).[16] We have shown that surgical removal of the superior cervical ganglion, which eliminates sympathetic neurotransmission to all cranial targets, produces increased basement membrane thickening and pericyte loss in the retinal vasculature and leads to the activation of Müller cells (glial cells of the retina).[17,18]

The functional role of α-adrenergic receptors and β-adrenergic receptors in the eye are target-specific. Within the choroid and iris, α-adrenergic receptors mediate vasoconstriction, while β-adrenergic receptor stimulation produces vasodilation of the vasculature.[19,20] A key role for both α-adrenergic receptor and β-adrenergic receptor pharmacology in the eye is in the management of glaucoma. For the management of glaucoma, agents can be used to either decrease the production of aqueous humor (β-adrenergic receptor antagonists or α-adrenergic receptor agonists) or increase outflow of aqueous humor (prostaglandins).[21–23] The functional role for adrenergic receptor signaling in the retina is not clear.

The signaling of adrenergic receptors has been known for several decades. Stimulation of β-adrenergic receptors by norepinephrine induces an increase in cAMP levels and phosphorylation of protein kinase A (PKA). PKA is then able to transduce into the nucleus of the cell to bind cAMP-responsive element binding (CREB) complexes to induce transcription of a number of proteins. In contrast to β-adrenergic receptors, which signal predominantly through G-protein stimulatory (Gs), α-adrenergic receptors activate Gq, producing an increase in phospholipase C and calcium signaling. The ability of adrenergic receptors to regulate aqueous humor production and blood flow in the eye is directly related to which receptor subtype is activated. Based on the increased understanding of adrenergic receptor signaling, a large number of pharmaceutics have been developed for the eye. Fortunately, the eye allows for topical delivery, which eliminates problems associated with systemic effects of adrenergic receptors on the vasculature of other critical organs (heart, lungs).

Based on our understanding of the cellular localization, functions, and signaling of both NGF and the neurotransmitters of the sympathetic nervous system, pharmaceutical companies have developed topical delivery methods for both NGF and adrenergic receptor agents for a number of disabling ocular disorders.

Cornea

The highest interest in topical NGF therapy is for corneal disorders. A number of studies have been done in both humans and in animal models to investigate the ability of topical NGF to accelerate the healing process in cornea ulcers. Investigations in dogs demonstrated that NGF and TrkA are present in normal dogs tears, corneal epithelium, and the lacrimal gland.[24] In dogs, topical treatment with NGF did not modulate corneal healing rates.[24] In contrast to the work in dogs, Esquenazi et al (2005) found that topical NGF to rabbits in combination with docosahexenoic acid (DHA) after photorefractive keratectomy (PRK) was associated with increased corneal epithelial cell proliferation and increased corneal surface area, in contrast to no treatment, NGF treatment alone, or DHA treatment alone.[25]

While topical NGF has been met with some obstacles for corneal healing in animal models of ocular disease, work on humans with corneal disorders has provided much more promising results. Lambiase et al (2007) reported that corneal ulcers produced from a variety of insults (diabetes mellitus, radiotherapy, trauma, etc), which were unresponsive

to traditional therapies for neurotrophic ketatopathy, were treated with topical murine NGF (200 ug/ml) for two hours for the first two days, followed by one drop six times daily until the ulcer healed.[26] Remarkable results were obtained in these patients; all patients has resolution of the corneal ulcer after 26 ± 11 days of NGF treatment. Limited side effects were observed and only two patients had a relapse of the ulcer during follow-up, which occurred over 72 months.[26] In a recent review by Aloe et al (2008), data on over 200 patients treated with topical NGF was presented which clearly demonstrates that NGF treatment promotes an accelerated healing action in the cornea without side effects.[27]

The role of sympathetic nerves and neurotransmission has also been investigated for their ability to resolve corneal ulcers. Work in rats undergoing surgical superior cervical ganglionectomy revealed that ocular sympathetic nerves stimulate corneal epithelial cell proliferation under both normal conditions and during times of corneal wounding. This increase in proliferation appears to be related to release of norepinephrine.[28] Since work in animal models suggested that adrenergic receptor agents may promote corneal wound healing and the cornea possesses an abundance of β-adrenergic receptors in both the epithelium and endothelium of the cornea,[29] work had been done to evaluate the ability of β2-adrenergic receptor antagonism to regulate corneal wound healing. In fact, β2-adrenergic receptor antagonism increased corneal wound healing by 16%.[11,30] While the mechanism is not clear since norepinephrine is suggested to increase epithelial cell proliferation in animals, work in humans suggests that a blockade of β2-adrenergic receptors in the cornea with topical agents may provide a novel therapeutic option for corneal ulcers.

Glaucoma/aqueous humor production/outflow

Glaucoma remains one of the most common and damaging ocular diseases. The pharmacological management of glaucoma has increased immensely in the last several decades, improved by the increased understanding of the ocular hemo/aqueous humor dynamics. NGF has been reported to reduce retinal ganglion cell loss, which, left unchecked, will lead to loss of retinal functions. Elevated intraocular pressure (as occurs in glaucoma) in rats produced a significant loss of NGF, which was correctable through topical NGF treatment.[31] To induce glaucoma in a rat model, hypertonic saline is injected into the eye, raising intraocular pressure (IOP) to 35 mmHg as compared to 24 mmHg in the untreated eye.[32] In the rat model of glaucoma, topical NGF (200 ug/ml) applied four times daily for seven weeks prevented retinal ganglion cell death likely through an increase in the Bcl-2/Bax ratio.[32] In the same study, three human patients with severe glaucoma were treated with topical NGF for three months. NGF treatment improved the functional responsiveness of the retina, as measured by PERG P50 and VER P100 values, with better neural conduction. These positive responses were maintained for an additional three months with no treatment. This positive response to NGF treatment likely occurs as TrkA phosphorylation increases Bcl-2 levels, a key anti-apoptotic protein.

The standard of care for many glaucoma patients are adrenergic receptor agents. This has occurred since work in monkeys demonstrated with timolol (a typical nonselective β-adrenergic receptor antagonist) reduced aqueous humor levels by 50%.[33] These results suggest that antagonism of β-adrenergic receptors reduced aqueous humor levels, producing a decrease in intraocular pressure. α-adrenergic receptor agents act in much the same way as β-adrenergic receptor antagonists to reduce aqueous humor levels in the eye.

Choroid

Little work has been done on topical NGF in the choroid. We have previously demonstrated that NGF treatment of human choroidal endothelial cells in culture promoted cell proliferation and migration through the PI3K and ERK1/2 pathway.[8]

In contrast to the limited information on NGF in the choroid, much work has been done on adrenergic receptor agents in the choroid, likely due to the predominant vasculature of the ciliary arteries of the choroid. We have demonstrated that stimulation of α-adrenergic receptor agonists on the vortex vein region of the choroid produced vasoconstriction.[20] Work in the rabbit has shown that neither betaxolol nor timolol had a significant effect on choroidal pressure-flow relationships, but that both do reach the systemic circulation.[34] Work on the posterior ciliary microarteries (many of them flowing through the choroid) demonstrated that these particular vessels do not have appreciable β-adrenergic receptor activity. However, betaxolol and propranolol likely produce vasorelaxation through blockade of Ca^{2+} channels, rather than true β-adrenergic receptor activities.[35] These results suggest that adrenergic receptor agents, particularly β-adrenergic receptor antagonists, may be used to influence choroidal blood flow.

Retina

Much of the work on NGF in the retina is focused on its potential to protect retinal ganglion cells and the optic nerve head in diseases, such as diabetes, carotid artery occlusion, and glaucoma. Topical conjunctival administration of NGF

(200 ug/ml) prevented cell death of retinal ganglion cells induced by hypertension or ischemia in rats.[36] NGF may reach the retina as a topical treatment since the final protein product NGF is only approximately 13–15 kD. This size of protein may be naturally transported into the retina with ocular fluids. However, the exact mechanism of NGF movement into the retina has not been investigated. Using the type I diabetic rat model, Ali et al (2008) demonstrated that peroxynitrite produced retinal degeneration through inhibition of NGF prosurvival activities.[37] The increased glucose in diabetes produces free radical and peroxynitrite in the retina. These authors found that increased peroxynitrite led to nitration of the TrkA receptor and enhanced $p75^{NTR}$ expression, producing increased apoptosis.[37] Similarly, work on a carotid artery occlusion model in rats demonstrated that an intravitreal injection of NGF protected retinal ganglion cells through modulation of the Bax/Bcl-2 ratio to promote the prosurvival actions of NGF.[38] While NGF does promote neuronal survival, some negative effects have also been reported. Using the oxygen-induced retinopathy model of proliferative diabetic retinopathy, Liu et al (2010) found that NGF promoted retinal neovascularization, which was mediated through activities of the TrkA receptor.[39] Since retinal neovascularization is a negative consequence in most cases, NGF actions in this setting are not positive. Nonetheless, work has increased on the role of NGF to prevent retinal neuronal apoptosis. Since this is likely dependent on which NGF receptor is activated (TrkA over $p75^{NTR}$), additional work is needed. All current work on NGF actions in the retina have been done following intraocular injections. The ability of NGF to reach the retina as a topical treatment is still under investigation.

The role of topical adrenergic receptor agents in the retina has been previously focused on blood flow regulation. Work in humans has demonstrated that epinephrine treatment increased macular blood flow by 8%.[40] Work using different classes of β-adrenergic receptor antagonists showed that all classes increased blood velocities in retinal and epiretinal capillaries of healthy human volunteers.[41] While β-adrenergic receptor antagonists increase retinal blood flow, work has also suggested that this may be due primarily to the reduction in intraocular pressure and Ca^{2+} channel actions, rather than a direct action on the β-adrenergic receptors.[42]

The role of β-adrenergic receptors in the regulation of glaucoma is not clear. One of the mechanisms of action for betaxolol, one of the most potent β-adrenergic receptor antagonists for glaucoma, is its ability to alter calcium levels.[43,44] In a recent review, Osborne reported that the beneficial effects of β-adrenergic receptor blockers in glaucoma therapy do not appear to involve their actions on β-adrenergic receptors.[45] These findings suggest that the role of β-adrenergic receptor antagonists as neuroprotective drugs for glaucoma therapy may be related to non-β-adrenergic receptor actions of these agents. In diabetic retinopathy, two studies have shown that β-adrenergic receptor antagonists do not reduce diabetic retinopathy, in spite of reduced systemic blood pressure.[46,47] Therefore, previous work on β-adrenergic receptor antagonists in the retina suggests that they do not prevent diabetic retinopathy in rodents. Nonetheless, the downstream effects of β-adrenergic receptor blockade mediated by receptor signaling in the retina are not known. Work in our lab has recently reported that blockade of β-adrenergic receptor signaling in the normal retina is detrimental, as evidenced by the reduced b-wave amplitude of the electroretinogram (ERG). This agrees with previous work in our lab, which showed positive effects on the retina following application of β-adrenergic receptor agonists in reducing inflammatory marker levels.[48–51] While we have data that systemic application of β-adrenergic receptor antagonists produce retinal dysfunction, we have also recently found that β-adrenergic receptor agonists are beneficial to the retina. Using the type I diabetic rat model, we demonstrated that topical isoproterenol (50 mM given once daily) inhibited the functional, biochemical, and histological manifestations of diabetic retinopathy. Isoproterenol inhibited the formation of degenerate capillaries, prevented apoptosis of cells in the ganglion cell layer, and decreased tumor necrosis factor (TNF) α levels over the 8-month study (Jiang et al in press).

Conclusions

Topical delivery of NGF and adrenergic receptor agents has provided physicians with new options for pharmacological management of glaucoma and corneal ulcers. Work is ongoing for the role of topical NGF or adrenergic receptor agents for the posterior eye, including the choroid and retina. Recent work is investigating a potential role for NGF in therapies for diabetic retinopathy through direct actions or through actions using stem cells for prevention of retinal ischemia ($CD34^+$ positive cells). Topical adrenergic receptor therapies for the retina in diabetic retinopathy are ongoing, as well as increasing the understanding of the mechanisms of β-adrenergic receptor antagonist effectiveness in ganglion cell health in glaucoma. Hopefully in the future, work on topical NGF and adrenergic receptor agents in the retina can be translated to human patients as easily as it has for corneal ulcers and glaucoma therapy.

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Disclosure

The author reports no conflicts of interest in this work.

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