Estrogens and neuroprotection in retinal diseases

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Estrogens play a critical role in the normal growth and development of humans and in recent years our understanding of their effects in the central nervous system (CNS) have been advancing rapidly. It is now known that estrogens influence synaptic plasticity, brain development, and memory. In addition, estrogens have been shown to be neuroprotective in degenerative disorders. The understanding of the influences of estrogens in the retina, as a component of the CNS, has not kept pace with the advances in understanding of the brain. Studies that have addressed the effects of estrogens on the retina, specifically those focusing on glaucoma, are examined here in the hope that estrogen therapy may be a viable option for treating retinal dystrophies and optic neuropathies.

Glaucotamatos visual field deterioration is a neurodegenerative process commonly associated with an insidious increase in intraocular pressure (IOP), with characteristic optic disc and retinal ganglion cell (RGC) nerve fiber layer damage [1,2]. World Health Organization statistics indicate that glaucoma accounts for blindness in at least 5 million people, causing 13.5% of worldwide blindness [3]. In the United States, glaucoma is the second leading cause of blindness and the second most frequent reason for ambulatory visits to physicians by U.S. Medicare beneficiaries [4]. Extrapolating to the entire Medicare population, blindness and vision loss are associated with greater than $2 billion in non-ophthalmologic related costs. The staggering healthcare and economic burdens make vision loss a medical imperative [5].

In recent years, there has been compelling evidence to support the use of estrogens as neuroprotectants [6-13]. However, the Women’s Health Initiative Study concluded that conjugated equine estrogens and estrogen plus medroxyprogesterone acetate increase the risk for ischemic stroke in generally healthy postmenopausal women [14,15]. Additional findings indicate that low doses of 17β-estradiol may protect against ischemia and reperfusion injury [16,17]. A correlation between glaucoma and ophthalmic artery blood flow changes with hormone therapy (HT), female reproductive factors, as well as HT and IOP clearly illustrate that estrogens influence the health of the retina [18-22]. Nonetheless, the use of estrogens as neuroprotectants is complicated by the natural feminizing sequelae that would be undesired in male patients, and therefore it becomes important to investigate use of synthetic non-feminizing estrogen analogs. With an emphasis on glaucoma as the primary retinal neurodegenerative disease, this review examines whether estrogens can be effective neuroprotectants in the retina.

Estrogen receptor expression in the retina: Estrogen receptors α and β (ERα and ERβ) are involved in classic receptor-mediated genomic pathways as well as alternative mechanisms of orchestrating neuroprotection [12,13,23]. Bovine and rat retinas have been shown to express both receptor isoforms (ERα predominates in rat) throughout the retinal thickness, seen in greatest density within the ganglion cell layer, the inner nuclear layer, and the outer portion of the outer nuclear layer [24,25]. Human donor eyes from premenopausal women have been found to express ERα in the neurosensory retina and retinal pigment epithelium, but receptors have not been detected in tissues dissected from men and postmenopausal women [26]. Another study of ER expression in humans showed that ERβ protein was localized to the ganglion cell layer and choroid. However, at the transcriprional level both ERα and ERβ were observed with local differences in expression, suggesting variability in the ratio of ERα:ERβ [27]. ERs have also been detected in the neuroendocrine secretory and metabolic ciliary epithelium. 17β-hydroxysteroid dehydrogenases (17HSDs), involved in the biosynthesis and inactivation for sex steroids, were shown to be under direct paracrine influence of 17β-estradiol, evidence of estrogen modulating its own fate within the eye [28]. The presence of estrogen receptors and metabolic machinery within the eye suggest that estrogens play more than a passive role in the retina.

Retinal estrogenic effects: Given the intimate association between estrogens, their receptors, and their metabolism in the eye, is it possible that estrogens could have deleterious effects similar to those seen with HT in the Women’s Health Initiative Study? Rather than producing hypercoagulability, it appears that HT in older women does not have a long-term vasodilatory effect on retinal arterioles. These studies were based on the coronary vessel and incidence of myocardial
infarction studies in fertile women, and does not support the hypothesis that exogenous estrogen exposure accounts for greater observed retinal arteriolar diameters in women [29]. Curiously, blood viscosity is decreased and ocular vascularity is improved in women with glaucoma who receive HT [19]. Neither of these studies clearly indicate what role, if any, HT plays in glaucoma. Is it possible then that gender differences or exposure to endogenous feminizing hormones could affect IOP and the development of glaucoma? This question was the basis for investigations that examined the effect of HT on IOP in a single case study and three large international studies examining female reproductive factors as risks for the development of open angle glaucoma (OAG). An early case study examined IOP changes with initiation of HT through 12 weeks of treatment. After the initiation of HT treatment of one glaucoma patient who had menopausal symptoms, it was found IOP was reduced by an average of 4.5 mmHg at four weeks and by an average of 4 mmHg at 12 weeks [20]. In a larger cross-sectional controlled study, 107 women receiving HT and 107 women serving as controls underwent IOP assessment and cup-to-disc ratio assessment. In addition, they completed comprehensive medical and family history questionnaires. The two groups were found to not differ in mean IOP, cup-to-disc ratios, prevalence of elevated IOP, or prevalence of glaucoma. A personal history of ischemic heart disease was the only clear risk factor for increased IOP [30]. The Rotterdam Study published findings on the association between early menopause and the development of OAG. This population-based study concluded that early menopause (≤45 years of age), age-adjusted and controlled for HT use, was a significant risk factor for the development of OAG [22]. The Blue Mountain Eye Study, which examined the association between endogenous estrogen exposure and OAG, determined that early menarche (age ≤ 12 years) and increasing parity significantly increased the risk for OAG [22]. A similar study conducted in rural southern India found no connection between female reproductive factors and OAG [21]. This disparate find may be explained by significantly greater phytoestrogen content in the predominantly vegetarian south Indian diet, possibly masking what may otherwise have been an association between female reproductive factors and OAG. This theory was supported by a study that examined retinal thickness in male and female rats whose diet was supplemented with soy phytoestrogens. Male rats fed a phytoestrogen-fortified diet showed an increase in retinal thickness, while female rats showed a decrease in retinal thickness compared to control diet-fed animals [33]. These studies equivocally suggest that HT and length of endogenous estrogen exposure may influence the development of OAG.

Although not directly relevant to retina, the risk of cardiovascular disease (CVD) in postmenopausal women receiving estrogen and progesterone has been proposed to be due to increased levels of pro-inflammatory cytokine TNF-α [31]. However, estrogen can also lower markers of vascular inflammation (IL-6 secretion), and thus render protective effects of lower doses of 17β-estradiol in combination with trivalent chromium therapy in diabetic monocytes [32].

**Estrogens as neuroprotectants:** Estrogen receptor independent and dependent neuroprotective mechanisms have been well established in the brain [7,12,13,34,35], but similar studies in the retina either are limited or have not been conducted. Those studies that demonstrate neuroprotective efficacy in models of retinal disease are examined here.

One of the pioneering studies in estrogen-mediated retinal neuroprotection examined the efficacy of a synthetic estrogen analog in an in vivo model of retinitis pigmentosa (RP) and in an in vitro model of N-methyl-D-aspartate (NMDA)-induced excitotoxic glaucomatous RGC death. In the RP model, treatment with an estrogen analog at postnatal day 9 yielded an outer nuclear layer, containing the photoreceptors cells lost in RP, that was nearly twice the thickness in untreated controls [36]. In the glaucoma model, primary RGCs treated with the same estrogen analog were protected from NMDA-induced excitotoxic death [36]. Furthermore, the in vitro study demonstrated maintenance of mitochondrial stability and inhibition of lipid peroxidation. These findings were in accord with previous reports from brain parenchymal studies, offering a novel approach to neuroprotection in degenerative diseases of the retina [36].

Another study examined protection of cultured retinal pigment epithelial cells (RPE), the pathologic target in age-related macular degeneration (AMD) vision loss, by 17β-estradiol against hydrogen peroxide-induced cell death. This study not only demonstrated significant protection of RPE cells, but also showed that 17β-estradiol quenched hydrogen peroxide-induced upregulation of apoptosis-related proteins [37]. The large cross-sectional Salisbury Eye Evaluation Project supported these in vitro findings. This study evaluated the effects of HT and female reproductive factors on AMD, showing that current HT was associated with lower odds of large drusen predictive of advanced AMD [38]. Together, these studies suggest that estrogen treatment may be effective in AMD, in addition to RP and glaucoma as described in the previous section.

Oxidative stress, induced by ischemia-reperfusion or other perturbations, is a ubiquitous pathway of degenerative vision loss. The effects of 17β-estradiol on leukocyte accumulation have been evaluated during ischemia–reperfusion injury and retinal damage after transient retinal ischemia. Treatment with 17β-estradiol significantly inhibited leukocyte accumulation and subsequently improved retinal function as assessed by electroretinogram [39]. Another ischemic retinal study showed that 17β-estradiol protected RGCs from early changes in synaptic connections that are associated with ischemia preceding apoptosis and ischemia-induced global apoptosis [40].

These studies created the foundation for the field and led to the examination of estrogen-mediated neuroprotective
mechanisms. Estrogens are classically known to effect transcriptional modulation via receptor-mediated genomic pathways, but they may also alter intracellular signaling cascades [35]. One study approached this subject by examining estrogen-mediated retinal neuroprotection in an in vitro hydrogen peroxide-induced model of retinal neurodegeneration along with an in vivo model of light-induced photoreceptor degeneration. Both 17β-estradiol and 17α-estradiol protected retinal neurons in vitro, with 17β-estradiol activating the phosphoinositide 3-kinase (PI3K) pathway, transiently increasing phospho-Akt levels. The estrogen receptor antagonist tamoxifen did not reverse the protective effect of 17β-estradiol, but inhibition of the insulin receptor beta blocked the PI3K-mediated protective effects. This finding suggested that neuroprotection with 17β-estradiol may be independent of its receptors, but dependent on the PI3K-signaling pathway that is known to promote neuronal survival. Systemic administration of 17β-estradiol, in the in vivo arm of the study, demonstrated activation of insulin receptor beta as well, with a transient increase in PI3K activity and phosphorylation of Akt, protecting rat photoreceptor cells [41].

Similarly, two recent studies addressed the neuroprotective effect of 17β-estradiol via extracellular signal-regulated kinase (ERK) pathway induction. In the first, retinas were harvested from female rats, both those that had received oophorectomy and those that had not, to determine the effect of optic nerve transection on RGC survival. It was observed that RGC survival was significantly decreased in rats that had received oophorectomy, but RGC loss was reduced with intravitreal 17β-estradiol injection [42]. Protection was mediated via the ERK signal transduction pathway. Interestingly, ERK inhibitor U0126 inhibited the neuroprotective effect observed [42]. In the second study, the neuroprotective effect of 17β-estradiol against NMDA-induced retinal neurotoxicity was examined. In that study, retinal pretreatment with 17β-estradiol silastic implants attenuated RGC death due to intravitreal injection of NMDA [43]. However, co-administration of U0126 or estrogen receptor antagonist 13-methyl-7-[9-(4,4,5,5,5-pentafluoropentylsulfanyl)monyl]-7,8,9,11,12,13,14,15,16,1 7-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (ICI) 182,780 with NMDA completely abolished the protective effect of 17β-estradiol. Moreover, NMDA treatment alone significantly increased the levels of phosphorylated ERK (p-ERK) that increased further with 17β-estradiol pretreatment. 17β-estradiol-induced increases in p-ERK levels were attenuated with administration of U0126 and ICI 182,780 [43].

Our own work has shown that 17β-estradiol and the synthetic estrone-derived non-feminizing estrogen analog 2-(1-adamantyl)-3-hydroxyxyestra-1,3,5(10)-triene-17-one (ZYC)-3 are effective neuroprotectants against glutamate-induced cytotoxicity of RGC-5 rat RGCs [25]. 17β-estradiol and ZYC-3 afforded complete protection against glutamate-induced RGC-5 cytotoxicity. As opposed to 17β-estradiol, ZYC-3 had no appreciable affinity for either estrogen receptor isoform, but was nearly 20-fold more effective at inhibiting lipid peroxidation [25]. This study also demonstrated that ZYC-3 pretreatment of glutamate-insulted RGC-5 cells elicited activation of a host of anti-apoptotic signal transduction pathways.

Signal transduction pathways that are known to affect the survival of stressed neurons were examined in RGC-5 cells treated with glutamate. Phosphorylated-Akt (P-Akt), also known as PKB or Rac, plays a critical role in the balance between survival and apoptosis and is activated by a host of survival factors [44-46]. The p42 (Erk1) and p44 (Erk2) MAP kinase proteins (activated by phosphorylation) also play critical roles in growth and differentiation regulated by a host of factors including neurotransmitters and neurotrophins [47-50]. Activation of phosphorylation cascades in these pathways has been shown to promote survival of RGCs in a variety of models of RGC injury and protection [51-54]. The p90 S6 ribosomal kinase family of proteins responds to numerous growth factors and is activated by phosphorylation downstream of Erk1 and Erk2 [55,56]. Additionally, phosphorylation of p53 was increased with ZYC-3 pretreatment. Phosphorylated activation of tumor suppressor protein p53 plays a major role in response to DNA damage by arresting the cell cycle and initiating DNA repair or apoptosis [57-59]. This may indicate cell cycle arrest for DNA repair to promote cell survival. The pro-apoptotic p38 MAP kinase pathway serves as a gateway to cytokine and stress responses, with activation via phosphorylation leading to cell death [60-63]. In models of axotomy-induced apoptosis, phosphorylation of p38 peaked at one day postaxotomy, inhibition of p38 phosphorylation attenuated RGC loss, and selective inhibition of NMDA receptors showed dose-dependent attenuation of p38 activation, resulting in protection of RGCs [64,65].

These studies demonstrate that natural estrogens and synthetic analogs are effective neuroprotectants, an outcome classically attributed to receptor-mediated gene expression. These effects may also be due to receptor-independent activation of signal transduction pathways. While estrogens may hold promise in treating degenerative retinal disorders including AMD, RP, and glaucoma, most of the studies discussed were conducted with natural estrogens. As a consequence, these studies are subject to criticisms that have led to a fear of HT within the general population. An alternative approach would be to exploit the neuroprotective virtues of estrogens while attenuating their classic receptor-mediated feminizing effects. One of the key receptor-independent mechanisms by which estrogens afford neuroprotection occurs through scavenging and resonance stabilization of free radicals.
The future neuroprotection: Oxidative stress is a fundamental pathogenic mechanism of RGC death in glaucoma [66-68]. Conversely, estrogens have been identified as potent antioxidants [35,69-73]. Simple principles of organic chemistry suggest that synthetic estrogens have a future as designer neuroprotective drugs. Key to their receptor-independent neuroprotective effects is their ability to function as direct antioxidants by quenching free radicals and terminating their propagation [74-76]. This mechanism involves donation of a hydrogen atom from the phenolic group found on the A ring of natural estrogens (Figure 1B), to the free-radical (Figure 1C), resonance stabilizing the free radical and terminating chain reactions (Figure 1D). This process leaves behind a phenoxy radical that can be regenerated in vivo [70]. Regeneration of phenolic antioxidants occurs via three known pathways using ascorbic acid, glutathione-dependent free-radical reductase, and a recently discovered NADPH-mediated reductive aromatization (Symbol X in Figure 1) [69,77,78]. This mechanism is key to their function as direct antioxidants.

Based on these principles, the efficacy of synthetic estrogen derivatives designed to harness the antioxidant capabilities of estrogens without their receptor-mediated feminizing effects will be the choice of future neuroprotective drugs in this class of compounds (Figure 2). Prodrug quinolic variants of some compounds have also been shown to attenuate estrogen receptor binding and are believed to enhance the ability of the compound to traverse the lipid bilayer (Figure 1A) [69]. These studies were conducted based on previously established protocols for treatment of RGC-5 with glutamate to induce an increased oxidative burden to examine the neuroprotective efficacy of novel compounds [25]. The drug ZYC-3 was identified as the most effective neuroprotectant and its mechanisms of action are the subject of a portion of our work in this field.

CONCLUSIONS

Estrogens clearly have an impact on the health of the retina.

Figure 1. Termination, stabilization, and recycling of free-radical by phenolic estrogen derived drugs. Structure A represents the quinolic ring that can spontaneously convert to a phenolic ring found in the structure of estrogens. The phenol (B) can then scavenge free radicals (C) and resonance-stabilize them (C-D) until reduced (X). Reduction of phenoxy radicals (C) is an enzyme-mediated process that uses ascorbic acid, glutathione-dependent free radical reductase, and a newly discovered NADPH-mediated reductive aromatization. This illustration was loosely based on work of Prokai et al. [79].

Figure 2. Examination of the neuroprotective efficacy of estrogens and analogs. Maximal efficacy, with dose of drug, was examined in a glutamate-induced cytotoxicity model of in vitro retinal ganglion cell death using the retinal ganglion cell-5 cell line. This screening process was used to guide drug selection and design, and to identify highly efficacious compounds for detailed studies of mechanism of action. Abbreviations: 17β-estradiol (E2) is 1,3,5(10)estratriene-3,17 beta-diol; E2Q is 3,5(10)estratriene-3,17 beta-diol-quinol; Estrone (E1) is 3-hydroxyestra-1,3,5(10)-triene-3,17 beta-diol; E1Q is 3-hydroxyestra-1,3,5(10)-triene-3,17 beta-diol-quinol; 17α-estradiol is 1,3,5(10)estratriene-3,17 alpha-diol; 17α-estradiol-quinol is 1,3,5(10)estratriene-3,17 alpha-diol-quinol; ADAEI is 2-adamantyl-3-hydroxyestra-1,3,5(10)-triene-17-one; ADAEIQ is 2-adamantyl-3-hydroxyestra-1,3,5(10)-triene-17-one-quinol; THN is 1,3,6,8-tetrahydroxynapthalene; THNQ is 1,3,6,8-tetrahydroxynapthalene-quinol; ZYC1 is 17β estratriene-3,5(10), 9(11)-tetrahydroxy-1,3,5(10)-triene-17-one; ZYC10 is 2-(1-adamantyl)-3-hydroxyestra-1,3,5(10)-triene-17-one; ZYC-3 is Enantiomer of ZYC1.
Current evidence indicates that estrogen receptors are found throughout the retinal thickness, concentrated prominently in the RGC and nerve fiber layers. At least in part, estrogen receptors mediate the influence of estrogens on blood viscosity and ocular vascularity. Estrogens and female reproductive factors may also have an impact on IOP and the development of OAG. The observation that estrogens may protect against degenerative vision loss in RP, AMD, and glaucoma is suggestive of their retinal neuroprotective capabilities, but also paves the way for the development of non-feminizing estrogen-like drugs that can be used in patients regardless of sex or health predispositions. Although not all novel drugs examined produced complete protection, even partial protection shows that outcome-directed drug design can be an effective method of developing neuroprotective drugs. These results may then be used to refine the design of compounds to improve their efficacy. The capabilities of medicinal, quantitative, and computational chemistry can be used to model free radical scavenging and resonance stabilization to refine the design of synthetic estrogens. Reexamination of such compounds in selective high throughput in vitro experiments could rapidly produce a host of efficacious drugs.

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