UNPRECEDENTED PROTECTION OF THE RETINA FROM GLAUCOMA-INDUCED NERVE DAMAGE BY TOPICAL ADMINISTRATION OF A NOVEL PRODRUG

BACKGROUND AND AIMS

> Estrogens are powerful neuroprotectants and have been considered potential drugs to protect the retina against neurodegenerative effects caused by glaucoma [1]. The "neurotrophic" effect of estrogens after systemic exposure and their poor ocular bioavailability, however, prevent their development as local ocular drugs.

> We report here our discovery that a novel type of prodrug [2] for 17ß-estradiol (E2), 10ß,17ß-dihydroxyestra-1,4-dien-3-one (DHED), overcomes these obstacles due to its improved ocular pharmacokinetics (compared to that of E2) and its selective in vivo conversion to E2 inside the eye.

EXPERIMENTAL METHODS (Pharmacology)

> DHED was prepared from E2 by microwave-assisted organic synthesis using lead(IV) acetate oxidation [3].

> Selected physicochemical properties impacting transcorneal permeability (lipophilicity and water-solubility) were measured experimentally. Affinities to estrogen-receptor (ER) binding were determined by competitive ligand-binding assays using recombinant ERs.

> In vitro conversion of DHED to E2 utilizing NAD(P)H was demonstrated by incubating DHED and the cellular reductant in PBS at pH 7.4 and 37°C, followed by liquid chromatography–tandem mass spectrometry (LC/MS/MS) assay [2].

> Metabolic conversion rates in various eye compartments were measured by incubating DHED in tissue homogenates (rat and pig) followed by LC/MS/MS assay.

> Evasion of pro-oxidant effect from DHED treatment was proven in ovxestrousized (OVX) rat retina homogenate by measuring H2O2 production as a marker of pro-oxidant (toxic) effects. Estradiol-1,5(10)-dien-3,4,17-trione (E1Q, a profoundly pro-oxidant estrogen metabolite) was used as a positive control.

> Lack of systemic effect after exposure to DHED was confirmed by its subcutaneous (s.c.) administration to OVX rats every 48 hours for a period of two weeks for a total of 7 injections (100 µg/kg body weight each) and, then measuring urinating wet weights. E2 (administered analogously to that of DHED) was used as a positive control.

RESULTS

> We used a widely accepted rat model of glaucoma realized through chronic pressure-induced optic nerve damage, which is generated by invasively induced, surgical inhibition of aqueous humor outflow, and characterized by a gradual elevation of IOP [4]. IOP elevation was induced in OVX Brown-Norway rats by injection of hypertonic saline solution in the episcleral vein of the ipsilateral eye using a micro glass needle with an injection pump.

> Using this model of glaucoma, intervention treatments (DHED and E2, respectively, in a vector that affords enhancement of transcorneal penetration) were administered as eye-drops in a volume of 10 µl daily for 14-30 days. IOP was monitored 2-3 times weekly with a Tono-Pen XL tonometer (Mentor, Norwell, MA) on conscious animals in the presence of a topical anesthetic in both the ipsi- and contralateral eyes for 10-14 days until stabilized elevation of IOP in the ipsilateral eye was achieved.

> Histology and TUNEL assays were used to measure cell death in the retinal ganglion cell layer (GCL) after the rats were sacrificed 19-30 days after IOP elevation.

> Using the OptoMotry system (CerebralMechanics, Leithbridge, Alberta, Canada) developed by Prusky et al., we measured functional effects of our small-molecule intervention approach on visual performance. In rats, we took advantage of the optomotor response in which an animal reflexively follows a moving visual stimulus with its eyes.

> In conclusion, our pharmacological approach has great promise for the development of a novel alternative and/or complementary approach to glaucoma therapy.

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