Protection of blue light induced retinal degeneration by the free radical scavenger Phenyln-t-ert-butylinonitrone and a serotonin receptor 5-HT1A agonist in rats

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Introduction
Retinal degenerations are among the major causes of severe visual impairment in industrialized countries. Age-related macular degeneration (AMD) is the most prevalent, but other inherited retinal degenerations, including retinitis pigmentosa (RP), also contribute to visual loss. Apoptosis of photoreceptors and RPE (Retinal Pigment Epithelium) is the final cell death pathway in RP and AMD (1). Exposure to excessive levels of light especially from short wavelengths induces photoreceptor apoptosis (2) and has previously been used as a model for the study of retinal degeneration. The mechanism by which light induces retinal degeneration is not completely understood. Rod photoreceptor is essential for light induced retinal degeneration, indicating that the signal flow through phototransduction cascade is necessary to mediate the damaging effect of light (3). A diverse range of agents has been used to prevent photoreceptor apoptosis in the light induced retinal degeneration. These include calcium channel blockers (4), antioxidant such as dimethylthiourea (5), free radical spin trapping agents such as phenyl-n-t-ert-butylinonitrone (PBN) (6), caspase inhibitors (7), drugs that upregulate endogenous neurotrophic factors such as alpha 2 agonist (8) and more recently the serotonin receptor 5-HT1A agonist (9).

The aim of this study was to compare efficacy of an anti-oxidant, PBN, and a serotonin receptor agonist, 8-Hydroxy-2-(di-pyrilamido)tetralin hydrobromide (8OHDHPAT).

Material and method

Animals and treatment
Twenty four male Spawale Dawley rats (350-450g) were randomized in three groups. Rates of the first group received subcutaneous injection of 8OHDHPAT, 500µg/kg (once a day, from two days before to two days after the light exposure), the second group was injected intravitreally with 0.5µg/kg 4 times during the exposure days, rats of the third group were injected with DMSO 0.9%.

Animals were housed and handled according to the AVCO Statement for the Use of Animals in Ophthalmic and Vision Research. Food and water were available ad libitum. Rats were kept under 12 light (500-100 lux) and 12 dark cycle.

Results

Electroretinogram (ERG)
Scotopic ERG was recorded, using system RETI-animal (Roland Consult) in both eyes after overnight dark adaptation. Standard flash ERG was obtained using a white flash of 3 ms duration and 3 cd·s/m² on the eyes, on days 7 and 14 after light exposure.

In vivo imaging
SD-OCT imaging was performed with a Spectralis TM (Heidelberg Engineering) 14 days after the light damage. retinas were observed for autofluorescence and infra red imaging. Blue laser autofluorescence was used to detect the natural fluorescence emitted by retinal pigment cells, infrared laser provided high resolution images of the retina.

Study Termination and Measurement of the ONL Thickness
After 14 days animals were euthanized and enucleated, embedded in paraffin. Vertical sections (5 to 7 µm thick) were cut through the optic nerve and stained with hematoxylin/eosin. ONL thickness was measured every 500 µm from the optic nerve to the peripheral retina in vertical sections.

Electroretinography
Blue light exposure induced a 60% decrease of the a-wave (photoreceptor function) and b-wave (mixed response: photoreceptor and bipolar cell response) amplitudes in placebo group 7 and 14 days after the induction.

A and b-wave response was significantly higher in 8OHDHPAT and PBN treated groups at both time-points. Both compounds induced almost the same recovery from the baseline response for a- and b- waves: 77 and 84% of a-wave baseline values 7 and 14 days after exposure and b-wave, 78 and 95%, 7 and 14 days after exposure respectively.

In vivo retinal imaging
Retina of rats treated with placebo showed large hyperfluorescent area typical of suffering RPE (a). These lesions are considerably reduced or even absent in 8OHDHPAT (c) and PBN treated rats (e). SD-OCT scans provided qualitative images of retinal layers with complete disappearance of ONL in most of placebo-treated rats (a) and preservation of retina integrity with 8OHDHPAT (b) or PBN (c) treated rats.

ONL thickness
To assess whether the treatment to preserve photoreceptor structure, the ONL thickness was evaluated by histology 14 days after exposure. Photomicrograph showed massive degeneration, with many photoreceptor nuclei missing (B) compared to untreated retina (A).

Conclusion
Significant protection of retina from light induced degeneration was achieved with 8OHDHPAT and PBN, which effectively rescued a-wave and b-wave amplitudes of ERG responses and preserved photoreceptor layer thickness.

The serotonin receptor 5-HT1A could be a valuable target for AMD and other retinal degenerative diseases.

References

Hematoyxlin/eosin labeled section of retina. Control retina (A) and light injured retina (B).