Topical administrations of Pilocarpine, Ramiprilat and N-Ac-Leucine demonstrates neuroprotective effects in N-nitroso-N-methylurea (MNU) induced photoreceptor degeneration.

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Introduction

Retinal degeneration (RD) is a leading cause of irreversible blindness worldwide. The common forms of RD mainly include retinitis pigmentosa, age-related macular degeneration, and retinal dystrophy. The pathogenic mechanisms of these diseases are not fully understood, and no effective treatments are currently available. Animal models are essential for understanding of the pathogenesis of retinal diseases and the development of new therapeutic strategies in humans. A number of animal models for studying retinal degeneration are available including those induced by physical or chemical treatments, natural mutants, or genetically engineered animals. Among them, retinal injury induced by DNA alkylating agents, such as N-methyl-N-nitrosourea (MNU), has been widely used as a model of retinitis pigmentosa[1,2]. In the model, PR degeneration is mainly induced by apoptosis, as the inhibition of initiator and effector caspases protects the structures and functional properties of the PR. But caspase-independent apoptosis has also been shown in the MNU model. Alteration of other mechanisms such as autophagy has been investigated, and the importance of non-apoptotic cell death mechanisms and autophagy have been confirmed *in vitro* and *in vivo* in the MNU-induced photoreceptor degeneration[3]. The regulation of autophagy is thought to have potential implications for neurodegenerative disease therapies.
In this context, we investigated the neuroprotective effect of three compounds (pilocarpine, ramiprilat and N-Ac-leucine (N-Ac-Leu)) suspected to act on the mTOR signaling in a mouse model with N-nitroso-N-methylurea (MNU) induced photoreceptor degeneration.

Material and Method

Induction of retinal photoreceptor degeneration

Photoreceptor degeneration was induced in forty albino rats (Sprague Dawley) by intraperitoneal injection of 60 mg/kg N-nitroso-N-methylurea (MNU). Animals were handled and cared for according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Treatment

Rats were randomly allocated in 4 groups: 1) MNU+Vehicle; 2) MNU+Ramiprilat (2%); 3) MNU+N-Ac-Leu (0.1%); 4) MNU+Pilocarpine (1%). Treatments were administered 3-times daily instillations from the induction to Day 7.

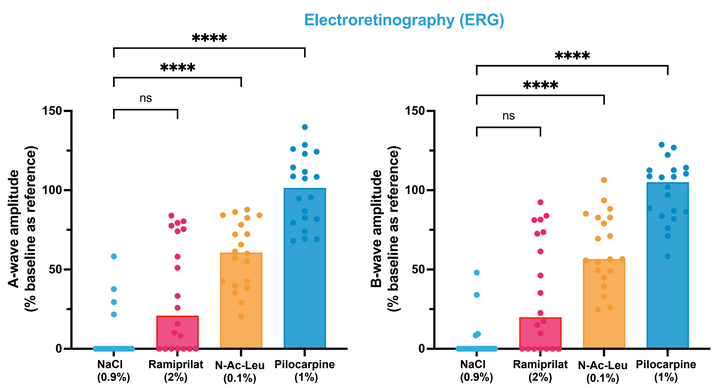
Clinical Evaluations

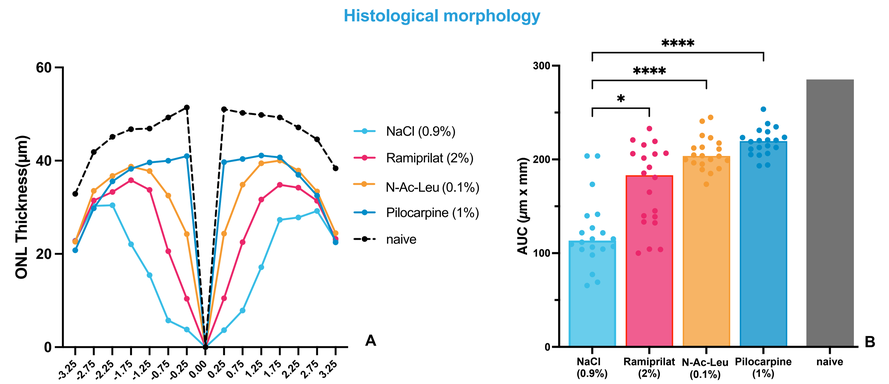
Before induction and on Day 7, retinal functionality was assessed by ERG on both eyes in scotopic condition using a flash intensity of 3 cd.s/m^2 (system RETI-animal® from Roland Consult). The amplitude of A- and B-wave were measured.

Retinal morphology by histology

On Day 7, after euthanasia, eyeballs (EB) were sampled, fixed in Davidson's solution, and embedded in paraffin. Sections (5 to 7 µm thick) were performed along the vertical meridian and stained with Hematoxylin/ Eosin (HE) stain. The vertical meridian included the optic nerve. The thickness of outer nuclear layer (ONL from photoreceptors) was measured at 7 different locations from the optic nerve head on both sides. Results were expressed as the outer nuclear layer (ONL) area under the curve (AUC).

Results





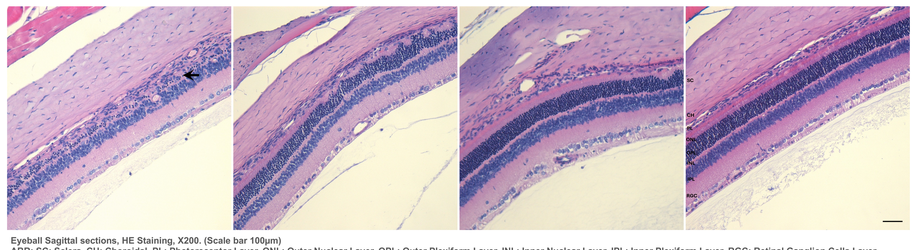
Electroretinography

One week after injection, MNU induced more than 90% decrease of the A-waves (photoreceptor function) and B-waves (mixed response: photoreceptor and bipolar cell response) amplitude in NaCl group.
Multiple ocular administrations of Ramiprilat (2%) or N-Ac-Leu (0.1%) for 7 days after exposure to MNU showed partial recovery of A-wave amplitude, with 20.9% and 60.7% of baseline level, respectively. This recovery was statistically significant for the N-Ac-Leu-treated group when compared to NaCl-treated group. Ocular administrations of Pilocarpine (1%) for 7 days after exposure to MNU showed statistically significant and complete recovery of A-wave amplitude.

ONL thickness

To assess the ability of treatment to preserve photoreceptor structure, the thickness of the ONL was evaluated 7 days after induction by histology.
Photomicrographs showed massive degeneration, with many photoreceptor nuclei missing in NaCl group (arrow).

Measurement of ONL thickness on vertical section (A) or Area Under the Curve (AUC) (B) shown that administration of Ramiprilat (2%), N-Ac-Leu (0.1%) and pilocarpine (1%) induced a significant protection of ONL compared with vehicle groups.



Eyeball Sagittal sections, HE Staining, X200. (Scale bar 100µm)
ABR: SC: Sclera, CH: Choroidal, PL: Photoreceptor Layer, ONL: Outer Nuclear Layer, OPL: Outer Plexiform Layer, INL: Inner Nuclear Layer, IPL: Inner Plexiform Layer, RGC: Retinal Ganglion Cells Layer.

Conclusions

Consistent with the ERG and histological results, Ramiprilat, N-acetyl-DL-leucine and Pilocarpine showed protective effect on MNU induced photoreceptor degeneration.
Pilocarpine is a well-known cholinergic agonist, used as a glaucoma therapy and recently as a treatment of presbyopia in US but never as a neuroprotector. The N-acetyl-DL-leucine, is treatment used for vertigo and poorly studied in degenerative eye diseases, if at all. Ramiprilat had already been studied for its action on ganglion cell survival in an ischemia-reperfusion model, but no activity on photoreceptor survival had yet been shown.
Although the mechanism of action underlying this retinal protection remains to be clarified, and particularly the activation of the mTOR pathway, the three test items may be a potential therapeutic agent for the treatment of retinal degeneration.

References

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