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Use of Polypeptide-Based Nanoconjugates as a Combination Therapy Approach to treat Retinitis Pigmentosa

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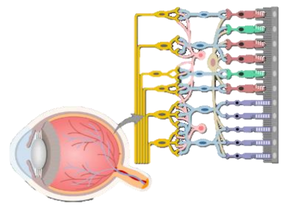
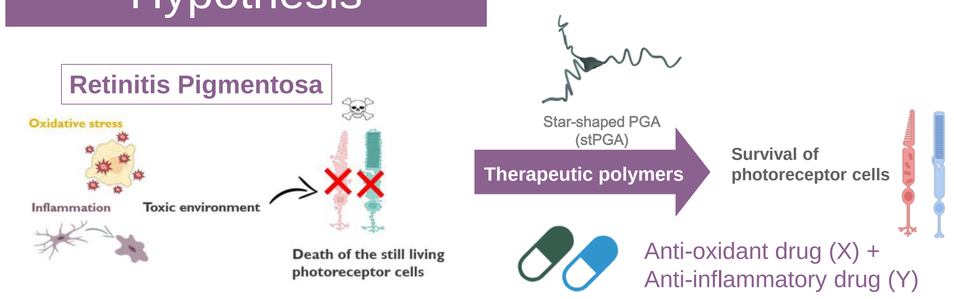
INTRODUCTION

Retinitis Pigmentosa (RP) is the most common form of inherited retinal degeneration with a prevalence of 1 in 4000 individuals worldwide. RP is the main genetic cause of blindness in the developed world. It presents a high clinical and genetic heterogeneity with more than **125 genes involved**. Currently, only one approved treatment, based on gene therapy for *RPE65* gene, is available. In spite of being a genetic disease, chronic inflammation and unbalanced redox status can influence its progression. We have previously described that, patients with RP and *rd10* mice, a murine model of autosomal recessive RP, showed altered redox status with lower antioxidant capacity (e.g., activity of antioxidant enzymes) and higher levels of oxidant molecules (e.g., lipid peroxidation or protein carbonylation). RP patients and *rd10* mice presented chronic inflammation including microglia activation. Previous findings support that antioxidant and anti-inflammatory therapies could offer a promising therapeutic approach based on neuroprotection.

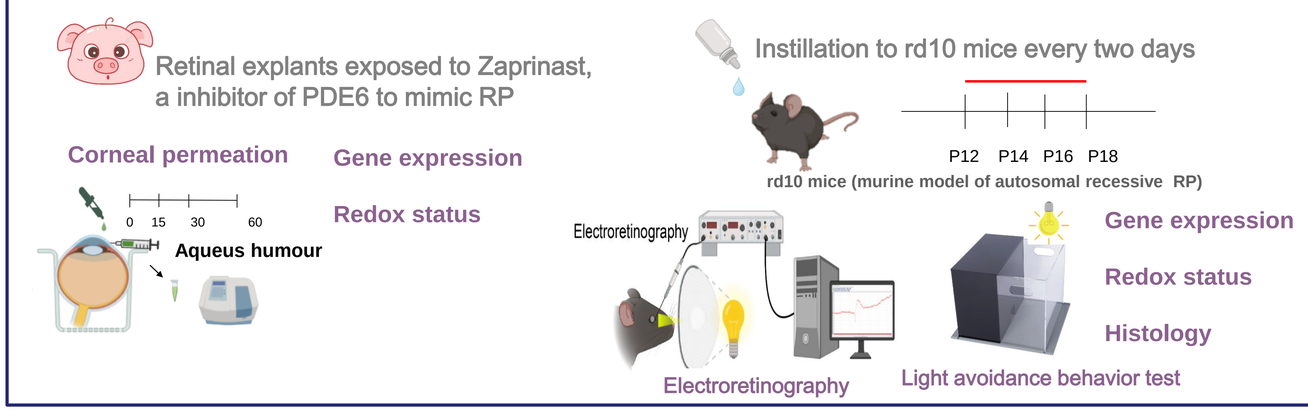


Recent advances in **Nanomedicine** seek to improve drug delivery to the retina by overcoming ocular barriers. To this end, non-invasive or minimally invasive delivery modalities are being developed. Due to their intrinsic characteristics, **therapeutic polymers**, and in particular **polypeptide-based combination conjugates**, represent **ideal delivery systems** for the combination therapies needed to address this pathology. Aiming to tackle the elevated oxidative stress and chronic neuroinflammation, we proposed the use of polypeptide-based combination therapy using poly-L-glutamic acid (PGA) based on the blockage of both pathways.

Hypothesis



METHODS



RESULTS - *EX VIVO* MODEL

Corneal permeation

We administrated St-PGA-Oregon green (StPGA-OG)at different times to pig's eyes to analyze the permeation. We extracted the aqueus humour to measure the fluorescence of fluorophore. StPGA-OG penetrated the cornea, where the limiting layers of trans-corneal permeation are the epithelium and the stroma, and consequently, enhanced topical drug delivery.

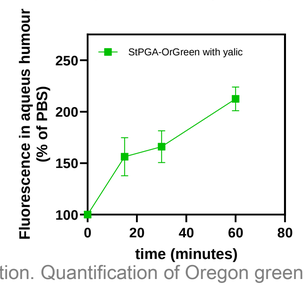


Figure 1. Corneal permeation. Quantification of Oregon green (fluorescence) in the aqueous humour.

Redox status and inflammation in an ex vivo model of retinal degeneration: Zaprinast-exposed retinal explants

We administered St-PGA-drug conjugates alone and in combination to porcine retinal explants exposed to Zaprinast to study its effect on both pathological processes involved in retinitis pigmentosa disease. Zaprinast reduced the antioxidant response by lowering, among others, CAT activity and increasing TBARS formation in treated explants compared to untreated explants. We did not detect significant changes in CAT activity nor TBARS formation after the exposure of retinal explants to PGA conjugates. We assessed gene expression of the cytokines TNFα, and IL6. The combination of St-PGA-drug conjugates significantly downregulated TNFα gene expression suggesting a synergistic effect of the drugs for this cytokine in zaprinast-treated retinal explants. We observed that St-PGA-drug conjugates alone reduced gene expression of IL6 without a synergistic effect in zaprinast-treated retinal explants.

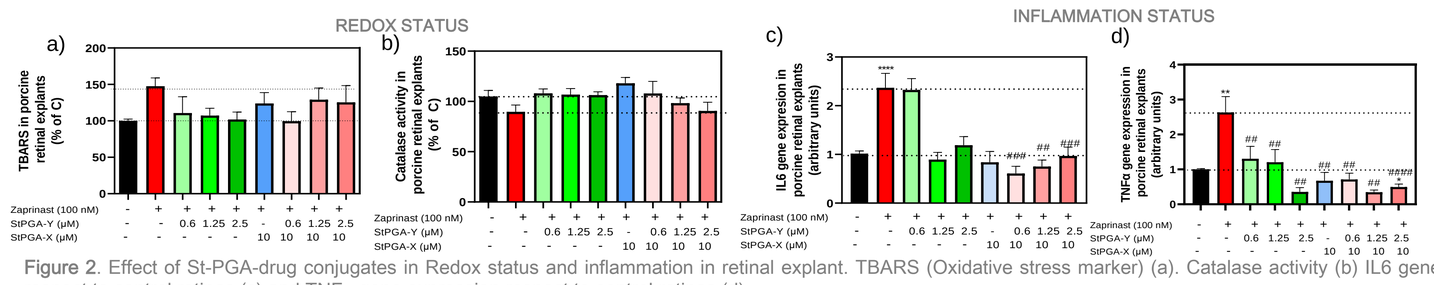


Figure 2. Effect of St-PGA-drug conjugates in Redox status and inflammation in retinal explant. TBARS (Oxidative stress marker) (a). Catalase activity (b) IL6 gene expression respect to control retinas (c) and TNFα gene expression respect to control retinas (d).

RESULTS - *IN VIVO* MODEL

In vivo visual function: ERG recordings and light avoidance test

We examined the innate aversion of mice to the light by the light avoidance test and the global retinal function through a scotopic full-field ERG in untreated rd10 mice, mice instilled with the StPGA-drug conjugates and control mice. rd10 mice showed a poor light perception but after instillation of the combination of StPGA-drug conjugates they partly restored the light perception (light test results). Under scotopic conditions, we detected a significant decline in b-wave amplitudes at all flash intensities in untreated rd10 mice compared to control mice (data not shown). Mice treated with the combination StPGA-drug conjugates showed better ERG recordings than untreated rd10 mice. StPGA-drug conjugates had no beneficial effect separately on either light avoidance test or ERG recordings.

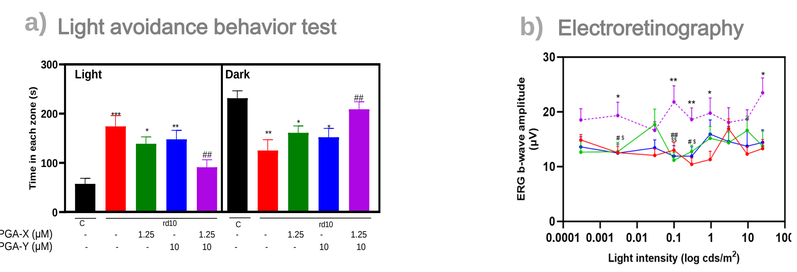


Figure 3. Eye drops of combination of St-PGA-drug conjugates improve on visual function. Light avoidance behavior test (a) and Electroretinography (b) from wild-type mice, C, untreated rd10 mice (rd10 mice) and rd10 mice instilled with St-PGA-drug conjugates.

Redox status and inflammation

rd10 retinas present and upregulation of cytokines such as TNFα, IL1β, or IL6. rd10 mice instilled with StPGA-X alone or combined with StPGA-Y (no synergistic effect) showed a significant reduction in GFAP, TNFα, IL6 and IL1β. Rd10 mice present also alterations in redox status including high levels of carbonyl adducts, a marker of protein carbonylation, and TBARS, a marker of lipid peroxidation, at PD18. rd10 mice instilled with StPGA-X alone, StPGA-Y alone or their combination (no synergistic effect) showed a significant reduction in carbonyl adducts compared to untreated rd10 mice.

a)

Gene	C	rd10	rd10 + StPGA-X	rd10 + StPGA-Y	rd10 + StPGA-X + StPGA-Y
Il1β	1.0 ± 0.01	69.5 ± 8.8****	27.9 ± 4.2$^{\#\#}$	29.9 ± 7.5$^{\#\#}$	40.3 ± 9.8**
Il6	1.0 ± 0.01	34.4 ± 4.9****	31.2 ± 6.6$^{***,\#\#}$	28.4 ± 6.4$^{**,\#\#}$	35.8 ± 4.4****
Tnfα	1.0 ± 0.01	136 ± 17****	72 ± 12$^{**,\#}$	62 ± 11$^{*,\#\#}$	96 ± 16***
Tnfr1	1.0 ± 0.06	7.2 ± 0.5****	4.8 ± 0.7$^{****,\#\#}$	5.6 ± 0.6****	5.0 ± 0.5$^{****,\#}$
Il10	No detectable	1.0 ± 0.02	2.2 ± 0.5$^{\#}$	1.0 ± 0.2$^{\$}$	3.3 ± 0.5$^{\#\#\#\#}$
Gfap	1.0 ± 0.06	27.4 ± 2.2****	17.0 ± 3.4$^{****,\#}$	20.2 ± 2.9****	21.2 ± 3.1****

b)

Variable	C	rd10	rd10 + StPGA-X	rd10 + StPGA-Y	rd10 + StPGA-X + StPGA-Y
TBARS formation (% of C)	100 ± 10	200 ± 48	167 ± 48	129 ± 28	185 ± 36
Carbonyl adducts (% of C)	100 ± 13	167 ± 14***	86 ± 8$^{\#\#\#\#}$	62 ± 11$^{\#\#\#\#}$	78 ± 5$^{\#\#\#\#}$

Table 1. Eye drops of combination of St-PGA-drug conjugates reduce gene expression of pro-inflammatory genes and markers of oxidative stress. Pro-inflammatory gene expression (a), evaluation of markers of oxidative stress (b) from wild-type mice, C, untreated rd10 mice (rd10 mice) and rd10 mice instilled with St-PGA-drug conjugates.

Retinal degeneration: ONL thickness and retinal inflammation

RP progression is accompanied by reduction the number of nuclei at the in outer nuclear layer (ONL), reactive gliosis (e.g. GFAP upregulation), microglia activation and migration toward ONL in rd10 mice. We observed that StPGA-Y conjugate alone or combined with StPGA-X conjugate (no synergy) significantly increased photoreceptor survival at the ONL, reduction in GFAP content and exhibited less microglia activation compared to untreated rd10 mice.

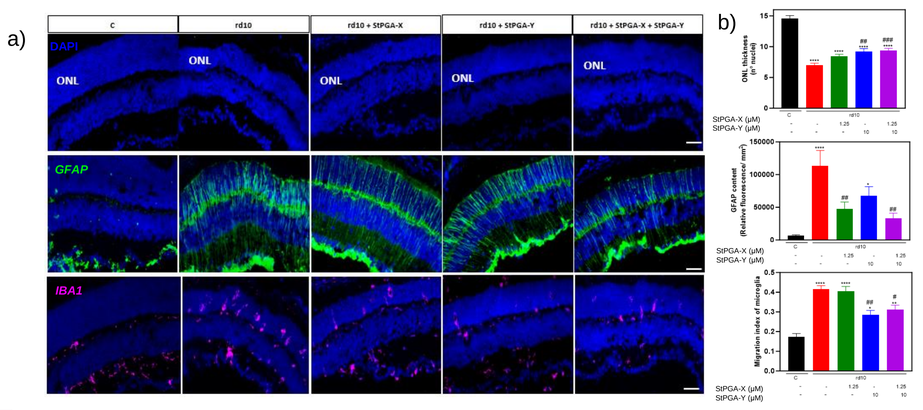


Figure 4. Eye drops of St-PGA-drug conjugates reduced photoreceptor degeneration and inflammation in rd10 mice at PD18. Photoreceptor degeneration and reactive gliosis were evaluated by histology. Representative photomicrographs of retinal sections showing DAPI (blue labelled) staining, GFAP (green labelled) or Iba1-positive cells (pink labelled) (scale bar: 50 μm) (a). quantification of the number of rows of the nuclei in the outer nuclear layer (ONL), quantification of GFAP relative fluorescence and migration index of microglia cells (b) from wild-type mice, C, untreated rd10 mice (rd10 mice) and rd10 mice instilled with St-PGA-drug conjugates.

Take home messages

*** This platform is a good approach to treat eye diseases.**
*** The combination of antioxidant and anti-inflammatory compounds have a synergetic effect to improve visual function of rd10 mice.**

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