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Prolonged prevention of retinal degeneration with retinylamine loaded nanoparticles

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ABSTRACT

Retinal degeneration impairs the vision of millions in all age groups worldwide. Increasing evidence suggests that the etiology of many retinal degenerative diseases is associated with impairment in biochemical reactions involved in the visual cycle, a metabolic pathway responsible for regeneration of the visual chromophore (11-*cis*-retinal). Inefficient clearance of toxic retinoid metabolites, especially all-*trans*-retinal, is considered responsible for photoreceptor cytotoxicity. Primary amines, including retinylamine, are effective in lowing the concentration of all-*trans*-retinal within the retina and thus prevent retina degeneration in mouse models of human retinopathies. Here we achieved prolonged prevention of retinal degeneration by controlled delivery of retinylamine to the eye from polylactic acid nanoparticles in *Abca4*$^{-/-}$*Rdh8*$^{-/-}$ (DKO) mice, an animal model of Stargardt disease/age-related macular degeneration. Subcutaneous administration of the nanoparticles containing retinylamine provided a constant supply of the drug to the eye for about a week and resulted in effective prolonged prevention of light-induced retinal degeneration in DKO mice. Retinylamine nanoparticles hold promise for prolonged prophylactic treatment of human retinal degenerative diseases, including Stargardt disease and age-related macular degeneration.

1. Introduction

Progressive retinal degeneration is a leading cause of irreversible vision loss worldwide. It potentially affects a broad age spectrum from Stargardt disease in juveniles to age-related macular degeneration (AMD) in individuals over 50 years old. Currently, there is no FDA approved treatments of the retinal degenerative diseases. Although diverse in clinical manifestations, many retinal degenerative pathologies are linked to impairments in all-*trans*-retinal (atRAL) clearance from the photoreceptor cells. This process depends on a metabolic pathway called retinoid (visual) cycle, a process involving continuous re-isomerization of atRAL back to its *cis* configuration [1–3]. Continuous recycling of the retinoids is essential for the regeneration of light-sensitive pigments required for vision as well as the health of photoreceptor cells [4]. Inefficient clearance of atRAL by the retinoid cycle results in transient accumulation of this toxic aldehyde and formation of related dimeric products including A2E-like derivatives, resulting in irreversible loss of photoreceptor cells [5–11]. Recent reports have shown that the sequestration of atRAL with primary amines or inhibition of retinoid isomerase (RPE65) can lower retina atRAL concentrations to a safe level and prevent retinal degeneration in *Abca4*$^{-/-}$/*Rdh8*$^{-/-}$ double knockout (DKO) mice, a model for human Stargardt disease and age-related macular degeneration (AMD) [12,13]. Retinylamine is an amine derivative of retinoids and acts as both an aldehyde scavenger and an inhibitor of RPE65 [14,15]. It shows the promise to effectively prevent light-induced retinal degeneration in DKO mice. Controlled and sustained delivery of retinylamine to the eye can improve its therapeutic efficacy and provide safe and prolonged protection against retinal degeneration.

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Drug delivery to the back of the eye has been a major challenge due to the unique anatomy and physiology of the eye [16–18]. The blood-ocular barrier is a major hurdle for effective intraocular delivery of therapeutics. Ocular implants and scleral plug drug delivery devices are used to overcome the barrier to achieve effective intraocular drug delivery. Unfortunately, these delivery systems are invasive to the eye and have poor patient compliance. Retinylamine is a retinoid derivative and can utilize vitamin A transport machinery including retinoid-binding proteins, receptors, and enzymes to overcome the blood-ocular barrier and to reach the retina after its release from the nanoparticles [19]. Thus, sustained release of retinylamine from a drug delivery system *in vivo* could effectively deliver the drug to the eye and maintain a relatively constant drug concentration in the retina to provide prolonged protect against retinal degeneration [20].

We aimed to develop a safe drug delivery system for controlled release of retinylamine to effectively protect the retina from irreversible degeneration. Polylactic acid (PLA) is a biocompatible and biodegradable polymer that was used in numerous clinical drug delivery systems [21–27]. Incorporation of retinylamine into PLA nanoparticles could provide controlled drug delivery and prolonged prolonged protection against retinal degeneration, Fig. 1. Here, We prepared PLA nanoparticles containing retinylamine and investigated drug delivery efficiency to the eye and hepatic pharmacokinetics in albino C57BL/6J-Tyr wild-type (WT) mice after subcutaneous administration. The therapeutic efficacy of the drug loading nanoparticles was examined in the *Abca4*$^{-/-}$*Rdh8*$^{-/-}$ mouse model *in vivo*.

2. Results and discussion

Retinylamine loaded PLA nanoparticles were formulated using single emulsion technique. The drug containing nanoparticles had an average diameter of 730 ± 310 nm with a loading capacity of 6.9 ± 0.3% (w/w), Fig. 2. The nanoparticles displayed an initial burst of release within the first 24 h and then exhibited zero-order drug release kinetics in PBS (pH 7.4) at 37 °C up to 4 weeks.

The effect of nanoparticle encapsulated retinylamine on modulation of the visual cycle chemistry was examined in concert with the pharmacokinetics of this drug in the eye after subcutaneous administration in 8-week-old albino WT mice. The kinetics of 11-*cis*-retinal (11c-RAL) recovery after light exposure was first investigated in WT mice. To establish a baseline for recovery of 11c-RAL after intensive light exposure, the mice were dark adapted for 24 h prior to 10 min light exposure at 6500 lux. This bright light caused ~90% bleach of the visual chromophore that reduced the at-RAL to all-*trans*-retinol, which was then converted into retinyl esters (Fig. 3a). Efficient regeneration of 11c-RAL was observed within the first few hours after light exposure, and full recovery was achieved after 16 h. These results suggest that any modulation of the visual cycle chemistry by the nanoparticles should be apparent after this time period.

The efficacy of the retinylamine-loaded nanoparticles was investigated in the WT mice according to the experimental procedures schematically depicted in Fig. 3b. Retinoid concentrations were measured 24 h after the light exposure in different treatment groups. At a retinylamine equivalent dose of 10 mg/kg, subcutaneous administration of retinylamine-loaded nanoparticles resulted in a prolonged constant concentration of retinylamide, a metabolic derivative indicative of the presence of retinylamine in the eye, Fig. 3c,d. It should be noted here that upon treatment with retinylamine, the enzyme lecithin:retinol acyltransferase (LRAT) amidated the drug by transferring a palmitoyl chain from phosphatidylcholine to form the corresponding retinylamide, a storage form of the drug [28]. The results here suggest that the zero-order sustained release of retinylamine from the nanoparticles maintained a relatively constant drug supply to the eye for up to 7 days (168 h), while retinylamide was not detected 3 days after administration of the free drug. The presence of retinylamine slowed down the retinoid cycle in the retina. Consequently, the concentration of 11c-RAL was maintained at a relatively low level and retinyl esters were at a relatively high level for 7 days with nanoparticles, but only 2 days for the free drug in the WT mice. The results indicate that sustained delivery of retinylamine to the eye by the nanoparticles resulted in more prolonged modulation of retinoid chemistry than the free drug at the same dose.

The hepatic pharmacokinetics of the retinylamine released from nanoparticles was examined in comparison with free retinylamine.

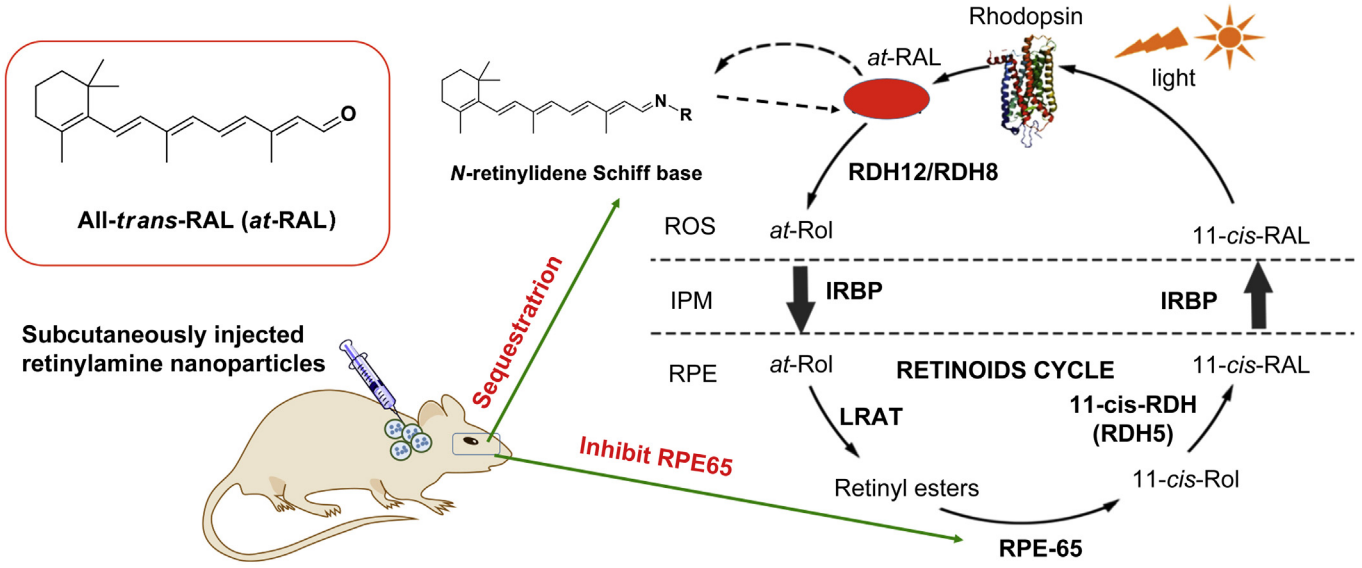

Fig. 1. Retinylamine loaded PLA nanoparticles for prolonged protection against light-induced retinal degeneration in *Abca4*$^{-/-}$*Rdh8*$^{-/-}$ mice. After subcutaneous administration of the biodegradable nanoparticles, retinylamine is gradually released in the body and a sufficient amount of the drug is available in the eye to sequester all-trans-retinal (*at*-RAL) for a prolonged period to effectively protect against light-induced retinal degeneration.