Experimental Eye Research 135 (2015) 59–66

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Topical cyclodextrin reduces amyloid beta and inflammation improving retinal function in ageing mice

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ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form
18 March 2015
Accepted in revised form 25 March 2015
Available online 25 April 2015

Keywords:
Retinal ageing
Cyclodextrin
RPE65
Retinal inflammation
Retinal amyloid beta
Lipid

ABSTRACT

Retinal ageing results in chronic inflammation, extracellular deposition, including that of amyloid beta (Aβ) and declining visual function. In humans this can progress into age-related macular degeneration (AMD), which is without cure. Therapeutic approaches have focused on systemic immunotherapies without clinical resolution. Here, we show using aged mice that 2-Hydroxypropyl-β-cyclodextrin, a sugar molecule given as eye drops over 3 months results in significant reductions in Aβ by 65% and inflammation by 75% in the aged mouse retina. It also elevates retinal pigment epithelium specific protein 65 (RPE65), a key molecule in the visual cycle, in aged retina. These changes are accompanied by a significant improvement in retinal function measured physiologically. 2-Hydroxypropyl-β-cyclodextrin is as effective in reducing Aβ and inflammation in the complement factor H knockout ($Cfh^{-/-}$) mouse that shows advanced ageing and has been proposed as an AMD model. β-cyclodextrin is economic, safe and may provide an efficient route to reducing the impact of retinal ageing.

1. Introduction

Ageing is associated with cellular decline which is partly linked to metabolic rate. The outer retina has the highest metabolic demand in the body required to maintain the oxygen demanding photoreceptor population (Linsenmeier and Padnick-Silver, 2000). Here with age there is progressive accumulation of extracellular material including neurotoxic amyloid beta (Aβ) (Isas et al., 2010), lipids (Curcio et al., 2005a,Curcio et al., 2005b,Wang et al., 2010) and proteins that are inflammatory such as complement (Hageman et al., 2001). These accumulate on Bruch's membrane (BM) restricting the exchange of metabolic nutrients between the outer retina and its blood supply. In mice these deposits are relatively linear along BM. In humans, deposits are focal and are called drusen. These are a key risk factor for age-related macular degeneration (AMD) when they accumulate in the central retina. With progressive deposition and inflammation 30% of the photoreceptor population is lost in both humans and rodents in normal ageing (Cunea and Jeffery, 2007,Curcio et al., 1993).

In humans, retinal ageing can develop into AMD where progressive inflammation and deposition result in central retina atrophy. Mice lack this area of specialisation and do not develop retinal atrophy but do suffer from similar deposition, inflammation and cell loss across the retina. AMD is the leading cause of blindness in those over 65 years in the West and is growing rapidly as populations age (Klein et al., 1997,Klein et al., 2004). In 50% of cases it is linked to immune vulnerability being associated with polymorphisms of complement genes (Edwards et al., 2005,Haines et al., 2005). Currently, there is no cure for this neurodegenerative disease, although systemic immunotherapeutic approaches have tried to reduce retinal Aβ load (Catchpole et al., 2013,DeMattos et al., 2001,Ding et al., 2011; Salloway et al., 2014). However, topical drug administration has largely been ignored as it was seen as unlikely to be effective due to drug dilution before it reached the retina. This assumes that drugs would have to pass through the anterior eye and vitreous before entering the retina. However, penetration of the drug may be obtained via the conjunctiva and sclera into the retina (Sigurdsson et al., 2007).

Cyclodextrins (CDs) are a family of cyclic polysaccharide compounds with a hydrophilic shell enclosing a hydrophobic cavity. This structure allows them to form water–soluble complexes with otherwise insoluble hydrophobic compounds. This has led to their utilisation as carriers to increase the aqueous solubility and stability of hydrophobic drugs (Loftsson and Duchene, 2007; Stella and He, 2008). They have undergone extensive safety studies and are

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http://dx.doi.org/10.1016/j.exer.2015.03.023

approved by the Food and Drug Administration (FDA) (Stella and He, 2008) for pharmaceutical use and dietary supplements. Topical administration results in their rapid retinal accumulation, presumably entering the eye via the conjunctiva (Loftsson et al., 2008; Sigurdsson et al., 2007).

Recently, CDs systemic delivery has shown efficacy in an Alzheimer's mouse model (Yao et al., 2012) reducing the size of Aβ plaques in the brain and upregulating genes associated with cholesterol transport and Aβ clearance. Further, systemic delivery significantly reduces lipofuscin deposits in the retina, which are an age related lipid rich pigmented deposit that accumulates in the retinal pigmented epithelium (Nociari et al., 2014). CDs are known to bind to cholesterol (Irie et al., 1992,Ohtani et al., 1989) and at high concentrations, they serve as a cholesterol sink. At low concentrations, CDs act as a cholesterol shuttle, transporting it between membranes (Atger et al., 1997; McCauliff et al., 2011). Hence, they can clear cholesterol which is known to be deposited on BM and whose presence has been linked to AMD (Pikuleva and Curcio, 2014).

Here, we ask whether topical CDs delivery has the ability to erode Aβ and reduce inflammation in the aged mouse retina and what impact this has on retinal function. This was based on our prior observation that CDs had the ability to enter the retina via the conjunctiva. We explore this in normal aged mice but also ask if it has similar abilities in terms of Aβ and inflammation alone in aged complement factor H mice ($Cfh^{-/-}$) that have been proposed as a murine model of AMD as it shares a genotype with 50% of AMD patients (Coffey et al., 2007). While there remain significant questions regarding mouse AMD models due to the absence of a macular, the aged $Cfh^{-/-}$ mouse does experience elevated deposition and inflammation and has reduced photoreceptor numbers and compromised visual function (Hoh Kam et al., 2013).

2. Materials and methods

2.1. Animals

8-9 months old C57BL/6 and 6–7 months old $Cfh^{-/-}$ mice which were backcrossed onto C57BL/6 genetic background for more than 10 generations were used. Animals were housed under a 12/12 light dark cycle with access to food and water ad libitum. All animals were used with University College London ethics committee approval that conformed to the United Kingdom Animal License (Scientific Procedures) Act 1986 (UK). UK Home Office project license (PPL 70/6571).

2.2. Treatment regime

C57BL/6 mice (n = 10) were treated with 3 μl of 10% 2-Hydroxypropyl-β-cyclodextrin (β-CD) (Sigma Aldrich, UK) in phosphate buffered saline (pH 7.4) as eye drops bilaterally 3 times daily for 3 months. Controls (n = 5) were untreated.

The $Cfh^{-/-}$ mice were divided into 3 groups. The first (β-CD LT) was treated with 3 μl of 10% 2-Hydroxypropyl-β-cyclodextrin (β-CD) (Sigma Aldrich, UK) as above as eye drops 3 times a day for 3 months (n = 10). The second (control) of $Cfh^{-/-}$ mice (n = 5) was left untreated. The third group (β-CD ST) was treated with 3 μl of 10% β-CD (Sigma Aldrich, UK) as above as eye drops 3 times a day for 3 days per month for 3 months (n = 5).

2.3. Electroretinogram (ERG)

After treatment C57BL/6 animals were given full field flash ERG to assess retinal function in response under scotopic and photopic conditions similar to Hoh Kam et al. (2013) using the ColorDome Ganzfeld ERG (Diagnosys LLC, Cambridge, UK). Mice were dark-adapted overnight for scotopic measurements and anaesthetised with 6% Ketamine, (National Veterinary Services Ltd, UK) 10% Dormitor, (National Veterinary Services Ltd, UK) and 84% sterile water at 5ul/g intraperitoneal injection. Pupils were dilated (1% Tropicamide, MINIMS, Bausch & Lomb, France) prior to recordings. Ground and reference subdermal electrodes were placed subcutaneously near the hindquarter and between the eyes respectively and the mouse placed on a heated pad (37 °C). Recording gold electrodes were placed on the cornea. ERG was carried out under scotopic conditions for both eyes simultaneously, with increasing stimulus strengths using a 6500 K white light at; 3.5×10^{-6}, 3.5×10^{-5}, 3.5×10^{-4}, 0.03, 0.3, 2.8 and 28.1 cd s/m^2. After the scotopic series mice were adapted to a 20 cd/m^2 background for 20 min. Then photopic responses to white light flash stimuli of 0.3, 2.8, 28.1 and 84.2 cd s/m^2 were recorded with a background light of 20 cd/m^2. An average of 20–25 readings were taken for each intensity. Statistical differences between groups were evaluated by using random ANOVA.

2.4. Immunohistochemistry

After ERGs, C57BL/6 mice were culled by cervical dislocation as were the $Cfh^{-/-}$ mice from which recordings were not undertaken. Eyes were collected and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), pH 7.4, for 1 h, cryopreserved in 30% sucrose in PBS and embedded in optimum cutting temperature (OCT) compound (Agar Scientific Ltd). 10 μm cryosections were thaw-mounted on a slide and incubated for 1 h at room temperature in a 5% Normal Donkey serum in 0.3% (v/v) Triton X-100 in PBS, pH 7.4. This was followed by an overnight incubation with either a mouse monoclonal antibody to Aβ 4G8 (1:100, Covance), a mouse monoclonal antibody to RPE65 (1:500, Merck Millipore, UK), both were conjugated with an Alexa Fluor 568 (Invitrogen, UK), or a rat monoclonal antibody to complement C3b (C3b) (1:50, Hycult biotechnology) diluted in 1% Normal Donkey Serum in 0.3% Triton X-100 in PBS. For the $Cfh^{-/-}$ mice, we used a goat polyclonal to complement C3 (1:500, Cappel MP Biomedicals, Cambridge, UK). After primary antibody incubation, sections were washed several times in 0.1 M PBS then slides stained for active C3b were incubated in a secondary antibody, donkey anti-rat conjugated with Alexa Fluor 488 (Invitrogen, UK) and for a donkey anti-goat conjugated with Alexa Fluor 488 (Invitrogen, UK) for C3, made up in 2% Normal Donkey Serum in 0.3% Triton X-100 in PBS at a dilution of 1:2000 for 1 h at room temperature. Negative controls were undertaken by omitting the primary antibody. After secondary antibody incubation, sections were washed and nuclei stained with DAPI. Slides were then washed in 0.1 M PBS followed by washes in Tris buffered Saline (pH 7.5). Slides were mounted in Vectashield (VECTOR Laboratories) and coverslipped.

For lipid detection retinal sections were stained with a saturated solution (3%) of Sudan Black B in 70% ethanol for 1 h at room temperature and then washed in several changes of distilled water. Slides were mounted in glycerol and then coverslipped.

2.5. Western blots

Eyes were dissected on ice and the retina and RPE-choroidal tissues were snap frozen in liquid nitrogen. Protein was then extracted by homogenising the samples in 2% SDS with protease inhibitor cocktail (Roche diagnostics), and centrifuged at 13,000 ×g. The supernatant was transferred to a new microcentrifuge tube and will be used for Western blots of C3 and RPE65. Aβ was extracted from the resultant pellet with 70% formic acid and the mixture was then centrifuged at 13,000 ×g. The supernatant was then