CASE REPORTS

Refsum's disease: electron microscopy of an iris biopsy

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Abstract

Refsum's disease (phytanic acid storage disease) results in an accumulation of lipid as a result of an absence of mitochondrial hydroxylation of phytanic acid. We describe a previously unrecorded electron microscopic study of lipid deposition in the iris pigment epithelium of a patient with Refsum's disease, and lipid was present in other sites within the iris.

Refsum's disease (hereditary ataxia polyneuritis^{1,2}) is an autosomally recessive inherited absence of α hydroxylation of phytanic acid (3,7,11,15 tetramethylhexadecanoic acid), a breakdown product of chlorophyll found especially in dairy products. The disease is manifested by a chronic polyneuropathy and cerebellar ataxia with retinitis pigmentosa and a variety of other ocular signs (Table). Symptoms begin in the second or third decade with an insidious, occasionally remitting course.

Pathological reports on Refsum's disease have led to the findings of phytanic acid and lipid accumulation in the liver, kidneys, heart, and central nervous system.³ Peripheral nerve studies show loss of myelinated nerve fibres with hypertrophy and 'onion bulb' whorls of Schwann cells⁴ and perineural fibrosis. Phytanic acid in low concentrations has been recovered from peripheral nerves and in much higher concentrations from the retina and ciliary body.⁵

One necropsy report of the appearances within the eye showed complete loss of photoreceptors, thinning of the inner nuclear layer, and reduction in the number of ganglion cells of the retina.⁶ Sudan III and Sudan black stains demonstrated lipid loading in retinal vessels. Lipid had also accumulated in the pigment epithelium of the retina, ciliary body, trabecular meshwork round the canal of Schlemm, and in both sphincter and dilator muscles of the iris, but not within the pigment epithelium in this case.⁶

Case report

Iris of a 36-year-old man was obtained after an extracapsular cataract extraction and lens implant and broad iridectomy. A diagnosis of retinitis pigmentosa was made clinically 15 years ago after he had presented with night blindness and tunnel vision. There was no family history of the disease. Three years later he developed a mixed sensory-motor polyneuropathy. A raised

TABLE Ocular and non-ocular signs in Refsum's disease

Ocular	Non-ocular
Retinitis pigmentosa	Peripheral neuropathy
Cataracts	Cerebellar ataxia
Miosis	Deafness/anosmia
Ophthalmoplegia	Goitre
Ptosis	Ichthyosis
Glaucoma	Cardiomyopathy

serum phytanic acid and sural nerve biopsy confirmed the diagnosis of Refsum's disease.

Ocular examination prior to surgery revealed visual acuities of 6/60 in both eyes, unreactive miosis, bilateral posterior subcapsular cataracts, and extensive 'bone-corporcular' retinitis pigmentosa. The iris was fixed in formalin and glutaraldehyde and processed for light and electron microscopy.

Results

Vacuoles of lipid, manifested by empty spaces, were visible in the biopsy. These were partic-

Figure 1: Vacuoles of lipid (arrowed) dissolved out during processing, visible as spaces within the iris sphincter muscle, which is stained immunohistochemically with antibodies to desmin filaments; a capillary is indicated by the star. ($\times 450$.)

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Figure 2: A, B, C: Lipid droplets within the iris stromal cells and pigment epithelial cells. D: These droplets are of similar size to the larger melanosomes of the IPE. (EM, $\times 5200$ (A), $\times 24\,500$ (B, C, D).)

ularly easily seen within the sphincter muscle, especially when the immunohistochemical stain with desmin antibodies was used (Fig 1). Unfortunately routine processing with toluene and alcohols removes lipid, and, though histochemical techniques, such as Nile blue sulphate and Sudan III and black were employed, unsurprisingly no specific staining was recordable. The electron microscopy of post-fixation osmified tissue confirmed lipid deposition in the anterior border cells and stromal cells (Fig 2A, B, C). Similar lipid was shown among the melanosomes of the iris pigment epithelium (IPE) (Fig 2D), a site at which no previous workers had demonstrated such abnormal lipid droplets, though ciliary body and retinal pigment epithelial

deposits have been shown.⁶ The deposits of fat within these IPE cells were of similar size to the larger melanosomes found within the pigment epithelium (Fig 2D). As it could be supposed that such lipid deposits were secondary to cataract formation, a further review was undertaken of a series of iris biopsies from patients with Fuchs's heterochromic cyclitis with cataract and their controls, five of each. These 10 samples were previously⁷ shown to have pigment epithelial cells covering a total of 53 grid squares, and a mean of 221 melanosomal areas were counted, per case, necessitating examination of many more. In this series no similar lipid inclusions to those seen in the Refsum's case were seen, though some of the control patients were noted to

Figure 3: Swollen and damaged unmyelinated axon lying within iris stroma, close to the sphincter. (EM, $\times 26\,000$.)

have the intragranular vacuoles illustrated in Zinn's classic paper on the melanosomes of the iris.⁸

Axonal damage was seen within an unmyelinated nerve fibre (Fig 3) lying close to the anterior border, and fixation appeared adequate, with organelle preservation elsewhere, so that this appearance is unlikely to be artefactual. Such myxoid damage is usually associated with myelinated nerve fibres in other sites in the body in Refsum's disease, but no myelin figures were seen in this biopsy.

Discussion

This demonstration of lipid droplets in the iris pigment epithelium shows lipid can be deposited in any part of the layer of pigment epithelium

derived from neuroectoderm. Although the role of the RPE as a phagocytic cell tissue devouring effete rod outer segments is incontestable, it is not usual to ascribe this function to the pigment epithelia of the ciliary body and iris. The effects of accumulation of phytanic acid residues in nervous tissues may be primary, as is thought to be the case in myelin synthesis⁹ with incorporation of a 'thorny' molecule instead of straight chain fatty acids. A similar mechanism may occur in the retina in membrane lipids, with consequent low lineoleate levels, known to influence electroretinograms.¹⁰ The accumulation in pigment epithelia might be unrelated to the primary disorder or secondary effect which produces a clinical pattern similar to classical retinitis pigmentosa.

The ocular effects of this disease may therefore be primary leading to ptosis, miosis, ophthalmoplegia, and retinal malfunction, or secondary with further retinal damage due to RPE disease. Cataract may also be secondary to the accumulation of lipid within the lens epithelium itself or to disruption of the function of the ciliary body, and physical disruption is seen in glaucomatous eyes.

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