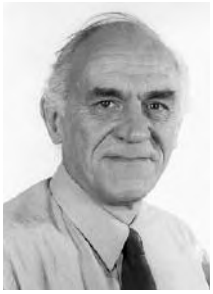
ORIGINAL ARTICLE



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Retinal Fundus Autofluorescence

It has been known for several years from histopathological studies that autofluorescence is present in the retinal pigment epithelium (RPE) due to the presence of lipofuscin. Lipofuscin is the name given to brown pigment granules composed of lipid-containing residues of lysosomal digestion.

The demonstration that the excitation spectrum of the "orange-red" fluorophores extended into the visible range indicated that imaging of lipofuscin was accessible to in-vivo excitation.[1] However, in-vivo human autofluorescence recording using spectrophotometric techniques[2] and imaging with a confocal scanning laser ophthalmoscope[3] (cSLO), are relatively recent. Several lines of evidence indicate that the image is derived from lipofuscin at the level of the RPE. The blue-green excitation light used at 488 nm and a short wavelength cut-off filter at 521 nm are appropriate for detecting autofluorescence from lipofuscin.[2,4] Delori demonstrated that the spectral characteristics of in-vivo fundus autofluorescence are consistent with that of lipofuscin,[2] with reference to the identification of individual lipofuscin fluorophores by Eldred.[5] The confocal nature of the optics of the scanning laser ophthalmoscope ensures that the autofluorescence recorded is derived from the ocular fundus provided that the focus is on the retina. That the source of autofluorescence is located external to the neurosensory retina[2] is supported by the decrease of autofluorescence at the macula due to absorption by neurosensory retina bearing luteal pigment and its increase in macular holes.[6] The lack of haemoglobin spectral lines indicates that the signal is derived from internal to the choriocapillaris. The distribution and intensity of fundus autofluorescence imaged with the cSLO is consistent with our knowledge of lipofuscin distribution derived from histologic studies.[7-9] The autofluorescence intensity is highest at the macula, has a foveal dip and decreases towards the periphery. The finding that autofluorescence intensity as measured by in-vivo spectrophotometric measurements at the fovea and at 7° temporal to the fovea[1] increases with age also corresponds with the knowledge derived from histologic studies.[7-9] Finally, deviation from the normal levels occurs with disease in an expected manner.

The source of autofluorescence is from various combinations of two molecules of all-trans retinal and one of ethanolamine (A2E).[10] This forms in the photoreceptor outer segment and is likely to be greatest in the distal discs.[11] The fluorophore is ingested by the RPE at the time of shedding and is present in the phagosome. It is resistant to lysosomal degradation accounting for the source of autofluorescence being in the long-term RPE phagolysosomes.

It is believed that the level of autofluorescence represents a balance between accumulation and clearance of lipofuscin. Accumulation of fluorescent material in the RPE reflects the level of metabolic activity which is largely determined by the quantity of photoreceptor outer segment renewal. Abnormally high levels of autofluorescence are thought to be due to RPE cell dysfunction or to the RPE being subjected to an abnormal metabolic load as occurs in Stargardt disease in which the discs would have abnormally high levels of A2E.[12] Evidence of clearance is derived from the observation that outer retinal degeneration is associated with decreased autofluorescence. This could be due to a variety of factors. There appears to be constant degradation of residual bodies in the retinal pigment epithelium.[13,14] There is evidence of photodegradation of A2E[15] and in addition, long-term phagolysosomes may be discharged from the RPE cells into the extracellular space.

The increase of RPE autofluorescence and residual body content with age are best approximated by a quadratic model.[16] The levelling off or reduction after the age of 70 years is predictable since the number of photoreceptors decreases in the elderly. There is a direct relationship between residual body content and autofluorescence, which is not surprising since the autofluorescence is derived from the residual bodies but the relationship, is not close with a low R^2. This could be explained by variation in dietary vitamin A. Rodents given a low vitamin A diet have little autofluorescence when compared with those on high diet yet the residual body content is little different between the two.[17]

It is now clear that autofluorescence imaging is useful for diagnosis in patients with visual loss and that certain inherited disorders have distinctive patterns of change.[18]

Perhaps, more important, is the ability to assess the state of the RPE/photoreceptor complex in ageing.[19] Until recently, the only index of ageing was the state of Bruch's membrane as indicated by the number, size and distribution of drusen. It is now possible to assess changes in the RPE, and it has been recognised for some years that the RPE plays a crucial role in the pathogenesis of age-related macular disease (AMD).[20] In AMD it is reported that geographic atrophy is preceded by focal increases in autofluorescence,[19,21] and this has given rise to concepts as to pathological processes in this form of late disease.[22] Lipofuscin is a free radical generator when illuminated with blue light.[23] It also acts as a surfactant that causes leakage of membranes and a rise in the pH of phagolysosomes with consequent predictable loss of activity of degradative enzymes.[24,25] In turn, lack of recycling from phagosomes may result in the lack of material for outer segment renewal and photoreceptor cell death.[26] In choroidal neovascularisation the likely outcome may be determined by the integrity of the RPE/photoreceptor complex prior to treatment. This can be verified on the basis of autofluorescence imaging.[27] If it is shown that therapeutic benefit can be predicted by autofluorescence imaging, it should become a routine part of the management of such cases particularly in the light of the therapeutic results of the new biological agents.

The value of autofluorescence imaging has been shown in clinical practice to give information that cannot be obtained by any other means and should be available to all clinicians who see patients with retinal degenerative diseases.

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