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The occurrence of cone inclusions in the ageing human retina and their possible effect upon vision: An electron microscope study

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Abstract

During normal ageing, photoreceptors of the human retina undergo various structural changes. We examined retinas from 33 donors (56 eyes; age span 13–94 years) by electron microscopy to see morphological changes in the cones with ageing. We show mitochondrial alterations and occurrence of electron-dense globules in the cone inner segments from the fifth decade of life. The globules are more prevalent in the macular cones than those in the mid-peripheral or nasal retinas ($p < 0.05$) and absent in peripheral retinal cones and rods. They peak in the sixth decade and then decline in the seventh decade ($p < 0.05$), from seventh to ninth decade, however, there was no significant change in their occurrence in the cones. We also show a type of inclusion, made up of bundled microtubules, which occur exclusively in the macular cones at the eighth decade of life. Evidence suggests that altered cone mitochondria with cristae remnants and dense matrix participate in globule formation in the ageing retina. Such mitochondrial changes may cause energy depletion, and bundling of microtubules (to form filamentous inclusions) could result in decreasing intracellular transport, in which case cones may die in the long run. These factors may be responsible for reported cone loss in the human retina with ageing.

Keywords: Retina; Ageing; Photoreceptors; Mitochondria; Microtubules; Lipofuscin granules

1. Introduction

During normal ageing, the mammalian retina shows structural as well as quantitative changes in its neuronal components. The retinal layers thin out and show associated decline in cell density, especially the ganglion cells and photoreceptors [1,9–11,15,16,26]. Morphological alterations in the fine structure of the retinal pigment epithelium and photoreceptors are also apparent with normal ageing of the retina. Marshall et al. [18] found that in the human macula, there is increased formation of localised convolutions and disorganisation of discs in the rod outer segments, all which begin in the fourth decade of life. Studies have shown the occurrence of refractile globules [25] in the cones, and lipofuscin granules in both cones and pigment epithelium of human retina [6,8,14] with ageing. A number of cone inclusions of various shape and size are reported to occur in the human retina during normal ageing, though their exact source and types, if any, are not known.

In the present study, we examined the fine structure of the human retinal cones in donor eyes at different ages. The aim was to see various alterations in their structure with normal ageing. We show occurrence of dense globules and their frequency in the cones from the fifth decade of life onward, and peculiar filamentous inclusions made up of bundled microtubules, in the eighth decade of life. The possible consequence of those changes in the cones upon vision in the elderly is discussed.

2. Materials and methods

2.1. Tissues

Human eyeballs from 33 donors were procured from the National Eye Bank, Dr. Rajendra Prasad Centre for Ophthalmic Science, AIIMS, New Delhi. The details regarding the age of the donors, sex, cause of death and *post mortem* delay in fixation are summarised in Table 1. Information about the donors was available from the hospital records. The technical staffs of the eye bank collected information about the donors from the death certificates as well as from the relatives of the donors. The eyeballs selected for this study comprised largely of normal donors who had no history of ocular diseases. Prior consent from the relatives of the deceased was taken for use of the residual part of the eyeball in research (a requirement under Institute Ethics Committee). Permission to use

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Table 1
Information about the donors whose eyeballs were used

Decade	Age[a]	Sex	Cause of death[b]	Postmortem fixation delay[c]
2nd decade	13	F	Diabetic ketoacidosis	2
	21	M	Road-traffic accident	5
	28	F	Systemic lupus erythematosus	30 min
4th decade	42	F	Road-traffic accident	4
	45	M	Heart attack	3
	46	F	Heart attack	4
	48	F	Heart attack	1
	49	M	Road-traffic accident	2
5th decade	50	M	Haemorrhage	2
	52	M	Road-traffic accident	3
	54	F	Road-traffic accident	1
	56	M	Myocardial infarction	2
	59	F	Heart attack	1
6th decade	62	M	Cardiac arrest	5
	63	F	Retro-pharyngeal abscess	3
	67	M	Heart attack	1
	68	M	Heart attack	6
	68	F	Heart attack	2
7th decade	70	M	Myocardial infarction	2
	72	F	Heart attack	4
	74	M	Heart attack	2
	75	M	Cardiac arrest	3
	78	F	Heart attack	2
8th decade	80	M	Heart attack	5
	80	M	Heart attack	3
	80	F	Cataract	4
	85	M	Myocardial infarction	4
	87	F	Myocardial infarction	1
9th decade	90	F	Heart attack	3
	90	F	Heart attack	5
	91	M	Heart attack	2
	93	M	Myocardial infarction	6
	94	F	Myocardial infarction	3

M, male; F, female.
[a] In years.
[b] Information obtained from case registry.
[c] In hours.

the eyeballs for this study was available from the officer-in-charge of the eye bank. The study was approved by the Institute Ethics Committee. A total of 56 eyes were examined by electron microscopy. One of the two eyes (left or right) was examined from each donor under the age range 13–52 years ($N = 10$), since occurrence of cone globules was either absent or the least in their retinas amongst all donors. The remaining eyes (46) examined were both eyes from 23 donors (age range: 54–94 years).

2.2. Fixation

After removal of lens and vitreous body, the eyes were fixed in a mixture of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 5–7 h at 4 °C. After fixation, the retina was separated from the adherent choroid and washed in PB. The macula, mid-peripheral and far peripheral retina (eccentricity: 3–5 and 10–14 mm from the foveal centre, respectively) along the temporal direction, near nasal retina (3–5 mm from the optic disc), and superior and inferior retina (3 mm above and below the optic disc, respectively) were cut. The macula was defined as a circumscribed retinal region located 4 mm temporal to the optic disc and arbitrarily subdivided into three consecutive areas starting from the foveal centre (labelled as subregions 1–3), supposedly representing the foveal, parafoveal and perifoveal regions. The first subregion cut was smaller in length (approximately 1 mm) than the rest two, which were cut into larger size (approximate length: 1.5 mm). The exact anatomical identification of those three subregions as the foveal, parafoveal and perifoveal regions was confirmed in histological sections (see below). After wash in PB, the tissue samples were post-fixed in 1% osmium tetroxide for 2 h at 4 °C and washed. They were dehydrated in ascending grades of acetone, infiltrated and finally embedded in araldite CY 212 (TAAB laboratories, UK).

2.3. Light microscopy and counting of cone globules

Retinas from 48 eyes of donors from fifth to ninth decades were employed for counting of cone globules. The observer (SC) was not informed about the age of the donors during counting. The regions examined included the macular (foveal, parafoveal and perifoveal regions), mid-peripheral temporal retina and near nasal retina, as already defined. Identification of macular subregions was done in semithin sections under light microscope, using rod cell abundance as a guide. The part of the section from the subregion 1 containing exclusively cones was considered to represent the fovea, while the sections showing cones and rods in ratios of 1:1 and 1:3 in subregions 2 and 3 were representatives of parafovea and perifovea, respectively. Outside the macula, in the temporal mid-peripheral and nasal retina, the rod number exceeded and the rod to cone ratio was approximately 10:1 in each region. The retinal regions from the superior and inferior quadrants and far periphery were not included, to limit sample number in count-