Sex-linked retinitis pigmentosa: ultrastructure of photoreceptors and pigment epithelium

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An ultrastructural study of a postmortem donor eye from a 24-year-old male patient with sex-linked retinitis pigmentosa showed abnormalities in all remaining cones and rods. Central foveal cones were reduced in number by about 50% and had shortened and severely distorted outer segments. Cones from the parafovea through the midperiphery gradually decreased in density and had no organized outer segments. In the far periphery, cones and rods had only slightly shortened outer segments. Photoreceptors equidistant from the fovea in all quadrants showed similar changes. The virtual absence of organized cone outer segments from the parafovea through the midperiphery was conspicuous in that this patient had full visual fields with large test lights 3 weeks prior to death. The pigment epithelium contained abnormally large numbers of melanolysosomes and few free melanin granules from the fovea through the midperiphery and few melanolysosomes and many free melanin granules in the far periphery. Whether or not these observations in the pigment epithelial cells represent a primary defect in this disease or reflect changes secondary to a defect in the photoreceptor cells remains to be defined.

Key words: retinitis pigmentosa, retina, retinal degeneration, sex-linked, cones, rods, pigment epithelium, melanin

Male patients with sex-linked retinitis pigmentosa characteristically have reductions in cone and rod electroretinogram (ERG) amplitudes, delays in cone and rod ERG b-wave implicit times, and reductions in early receptor potential (ERP) amplitudes by age 15.1,2 Psychophysical studies have shown elevated cone2,3 and rod3,4 thresholds in young affected patients. In patients with this genetic type, elevations of rod psychophysical thresholds have been linearly correlated with the amount of rhodopsin remaining as measured by retinal densitometry.5 As the condition progresses, ERGs become nondetectable, psychophysical cone and rod thresholds become very elevated, and visual fields are markedly constricted. Affected male patients usually report vision reduced to count fingers or hand motions by 35 to 45 years of age. The ultrastructural findings that could account for these functional abnormalities have not been previously defined. This report describes the ultrastructure of the cones, rods, and pigment...
Fig. 1. Fundus photograph taken 6 months prior to death of the right eye of patient with sex-linked retinitis pigmentosa. Findings appeared identical 3 weeks prior to death. Bone spicule pigmentation can be seen in the retina at the edge of photograph (A) about 45° anterior to the fovea in the superonasal quadrant and in a representative area in the far periphery (B) 45° to 60° anterior to the fovea. The choroidal vessel pattern appears prominent.

epithelium across the retina in a 24-year-old male patient with sex-linked retinitis pigmentosa.

Methods

The donor eye studied in this report was obtained from a 24-year-old blond white male patient with sex-linked retinitis pigmentosa. The patient’s family history revealed a brother, age 35, with severe constriction of his visual fields and advanced retinitis pigmentosa, a maternal uncle who became blind around age 40 with retinitis pigmentosa, and a maternal aunt who had some night blindness by age 30 but retained vision until her death at age 65. The patient’s mother had no visual symptoms but showed a 1 to 2 disc diameter area of bone spicule-type pigmentation in the temporal midperiphery of her right eye. The paternal side of the family had no history of retinitis pigmentosa. The findings of the carrier state for sex-linked retinitis pigmentosa in the patient’s mother as well as the history of an affected brother and maternal uncle helped to establish the sex-linked mode of transmission in this family.

The patient was aware of night blindness at age 10, although he reported that his night vision was better in the far peripheral field than in the central field. He denied any decrease in visual field. Three weeks prior to his death, his visual acuity with contact lenses was OD 20/80 and OS 20/50; refractive error was OD −6.75 +2.50 ×100° and OS −6.50 +2.50 ×72°. Pertinent findings in each eye included clear lenses, attenuation of the retinal vessels, and depigmentation of the pigment epithelium with visible choroidal vessels (Fig. 1A and B). Bone spicule-type pigmentation could be seen in the peripheral fundus beginning approxi-
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Both eyes of this patient were enucleated within 15 min after death. The right eye was prepared for ultrastructural studies as described previously. In summary, the posterior portion of the eye was fixed in 1% formaldehyde–2% glutaraldehyde in a 0.1M Sorensen’s phosphate buffer, pH 7.35 for 8 hr, briefly washed in buffer, postfixed in 2% OsO₄ for 1 hr, and embedded in Epon/Araldite. All quadrants of the retina were systematically surveyed in the light microscope from the foveola to the ora serrata with 1 μm sections. Selected areas were examined with a JEOL 100C electron microscope (JEOL USA, Inc., Medford, Mass.). The left eye of this patient was used for biochemical and tissue culture studies. Eyes which served as controls were obtained from a 15-year-old girl 1½ hr after accidental death and a 24-year-old woman immediately after enucleation for a ciliary body melanoma; these eyes were fixed and examined in the same manner.

Results

In the donor eye with sex-linked retinitis pigmentosa, photoreceptor cells in the fovea (central 1.5 mm) are reduced in number by about 50%; the configuration of synaptic terminals reveals that all these remaining photoreceptors are cones. Their inner segments are approximately twice normal diameter and contain swollen mitochondria (Fig. 2). Outer segments are distorted, severely shortened, or absent (Fig. 3); some outer segments are so severely distorted that an accurate estimation of their total length could not be obtained. Discs within the outer segment are twisted and vesiculated (Fig. 3), and frequently only a cilium remains. Large autophagic vacuoles are seen in the perinuclear cytoplasm of many cones (Fig. 2). Pigment epithelial cells apposed to foveal cones contain large numbers of melanolysosomes, lysosomes, and few free melanin granules (Fig. 2). Their apical surfaces have bulbous protrusions that abut on remaining cone outer segments and on cone inner segments (Figs. 2 and 3). Some pigment epithelial cell nuclei are apically displaced.

The outer nuclear layer in the parafovea (annulus 1.5 to 3.0 mm in diameter centered on the foveola) is reduced to a single row of cells (Fig. 4). Remaining cones in this region have little or no inner segments distal to the

mately 45° anterior to the fovea (Fig. 1, A) and extending anteriorly to about 60° in all meridians (annulus 13.5 to 18 mm in diameter centered on the foveola). A representative peripheral fundus photograph (Fig. 1, B) shows the pigment extending anteriorly from the area of a vortex vein (45°).

Visual field testing on the Goldmann perimeter showed generalized constriction to the 5° to 10° isopter with a II-4 white test light and to the 30° to 40° isopter with a III-4 white test light in each eye; fields were full with a V-4 white test light. Dark adaptation testing with an 11° white test light in the Goldmann-Weekers dark adaptometer showed a monophasic curve with no evidence of a rod-cone break; final dark-adapted threshold after 45 min was elevated 4.0 log units above normal final dark-adapted rod threshold and 1.4 log units above normal final dark-adapted cone threshold with the patient looking directly at the test light. In the far peripheral field, the patient reported thresholds that were only slightly above normal dark-adapted rod thresholds, but the precise level was not quantitated. Large field ERGs obtained under dark-adapted conditions showed a non-detectable rod response to a blue light stimulus and a response reduced 90% below normal to single flashes of white light. An electro-oculogram showed an abnormal light-peak to dark-trough ratio of 1.1 in each eye (normal ≥ 1.8). More extensive psychophysical and electrophysiological testing could not be performed 3 weeks prior to his death because of the patient’s poor general physical condition.

Six months prior to his death, this patient was hospitalized at the University of Wisconsin Medical Center because of recent onset of shortness of breath. Chest x-ray revealed bilateral pleural effusions. A thoracentesis showed hemorrhagic fluid containing embryonal-type large malignant cells. Over the next 6 months, he received many medications to try to arrest his condition, but he continued to develop recurrent pleural effusions that required frequent thoracenteses. Medications included Adriamycin, Cytosan, 5-fluorouracil, vincristine, 1-(2-chloroethyl)-3-cyclohexyl-N-nitrosourea (CCNU), bleomycin, and 11-cis-platinum. He developed severe leukopenia and bacteremia 2 weeks prior to death. He received gentamicin, meticillin, and carbenicillin but expired as a result of respiratory failure and aspiration pneumonitis. Postmortem examination revealed that the patient had an anaplastic mesothelioma originating in the pleural lining of the lung with some extension into the diaphragm. He showed no evidence of generalized metastases.
Fig. 2. For legend see facing page.
Sex-linked RP; retinal ultrastructure

Fig. 3. Central foveal cones have outer segment discs (OS) that are vesiculated and disrupted. Rough endoplasmic reticulum is prominent in apical portions of pigment epithelial cells. Apical microvillus processes (asterisk) of pigment epithelial cells abut on Müller cell microvillus process (M). (×16,700.)

Fig. 2. Cones in the central fovea have enlarged inner segments (IS) and distorted remnants of outer segments (OS). Autophagic vacuoles (arrows) are seen in the perinuclear cytoplasm. Pigment epithelial cells contain large numbers of melanolysosomes (1), lysosomes (2), and few free melanin granules. Apical protrusions of these cells extend between cone inner segments. (×9600.)

external limiting membrane, no organized outer segments, and large autophagic vacuoles in the perinuclear cytoplasm (Fig. 5). Pigment epithelial cells have basally placed nuclei and numerous melanolysosomes and lysosomes. Bulbous apical portions of these pigment epithelial cells contain stacks of rough endoplasmic reticulum, some melano-
lysosomes, and few free melanin granules (Fig. 5).

More anterior to the parafovea, cone density gradually decreases with increasing eccentricity in an annulus 3.0 to 18.0 mm in diameter centered on the foveola (Figs. 6 and 7a). The intervening space between cone cell bodies is filled by enlarged Müller cell processes. Inner segments of remaining cones barely project past the external limiting membrane, and the Müller cell processes abut directly on the pigment epithelium. The outer plexiform layer gradually becomes indistinct, and increasing disorganization of the inner nuclear layer can be seen (compare Figs. 6 and 7a). In the zone of bone spicule pigmentation (annulus 13.5 mm to 18.00 mm in diameter), only occasional cone cell bodies are seen. Some pigment-laden macrophages are seen between the inner nuclear layer and the external limiting membrane (Figs. 7a and 7b). Macrophages are identified by the numerous vacuoles containing melanin granules in various stages of digestion. Similar pigment-containing macrophages were seen within the retina from the fovea through the midperiphery. Pigment epithelial cells throughout the zone of bone spicule pigmentation contain many melanolysosomes but are largely depleted of free melanin granules.

An increase in the numbers of photoreceptor cell nuclei in the outer nuclear layer can be seen at the anterior border of the zone of...
Fig. 5. Parafoveal cones have no organized outer segments, and small portions of their inner segments extend beyond the external limiting membrane (asterisk). Autophagic vacuoles (arrows) are present in cone cell bodies. Protrusions of the pigment epithelial cells extend proximal to their apical tight junctions (arrowheads). (×4200.)

Fig. 6. Cone density gradually decreases in an annulus 3.0 to 13.5 mm in diameter centered on the foveola. Cones (arrows) have inner segments which barely project beyond the external limiting membrane. (×740.)
bone spicule pigmentation (Fig. 8). Inner segments extend well beyond the external limiting membrane, and short outer segments are also apparent (Fig. 8).

In the far periphery, anterior to the zone of bone spicule pigmentation, large numbers of rods are seen in all quadrants (Fig. 9). Although outer segment length varies, most rods have outer segments reduced at least 25% in length (Fig. 9) when compared with those seen in a representative section taken from a normal eye in the same region (Fig. 10). Discs within the outer segments are generally well oriented along the long axis of the cell (Figs. 11 and 12). Rod inner segments are filled with long, well-ordered mitochondria (Fig. 12), and the cell bodies and synaptic terminals (Fig. 13) appear normal. Cones in the region anterior to the zone of bone spicule pigmentation have outer segments shortened at least 25% in length (Figs. 9 and 11), and outer segment discs appear disoriented and vesiculated (Fig. 14). In contrast to the fovea, mitochondria in the cone inner segments appear normal, and no large autophagic vacuoles are seen in cone cell bodies. Cone synaptic terminals appear normal (Fig. 15).

The pigment epithelial cells in the far periphery contain many free melanin granules in the bulbous apical portions and in the microvillous processes (Figs. 11, 12, and 14). Microvillous processes surround cone and rod outer segments, frequently up to the level of the inner segment, and they often are distended (Figs. 11, 12, and 14). Several phagosomes containing outer segment membranes and few melanolysosomes are seen in the pigment epithelium in this region.

Discussion

This ultrastructural study of an affected male patient with moderately advanced sex-linked retinitis pigmentosa has demonstrated that all remaining cone and rod photoreceptors across the retina were abnormal. Remaining central foveal cones had shortened and severely distorted outer segments. Cones gradually decreased in density from the parafovea to the midperiphery; remaining cones in this region barely projected beyond the external limiting membrane and had no organized outer segments. An increase in cone density occurred at the anterior border of the zone of bone spicule pigmentation in all quadrants. In the far periphery, anterior...
Fig. 7b. Pigment containing macrophage (M) within the zone of bone spicule pigmentation is located proximal to the external limiting membrane (arrows) and contains melanin in various stages of digestion. (×5800.)

Results of psychophysical and electrophysiological tests on this patient prior to death could be correlated with these ultrastructural findings. Three weeks prior to death, this patient showed no detectable rod function on dark-adaptation testing of the central retina.

to the zone of bone spicule pigmentation, remaining cones and rods had outer segments that were shortened at least 25% below normal. Photoreceptors equidistant from the fovea in all quadrants showed similar changes.
Fig. 8. Increase in the number of nuclei in the outer nuclear layer and the appearance of inner and outer segments of photoreceptors occur at the anterior border of the zone of bone spicule pigmentation (right of large arrow). Three outer segments are identified by small arrows. (×530.)

Fig. 9. Rod (R) and cone (C) are found in the far periphery of the donor eye with sex-linked retinitis pigmentosa. Rod and cone outer segments are reduced below normal in length; pigment epithelial cells are filled with melanin granules. (×750.)
Fig. 10. Representative section of retina in the far periphery (similar area to Fig. 9) from normal eye. R, Rod; C, cone. (x750.)

and no rods were seen in this area. The patient reported a minimally elevated rod threshold in the far periphery that was consistent with rods in the periphery that were slightly reduced with respect to outer segment length. Visual acuity was reduced to 20/80, and visual fields were constricted with small test lights but were full with large test lights; these findings were consistent with moderately reduced cone density in the central fovea and very reduced cone density in the midperiphery. Marked reduction in ERG amplitudes and a decrease in the light-rise to dark-trough ratio of the electro-oculogram could be accounted for by widespread impairment and loss of photoreceptor cells.

The virtual absence of cone outer segments from the parafovea through the midperiphery was conspicuous in that this patient had full visual fields with large test lights prior to death. Previous studies in rodless mice with hereditary retinal degeneration7–9 and in dystrophic10 and light-damaged11 rats have emphasized that visual discriminations could be performed with few photoreceptors and apparently no outer segment material. Remaining visual field in the parafovea and midperiphery in this patient appeared to depend primarily on the cell bodies and intact cone synaptic terminals rather than on organized outer segments.

Small autophagic vacuoles have been seen in normal photoreceptors and have been thought to represent a stage of normal degradative activity within these cells.12 Large autophagic vacuoles seen in foveal cones of this eye as well as foveal cones of a patient with probable dominant retinitis pigmentosa6 could represent increased degradative activity within these cells. Both patients had cancer and received antimetabolite therapy prior to death, so that a toxic effect of antimetabolite drugs on the foveal cones, lead-
Fig. 11. Representative cone (C) and rod (R) in the far periphery of donor eye with sex-linked retinitis pigmentosa show outer segments slightly shortened in length. Inner segments of photoreceptor cells appear normal. Swollen microvillous processes of the pigment epithelium extend up to the inner segments. Free melanin granules are prominent in the apical portion of the pigment epithelium. (×3200.)
Fig. 12. In the far periphery, well-ordered discs are seen in a shortened rod outer segment. Outer segments are surrounded by microvillous processes of the pigment epithelium, some of which extend up to the inner segments and are distended. (×19,700.)
Fig. 13. Rod synaptic terminal in the zone anterior to bone spicule pigmentation contains a synaptic ribbon (arrow) surrounded by vesicles. Many postsynaptic processes invaginate into the terminal and are filled with vesicles. (× 38,100.)

ing to large autophagic vacuoles, cannot be excluded. Electron microscopic studies of additional postmortem donor eyes with retinitis pigmentosa could help to determine whether or not large autophagic vacuoles are a characteristic pathological finding in all genetic types.

In this donor eye from a young patient with sex-linked retinitis pigmentosa, the pigment epithelial cells contained abnormally large numbers of melanosomes and few free melanin granules from the fovea through the midperiphery and many free melanin granules with few melanosomes in the far
Fig. 14. Cone outer segment discs are vesiculated and disoriented. Outer segments are surrounded by distended microvillous processes of the pigment epithelium. (×14,600.)

periphery (Figs. 2 and 11). Large numbers of melanolysosomes were also seen in the foveal pigment epithelium of an elderly patient with advanced dominant retinitis pigmentosa. Whether or not these observations in pigment epithelial cells represent a primary defect in these two genetic types or reflect changes secondary to a defect in photoreceptor cells remains to be defined.

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Fig. 15. Cone synaptic terminal in the far periphery has numerous synaptic ribbons and appears normal. (×11,000.)

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