Ceroid-Lipofuscinosis (Batten’s Disease)

Sequential Electrophysiologic and Pathologic Changes in the Retina of the Ovine Model

R. J. Graydon and R. D. Jolly

The sequential electrophysiologic and pathologic changes in the retina in ceroid-lipofuscinosis were recorded in a time-course study in the ovine model. Over a relatively short period in the course of the disease, a severe reduction in both rod and cone b-wave amplitudes developed with rod b-wave changes preceding those of cones. These changes paralleled a similar loss of rod and cone photoreceptor cells. In affected retinas, outer segments appeared shorter than normal. By 84 weeks of age, the outer nuclear layer was reduced to the width of a single nucleus. In addition to these changes, electronmicroscopy showed the formation of abnormal dystrophic rod and cone outer segments in photoreceptor cells. Most cells in the retina showed the accumulation of a fluorescent lipopigment, this being most prominent in ganglion cells. Ultrastructural studies showed them to be made of electron dense granular material and a variety of membranous and tubular arrays. Invest Ophthalmol Vis Sci 25:294-301, 1984

The ceroid-lipofuscinoses are a group of inherited storage diseases of humans and animals. They are characterized by the accumulation of fluorescent lipopigments in neurons and a wide variety of other cell types. In humans there are distinct subtypes such as the infantile form (Haltia-Santavuori), late infantile form (Jansky-Bielschowsky disease), juvenile form (Stengel-Batten-Spielmeyer, Vogt-Mayou-Sjogren disease), and an adult form (Kufs disease).

Clinically, the ceroid-lipofuscinoses are characterized by dementia, visual loss, ataxia, seizures, and premature death; but the entities vary in their age of onset, presenting signs, and age at death. With the exception of most adult forms, blindness is a constant feature, with loss of vision being the usual presenting sign in the juvenile form of the disease. Electroretinography has been performed in many patients with ceroid-lipofuscinosis with reduced or extinguished responses being recorded.

Pathologic descriptions of retinal changes in all forms of the disease have been from patients with terminal disease. There is a severe loss of rod and cone photoreceptor cells, but changes to the remaining layers of retina are minimal apart from the presence of characteristic lipopigment granules. Ganglion cells, in particular, contain the largest amounts of such storage pigment. Ultrastructural studies confirm light microscopic findings. Storage bodies are noted in all layers of the retina, including pigment epithelium.

In the late infantile and juvenile forms of the disease, these are largely made up of membranous, curvilinear and fingerprint profiles, and of granular osmiophilic material in the infantile disease. In the English setter canine model, severely reduced vision occurs late in the course of the disease. There is 30–63% reduction in a- and b-wave amplitudes, and the c-wave is replaced by a negative potential. The visual-evoked response is depressed. There is, however, no obvious loss of—or damage to—photoreceptors or pigment epithelium, even though these cells harbor massive amounts of autofluorescent lipopigment. The pigment epithelium contains peculiar circular, semicircular, and straight stacks of membranes not always surrounded by a limiting membrane.

From the Department of Veterinary Pathology and Public Health, Massey University, Palmerston North, New Zealand.

Supported by the United States National Institute of Neurological and Communicative Disorders and Stroke, Grant No. NS 11238.

Submitted for publication: June 20, 1983.

Reprint requests: R. D. Jolly, PhD, Department of Veterinary Pathology and Public Health, Massey University, Palmerston North, New Zealand.
In contrast to the English setter, the ovine model presents with loss of vision and, as such, resembles the juvenile form of human disease. Advanced cases may show slight attenuation of retinal vessels. Histologic examination revealed loss of photoreceptors and accumulation of lipopigment in various layers of the retina. In the present study, the sequential electrophysiologic and histopathologic changes in ceroid–lipofuscinosis are followed using affected lambs of different ages. The purpose was to understand the pathogenesis of the disease process in the retina and to develop an accurate means of monitoring stages of the disease.

Materials and Methods

Animals

The seven South Hampshire lambs with ceroid–lipofuscinosis used in this study were bred in an experimental flock of heterozygous ewes mated to a 9-month-old homoygously affected male. Diagnosis was made by histopathology of brain biopsies at 3–4 months of age prior to the onset of clinical signs. Control animals were six unrelated lambs matched for age and sex, but not breed.

Electroretinography

A corneal contact lens incorporating a silver-ring electrode was fabricated from 2.0 mm clear methacrylate sheet using a modification of a technique described by Witzel et al. For electroretinography, anesthesia was induced with intravenous sodium pentobarbitone and maintained with 1–2% halothane and oxygen. Dilatation of the pupil was obtained by instilling 1% cyclopentolate and 10% phenylephrine hydrochloride drops into the eye. The contact lens was filled with a methylcellulose-sodium chloride mixture to form a conducting salt bridge and inserted into the eye. A reference electrode was inserted under the midline skin on the bridge of the nose and a ground electrode was attached to the sheep’s ear.

Photostimulation was provided by a Grass PS22 photostimulator (Grass Instrument Co., Quincy, MA), which gave a flash of 10 μsec duration. The resulting retinal response was amplified 2000 times and the average of 32 responses displayed on a storage oscilloscope and photographed with a polaroid camera to provide a permanent record of the electroretinogram (ERG). The photostimulator was positioned 30 cm from, and at 90° to, the corneal surface. Photopic ERGs were recorded using white light at a relative intensity setting of 8 and a flash frequency of 2 per sec. The animal was then dark-adapted for 20 min before scopic ERGs were obtained using blue light at relative intensity 2 and a flash frequency of 1 per sec. ERGs were recorded from six affected sheep from 20–66 weeks of age to study the sequential pattern of retinal degeneration in ceroid-lipofuscinosis. Following electroretinography, the animals were allowed to recover from anesthesia, or anesthesia was maintained for surgical enucleation of an eye for histopathologic examination. The second eye was enucleated at a later date, immediately after electroretinography and euthanasia. Thus, a series of eyes was available for histopathologic study spanning the time period over which ERGs were recorded.

Preparation of Retina for Microscopy

The anterior segment of each enucleated eye was removed with the aid of a razor blade incision just posterior to the corneal-scleral junction. Vitreous humor was removed carefully and the posterior segment was fixed overnight in a mixture of cold 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate or cacodylate buffer pH 7.2. Following primary fixation, the tissue was washed three times with appropriate buffer. Small pieces of retina and the underlying choroid and sclera were obtained by a 2.0 mm punch from areas adjacent to the optic disc, as well as the mid and peripheral regions. Samples were taken from the tapetal and nontapetal portions of the nasal and temporal segments of the retina. These tissues were post-fixed for 2 hr in 1% osmium tetroxide in 0.1 M phosphate or cacodylate buffer before dehydration in graded alcohols and propylene oxide and embedding in epoxy resin (Durcupan–ACM, Fluka, Switzerland). Sections for light microscopy were cut at 0.5–1.0 μm and stained with 1% toluidine blue for 45 sec on a hot plate at 80°C. Appropriate areas for thin sectioning were cut at 70 nm and stained with saturated uranyl acetate and lead citrate.

The remaining posterior segment was divided in an anterior–posterior direction through the optic disc, and the two halves were embedded in paraffin wax by routine methods. Sections were prepared and stained by periodic acid-Schiff (PAS) and Sudan black methods. Unstained deparaffinised sections for fluorescent microscopy were examined with a Reichert Immunopan microscope under blue light of 500 nm wave length.

Cell Counting

The relative number of photoreceptor cells present in each retina was estimated by counting the nuclei
in the outer nuclear layer over a standard length provided by an eye piece graticle. Counts were done on toluidine blue stained epoxy embedded sections of retina adjacent to the optic disc.

Results

Electroretinography

The sequential change in cone dominant b-wave amplitudes in white light stimulated, light adapted sheep is plotted relative to age and cone b-waves of normal control animals (Fig. 1). All but one recording was below normal but a rapid decline in cone b-wave amplitudes did not commence until approximately 52 weeks of age, and they could still be measured at a greatly reduced amplitude at 66 weeks when the experiment was concluded. In contrast, rod dominated b-wave amplitudes (Fig. 2) measured in dark adapted animals declined rapidly at an earlier age and were virtually extinguished by 66 weeks of age.

Histopathology

Light microscopy of sections of retina from lambs of different ages showed a progressive loss of nuclei in the outer nuclear layer (Fig. 3). Their decline in numbers per unit length approximately matched the decline in b-wave amplitudes but some remained when rod dominated b-waves virtually were extinguished. These were presumed to be nuclei of cone photoreceptor cells, as cone inner segments were proportionally more common by this stage. In the retina of an additional sheep examined at 84-weeks-of-age, the outer nuclear layer was reduced to a single row of nuclei closely adjacent to the pigment epithelium. At all stages from 20-weeks-of-age, the rod receptor cell outer segments appeared shorter than those of normal controls (Fig. 3). In later stages, cone inner segments appeared shorter and rounder, this result being interpreted as a change due to loss of lateral support.

Initially, the degeneration and loss of photoreceptor cells was more severe in the central areas of the retina, but eventually all areas were involved. In contrast to the outer nuclear layer, there was no significant loss of cells contributing to the inner nuclear or ganglion cell layers in the period of study.

Sudanophilic, PAS-positive, autofluorescent granules were noted in almost all cell types in all the affected retinas studied. They were seen readily in both the nonpigmented tapetal area of the pigment epithelium, as well as the pigmented area after bleaching of melanin with hydrogen peroxide. However, they were relatively smaller and fewer in number than elsewhere in the retina. These storage bodies were most concentrated in ganglion cells and least often seen in the plexiform layers. At low power, they formed an obvious band of sudanophilic material in the outer nuclear layer adjacent to the outer limiting membrane. In contrast to the above storage lipopigment, a small number of smaller bodies with similar staining characteristics were
Fig. 3. Light micrographs of (a) normal lamb retina, (b) lamb with ceroid-lipofuscinosis at 48-weeks-of-age and (c) lamb with ceroid-lipofuscinosis at 66-weeks-of-age. There is progressive loss of nuclei in the outer layer and shortening and/or partial collapse of outer segment layer. Epoxy resin ×150.

Fig. 4. Electronmicrograph of a ganglion cell containing electron dense storage bodies of lipopigment ×22,000. The inset shows similar storage bodies at high magnification showing membranous, tubular and crystallloid arrays ×42,500.
tubular structures in a membranous matrix were found bodies, membranes formed a variety of whorled and crystalloid formations were noted occasionally (Fig. 4). They varied considerably in size and frequently were seen to be surrounded by a limiting membrane. Some of the larger irregular bodies appeared to be aggregations of smaller ones. In most bodies, membranes formed a variety of whorled and stacked profiles conforming to patterns commonly described as membranous arrays. In the ganglion cells, tubular structures in a membranous matrix were found and crystalloid formations were noted occasionally (Fig. 4).

As noted by light microscopy, there was a progressive loss of photoreceptor cells, with that of rod cells predominating. The resultant spaces were occupied by pale amorphous granular material, with few organelles interpreted as cytoplasm of supportive Müller cells. Rod or cone photoreceptor cells were seen only occasionally in the process of degeneration. The earliest, and most specific, change noted was formation of dystrophic outer segments in which the membrane layers were formed irregularly (Figs. 5a and b). In the two cells depicted and in others, the remaining cytoplasm and organelles were relatively normal, other than for slight dilatation of endoplasmic reticulum, Golgi apparatus, and some mitochondria. Kinked distorted outer segments were noted occasionally (Fig. 6).

There was no apparent loss of cells in the inner nuclear layer, but many contained typical storage bodies. In addition, some cells showed vacuolation associated with coarse membranous material dissimilar to that of storage bodies. This also was seen occasionally in photoreceptor cells (Fig. 7).

The pigment epithelium remained relatively normal throughout the disease process, apart from the spatial distortion of its processes, associated with the progressive loss of photoreceptor outer segments with which they normally interdigitate. Their cytoplasm contained numerous electron-dense membranous structures, and there was some difficulty in differentiating some storage bodies from partially digested outer segments. Storage lipopigment was also found in pericytes of the choriocapillaris.

Discussion

Clinically and morphologically ovine ceroid–lipofuscinosis has features most in common with the late infantile and juvenile forms of human disease. In terms of deteriorating vision as the presenting sign, it most resembles the juvenile disease. Atrophy of the optic disc, vascular attenuation, and abnormal pigmentation of the macula and peripheral fundus, which are common ophthalmoscopic findings in the human syndromes, were not features of the ovine disease. Slight attenuation of retinal vasculature was noted late in the disease but was not sufficient to be of diagnostic significance.

The ovine retina is of the mixed rod and cone type (Fig. 3a) and electronretinograms of sheep are similar to those from other mixed retinas. In the present time-course study, there was severe deterioration of both cone and rod dominant b-wave amplitudes relative to age, but rod changes were more severe and preceded those of the cones. These findings are in accord with those of the histopathologic studies that showed a loss of both types of cells, but that of rod photoreceptors predominated.

Although there may be significant variations in ERG amplitudes between recordings of individual sheep, or between sheep, the reduction in rod and cone b-wave amplitudes was rapid and took place within a relatively discrete time interval in the natural course of the disease. Sequential electroretinography, therefore, could provide a sensitive method of monitoring the course of disease, particularly, in experiments designed to test potential therapeutic strategies. These studies confirm and extend information currently available from case studies of human patients and mainly reflect late or end-stage retinal changes. The present data differ from that recorded in the English setter dog, the alternate well-developed animal model of the human ceroid–lipofuscinoses. In the dog, blindness is not a particular feature, although deterioration of vision is recorded late in the course of the disease. It is not associated, though, with any significant loss of photoreceptor cells.

Apart from the loss of photoreceptor cells, degenerative changes not previously reported in this group of diseases were noted. These were the shortening of outer segments (Fig. 3) and, less commonly, the presence of irregular dystrophic outer segments as depicted in Figures 5a and b. The shortening may have been more apparent than real and due to collapse and kinking of outer segments (Fig. 6) associated with loss of lateral support as cell numbers decreased. The fact that it proved difficult to obtain sections of affected retina showing the full length of outer segments in the one plane (Fig. 3b) supports this hypothesis.

There was no significant loss of cells in other layers of the retina that, apart from the presence of electron-dense storage bodies and in some cells vacuolation
with coarse aggregations of membranous material, were of normal histologic appearance. The storage bodies were similar in appearance to those described previously in the central nervous system and other organs of affected sheep5,6 and of human patients.22 Their morphologic variations have not been emphasized because of their great variability and the fact that the nosologic significance of these variations is unknown. Some of the larger bodies appeared to be aggregations of smaller bodies and many were clearly membrane-bound and compatible with the structure of secondary lysosomal storage bodies.

The underlying biochemical anomalies and pathogenesis of lesions in the ceroid-lipofuscinoses are not known. The accumulation of fluorescent lipopigment with tinctorial and staining properties similar to ceroid and lipofuscin gave rise to the name by which this group of disease commonly is known.23 It also resulted in the hypothesis that the pathogenesis of these disease entities, in some way, reflect the pathogenesis of these pigments.23 This is believed to be associated with free radical mediated peroxidative damage to unsaturated fats with breakdown to malondialdehyde and the formation of Schiff base polymers. Few would dispute this for the pigment known as ceroid, a yellow fluorescent lipopigment, which develops as a degenerative
change in abnormal accumulations of lipids. This is presumably due to local deficiency of free radical protective mechanisms. Despite the long-held belief that similar changes are basic to the pathogenesis of the so-called ceroid-lipofuscinoses, there is no strong experimental evidence to support this with the exception of the recording of a deficiency of a phenylene-diamine mediated peroxidase in human patients. Later studies showed that in leucocytes, there was a deficiency in a soluble peroxidase with concurrent increase in more tightly bound enzyme. This suggests more an abnormal partitioning of enzyme, rather than a genetic deficiency per se, and that the finding is an epiphenomenon. If ceroid is present in the storage lipopigment, then we consider it more likely to be the result of consequential oxidation of abnormal accumulations of lipid, rather than reflecting the basic biochemical anomaly. Its presence is not needed to explain the fluorescence of the storage material, as thin layer chromatographic studies show a number of fluorescent components. This is supported by unpublished observations on isolated storage material from the livers of affected sheep in which at least 12 fluorophores are noted.

Alternative hypotheses concern a possible anomaly of retinoid and dolichol metabolism. There is no experimental support for the former in the ovine ceroid-lipofuscinosis model, but we do confirm enrichment of dolichol in ovine storage material. However, this may also be an epiphenomenon as dolichols are found enriched in lysosomal preparations and also increase in brain with age. The pathogenesis of the ceroid-lipofuscinoses, therefore, still remains an enigma. As the dystrophic changes in the membranous photoreceptor cell outer segments may reflect a primary change of pathogenic significance, further electronmicroscopic and biochemical studies of this organelle are warranted.
Key words: ceroid-lipofuscinosis, Batten’s disease, retina, electroretinogram, pathology, photoreceptor cells, storage disease, lipopigment

Acknowledgments

The authors wish to acknowledge help and advice from Dr. D. H. Carr, Dr. R. S. Clemmett, Mrs. P. Slack, Mr. R. Faulding, Mr. T. Law, the Massey University farm staff and the D.S.I.R. Electronmicroscopy Unit.

References