Atypical band-shaped calcific keratopathy with keratocyte changes

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SUMMARY The clinical features of a patient with atypical band keratopathy are described. Histochemical and electron probe analyses indicate that the granular deposits in Bowman's layer contain calcium and phosphate. An unusual feature in this patient was the presence of severe keratocyte degeneration; its possible role in the pathogenesis of this condition is discussed. Exfoliation of the calcified Bowman's layer appears to have been the basis for severe attacks of recurrent ocular pain.

The mechanisms of ectopic corneal calcification, although the subject of much experimental work which has been reviewed by Obenberger and his coworkers,^{1,2} remain largely speculative. In most patients the clinical appearance of a horizontal band-shaped opacification of the Bowman layer is associated with a variety of local and/or systemic diseases. Calcification is often referred to as 'secondary' in these cases to provide distinction from the much rarer hereditary or dystrophic variety in which no such associations are found.

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We recently observed a patient with atypical calcific opacities which gave rise to recurrent attacks of irritation and pain. Biopsy indicated that these features were associated with histological and ultrastructural changes of the keratocytes. Since some of the clinical and cytological features appear to be unique, we have compiled the following clinico-pathological report.

Case history

A 41-year-old male Nigerian academic of the Yoruba tribe was first seen in January 1980. He complained of recurrent episodes of redness and photobia with

Fig. 1 Right eye. Annular opacity in the pupillary zone of cornea. Jagged inner edge outlines central clear area.

Fig. 2 Left eye. Discoid opacity with fissures. A greyish reticulum within the opacity imparts a mosaic appearance.

Fig. 3 Corneal opacity in right eye is seen as black silhouette against fundus glow.

severe pain which had affected the left eye almost weekly for the previous 4 months. Similar occurrences had affected the right eye for a period of 3½ years, but they had ceased abruptly 6 months ago. No similar eye disorder was known to have occurred in any other members of his family. The patient's health had been unremarkable until the start of these ocular symptoms. There was no history to suggest corneal exposure to chemical factors or of vitamin D intoxication.

Fig. 4 Left eye. After removal of 2 mm biopsy (arrows mark site) and debridement of most of the remainder, only a narrow rim of the original opacity remains.

Visual acuity was 6/9, N5 in each eye when mild mixed astigmatism had been corrected. Subepithelial discoid greyish-white lesions about 3·8 mm in diameter were present in the central zone of both corneas. On the right side the lesion was annular in form, a central clear area being outlined by a jagged inner edge (Fig. 1). The left corneal opacity, which was somewhat larger, appeared as a grey disc in which white lines formed a mosaic pattern (Fig. 2). An irregular fissure running vertically almost divided the disc. The temporal edge of the fissure was slightly elevated, and the overlying epithelium stained with both fluorescein and rose Bengal. At high magnification the lesions had a granular structure. Ophthalmoscopically the opacities were silhouetted in the red fundus glow (Fig. 3). Tear and meibomian secretions and ocular tensions were normal. Sensation over the corneal opacities was slightly diminished. No other ocular abnormalities were noted.

The attacks of ocular pain affecting the left eye became more severe and incapacitating. Since it seemed likely that the quiescent state of the right cornea had been achieved by a natural exfoliation of the central part of the lesion over several years, biopsy and debridement of the left corneal lesion were undertaken under retrobulbar anaesthesia. The opaque material was removed with ease. Within 48 hours the patient was symptom-free and the left vision corrected to 6/9+. The slit-lamp appearances following this procedure (Fig. 4) now closely resembled those seen on the right side. No further attacks have occurred over the past 9 months, vision and slit-lamp appearances remaining constant.

GENERAL INVESTIGATIONS

All studies to determine the aetiology of these lesions were uninformative. Fasting levels of serum calcium, phosphate, alkaline phosphatase, glucose, and lipids were normal on 3 occasions. The Kveim test and other investigations for sarcoid were negative. There was no radiological evidence of ectopic calcification.

METHODS

A 2 mm trephine was used to biopsy the left corneal opacity. The disc, which included the anterior 1/6th of stroma, was placed in ice-cold 3% glutaraldehyde. Some of the material obtained by superficially scraping away the remainder of the opacity was fixed likewise. After dehydration the specimens were osmified and embedded in methacrylate. After staining with uranyl acetate and lead citrate, sections were examined in a Phillips 301 electron microscope. Unstained sections were subjected to elemental analysis with an Edax analysing electron probe. Orientational sections cut at 0·5 µm were stained in 1% toluidine blue for light microscopy. The

Fig. 5 Corneal epithelium shows mild dysplasia with hydrops of some superficial squames. The Bowman layer contains intensely basophilic granules. Some of the superficial keratocytes are vacuolated. (Toluidine blue, $\times 580$).

remainder of the material was fixed in neutral formalin and subsequently embedded in JB4 compound (Polysciences). Sections from this material were subjected to routine histological stains, as well as certain histochemical procedures which will be indicated alongside the results obtained.

Observations. The trephined biopsy specimen and curetted material consisted of epithelium and superficial corneal stroma.

Fig. 6 Fissure in Bowman's layer, which may be traumatic (surgical) artefact, illustrates its friability. (Toluidine blue, $\times 467$).

LIGHT MICROSCOPY

In toluidine blue preparations the epithelium showed mild dysplastic changes (Fig. 5). Surface squames were often swollen and vacuolated. Nests of intensely basophilic cells were seen in the intermediate layers. In many places the basal cells were separated from Bowman's layer by a stratum of vacuolated amorphous material. Elsewhere the basal cells lay directly on the Bowman layer or on the basement membrane. The Bowman layer was studded with myriads of intensely basophilic spherules, the larger of which were about $1\ \mu\text{m}$ in diameter. Occasional spherules were found in the adjacent stroma. Both spherules and the basement membrane, where present, gave positive reactions with the von Kossa and alizarin red methods for phosphate and calcium respectively. The friability of Bowman's layer was indicated by occasional vertical cracks with displacement of the fragments (Fig. 6). Beneath the Bowman layer were patches of vacuolated amorphous material similar to those on its anterior surface. Throughout the stroma keratocytes showed a variety of changes which are described in infra-structural detail below.

ELECTRON MICROSCOPY

Epithelial cells which appeared dark blue in toluidine blue preparations were electron-dense and contained numerous tonofibrils. In other areas paler cells were undergoing hydropic degenerative changes, shown as cytoplasmic vesicles which were often prominently aligned under the cell membrane (Fig. 7). Similar

Fig. 7 A band of amorphous granular debris is found below Bowman's layer. A degenerated keratocyte (arrows) distended with clear vacuoles is seen below. (Electron micrograph, $\times 1470$).

changes were seen in the basal cells. The basement membrane-hemidesmosome system was absent in some sections, the basal cells lying either directly on the Bowman's layer or more commonly on the layer of vacuolated debris, which contained a variety of fibrillar materials, including normal and 'curly' collagen (Fig. 8) as well as a variety of organelles. Similar material was also patchily distributed below Bowman's layer (Fig. 7). When the basal cells lay directly on Bowman's layer, tufts of fibrils extended upwards, producing a sawtooth appearance in the cell membrane (Fig. 7).

Bowman's layer, which was present in all sections examined, contained numerous electron-dense

spherules which often showed a paler core and a denser rim, although sometimes the relative densities were reversed. The spherules consisted of tightly packed electron-dense granules which replaced or obliterated the random collagenous architecture of the Bowman's layer. Elemental analysis of the spherules by electron probe indicated that calcium and phosphorus were present in considerable quantities (Figs. 9A, B).

Degenerative changes in the keratocytes were especially severe in the vicinity of Bowman's layer; several stages of this process are suggested by their varying appearances (Figs. 10A, B, C). Firstly, the cytoplasm distends with vacuoles and electron-dense

Fig. 8 Heavily calcified portion of Bowman's layer shows concentric ring formations in some spherules. The basement membrane (also calcified) is thickened and electron-dense. A layer containing various presumed collagen fibrils separates the basement membrane from the basal epithelial cell. E. (Electron micrograph, $\times 32\,500$).

Fig. 9A Elemental analysis of one of the spherules in Bowman's layer shows high concentration of calcium (Ca) and phosphorus (P) within the spherule, to be compared with Fig. 9B, which indicates lower concentrations of these elements in the adjacent stroma. Copper (Cu) originating from the grid holding the section is the main component in both 9A and 9B.

Fig. 10A Keratocyte shows numerous vacuoles and granular inclusions. (Electron micrograph, $\times 14255$).

inclusions. Secondly, the cell membrane disintegrates. Finally the cytoplasm becomes increasingly electron-lucent and occupied by degenerating organelles, the nucleus becoming pyknotic or disintegrating. Ultimately the spaces occupied by dead keratocytes become empty apart from tonofibrils and debris. 'Ghost' cells of this latter type were especially common beneath the Bowman layer. Patches of vacuolated amorphous debris below the Bowman layer were the only other abnormality in the stromal collagen.

Discussion

The corneal opacities described in this report do not appear to have been previously documented. A superficial mosaic opacity, similar to that in our patient's left eye was noted by Valerio³ in both corneas of a young man whose father and other family members were afflicted with the primary (hereditary) form of band-shaped calcific opacity.

Histochemical and electron micro-probe elemental analysis of our biopsy material has indicated that the darkly stained spherules found in Bowman's layer contain calcium and phosphorus in considerable quantities, presumably as a calcium phosphate. An interesting finding is the presence of widespread degenerative changes in the keratocytes.

The present case should be differentiated from noncalcific band-shaped keratopathy in which sub-epithelial yellowish granules are the essential bio-microscopic features. Kloucek⁴ studied keratectomy specimens from 2 related patients with the rare primary form of this disorder. He observed that the yellow granules corresponded with electron-dense spherules in the Bowman's layer and contained a nonlipid protein but no calcium. He also noted that keratocytes in the superficial stroma had proliferated and undergone a variety of degenerative changes, similar—if less widespread—than those seen in our patient, leading him to consider that the spherules in Bowman's layer had been produced

Fig. 10B Keratocyte distended with electron-lucent vacuoles and granular debris. Nuclear membrane is present but plasmalemma has largely absorbed. (Electron micrograph, $\times 4610$).

by these cells, although the possibility that these changes were reactive rather than primary was acknowledged.

It is improbable that keratocyte degeneration seen in our case is merely a sequel to recurrent erosions of the epithelium and Bowman's layer, since the main biopsy was taken towards the upper periphery of the lesion, where such occurrences are rare. Moreover, Rice and his co-workers⁵ observed only inconstant keratocyte injury even in long-standing cases of Reis-Bücklers's dystrophy, in spite of recurrent epithelial breakdown and disintegration of Bowman's layer. Nor does it appear that calcification of the Bowman's layer would in itself account for such severe changes in the keratocytes, since no gross abnormality of the keratocytes was noted either by Koseki⁶ in his electron microscopic study of the calcified anterior stroma in a patient with band keratopathy and uveitis, or by Ticho and his coworkers⁷ in their examination under light microscopy of a keratectomy specimen from a patient with primary calcific band-shaped keratopathy. Artefactual change may be excluded on the grounds that cytological preservation of the specimen was otherwise adequate and that the changes were seen throughout the biopsy. Keratocyte

degeneration in this case may have been a response to a precursory unknown noxious stimulus, but there is no other clinical or ultrastructural evidence to support this contention. Thus, disease of the corneal keratocytes of the anterior stroma cannot be excluded as the primary disorder in our patient. It may be further hypothesised that such a breakdown of the superficial keratocyte system, liberating lysosomal enzymes including phosphatases, could produce a local increase in phosphate which, combined with calcium, has impregnated the Bowman's layer.

Dehiscence of this layer probably played a role in the natural exfoliation of the opacity, causing recurrent erosions. These attacks stopped spontaneously on the right side, presumably when most of the opacity had been eroded, and they have ceased on the left side following superficial keratectomy. We found no ultrastructural correlation with the greyish bars outlining the polygons seen in the opacity clinically. This is in contrast with the observations made by Tripathi and Bron,⁸ who in their case of secondary anterior crocodile shagreen of Vogt found calcified folds of the Bowman layer, corresponding with the mosaic.

Fig. 10C A 'ghost' keratocyte in which a few organelles and tonofibrils remain. (Electron micrograph, $\times 8415$).

Mr R. Cousins, the Charles Clifford Dental Hospital, Sheffield, was responsible for the photomicrographs.

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