The red eye of renal failure: a crystal induced inflammation?

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Abstract
Of 57 patients with chronic renal failure and calcification of the anterior membranes of the eye eight developed inflammatory reactions, clinically indistinguishable from pingueculitis. In three patients an acute inflammatory reaction of the episcleral tissue and the conjunctiva over it developed which we considered to represent the red eye of renal failure. In these patients massive shedding of calcium phosphate salts was clinically evident. Histopathologically in all these patients calcific deposits were observed. Although sporadically polymorphonuclear leucocytes were present in the inflamed tissues we could not demonstrate crystal phagocytosis. We believe that in the red eyes in renal failure and, more specifically, in the red eyes of renal failure a crystal induced inflammatory mechanism is not operative.

In patients with renal failure requiring regular haemodialysis, calcific deposits in the conjunctiva and cornea are common. As calcium phosphate salts are sparingly soluble and, as for the precipitation of the salt, the product of the concentration of the positively and negatively charged part of the salt is constant, an increase in either serum calcium or serum phosphorus concentration, large enough to exceed the in vivo solubility product, will lead to the deposition of calcium phosphate salts in the form of microcrystalline hydroxyapatite.1

An acute inflammatory response in the joints associated with the presence and phagocytosis of urate crystals has been described.2 Hydroxyapatite crystals also have a marked phlogistic potential.3 After being ingested by polymorphonuclear leucocytes they lead to cell death and to the release of large quantities of enzymes all capable of initiating inflammatory reactions. The crystals extruded in the extracellular tissue set in motion a new cycle of phagocytosis, cell death, and the release of inflammatory mediators continuing the inflammatory response.4

Berlyne5 formulated the skillful theory of crystal induced inflammation, a dose related and reversible inflammatory reaction, for the explanation of the mechanism of the red eyes of renal failure. The histopathological study of Berlyne demonstrated the presence of subconjunctival polymorphonuclear leucocytes and calcium phosphate crystals small enough to be phagocytosed, a prerequisite for the crystal induced inflammation theory. However, no mention was made of phagocytosis and he did not pursue the crystal induced inflammation concept to explain the red eyes in renal failure.

Berlyne and Shaw6 took a conjunctival biopsy of a patient they considered to present with the red eye in renal failure. However their biopsy may have been taken from an inflamed pinguecula as demonstrated by the figure shown in their report. We therefore studied biopsies from patients with a distinctively different type of inflammatory reaction, clinically characterised by a waxy-red hyperaemia of the episclera and the conjunctiva over it, patients who were biochemically characterised by a high calcium/phosphorus ratio.7 In addition we examined biopsies that were taken from patients without any inflammatory reactions of the outer membranes of the eye and also from patients with inflammatory reactions clinically indistinguishable from pingueculitis.

Patients and methods
The patient groups were identified both clinically and biochemically: group A consisted of 46 patients with calcium deposits but without the occurrence of an inflammatory reaction during the observation period, which was 6 years on average. Group B consisted of eight patients with calcification and inflammatory reactions associated with pingueculae. We considered these patients to represent the red eyes in renal failure. Group C consisted of three patients with an inflammatory reaction resembling diffuse episcleritis, extending well beyond the palpebral aperture, and an associated conjunctivitis which we considered the red eye of renal failure. Biochemically these patient groups were characterised on the basis of the relationship between the serum calcium and phosphorus concentration. Fifty healthy persons, matched in sex and comparable in age and weight, were used as controls.

 Conjunctival biopsies were taken from patients of the three main groups and were examined by light microscopy. The tissues were fixed in phosphate buffered neutral formalin (10%). The histological sections were processed according to standard methods. The histochemical stains used were haematoxylin and eosin, to reveal the general cell structure, Giemsa stain and toluidine blue for the study of
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Results

Serum calcium and phosphorus product
In Table 1 the average values of the serum calcium-phosphorus product of patient groups A, B, C, and the control group are shown. Statistically there was a significant difference between the patient groups and the control group. The patient groups all showed higher values than the control group. This is shown graphically in Figure 1. From this figure it is apparent that between the patient groups there was also a difference; statistically this difference was significant.

The highest calcium phosphorus product was observed in patients of group C. These patients had inflammatory reactions characterised by a diffuse waxy red episcleral and conjunctival hyperaemia. However in this group the average value of the serum calcium-phosphorus product exceeded the empirically observed serum calcium-phosphorus product value that, in the past, was associated with the red eye of renal failure.

Table 1 The average calcium/phosphorus product and the number of persons in groups A, B, C, and the control group (Co) are shown with the standard deviations

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>4.59</td>
<td>5.02</td>
<td>6.62</td>
<td>3.13</td>
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<tr>
<td>Number</td>
<td>46</td>
<td>8</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>SD (n-1)</td>
<td>1.29</td>
<td>1.41</td>
<td>1.62</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 2 The average serum calcium/phosphorus ratio, the number of patients and the standard deviation in patients of groups A, B, C, and the control group (Co)

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>1.51</td>
<td>1.44</td>
<td>2.00</td>
<td>1.90</td>
</tr>
<tr>
<td>Number</td>
<td>46</td>
<td>8</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>SD (n-1)</td>
<td>0.56</td>
<td>0.39</td>
<td>0.75</td>
<td>0.24</td>
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</tbody>
</table>

Histopathology
A conjunctival biopsy of a representative patient from group A showed marked subepithelial calcium deposits. There was a certain amount of elastic degenerated connective tissue. No inflammatory cells in or around the calcific deposits were observed, but perivascularly some polymorphonuclear leucocytes were seen. There was no distension of the conjunctival or episcleral vessels.

In the biopsies of patients from group B marked deposits of subepithelial calcium were also seen. The collagen fibres were degenerated. A localised, moderate increase in polymorphonuclear leucocyte infiltration was seen in almost half of the cases. Sporadic leucocytes were observed in 14% of the biopsies; however these inflammatory cells may be completely absent.

Serum calcium/phosphorus ratio
In Table 2 the average serum calcium/phosphorus ratios are shown for the patient groups A, B, C, and the control group. The control group and group C had the highest value. There was a statistically significant difference in the serum calcium/phosphorus ratio between the patient groups and the control group but not among the patient groups. The inability to demonstrate a difference between group A and B on the one hand and group C on the other was the consequence of the smaller number of patients in group C. The difference between the patient groups we observed, however, was found to be dependent on the contribution of the average serum calcium concentration to the ratio; the serum phosphorus concentration was constant, as shown in Table 3.
were not located in the areas of calcification (Fig 3). No crystal phagocytosis was observed. The histopathology of this group is compatible with primary, simple episcleritis. No complement activation was detected by immunofluorescence.

Discussion
After being phagocytosed microcrystalline hydroxyapatite is very toxic to leukocytes because these crystals are membranolytic. It is therefore not surprising that for Berlyne the development of the red eye in renal failure is none other than the mechanism of crystal induced arthritis. He looked at the conjunctival sac as a specialised joint in which the moving surfaces are the lids and eyeball; the conjunctiva being analogous to the synovial membrane. The presence of polymorphonuclear cells, mononuclear cells, as well as crystals small enough to be phagocytosed, being the conditions for the induction of a crystal induced inflammation, seems to favour such a mechanism.

Hydroxyapatite crystal deposition in soft tissues will occur when the serum concentrations of calcium and phosphate exceed the solubility product – that is, in a marked supersaturated solution. In vitro the serum calcium/phosphorus ratio determines the type of salt formed. In a high calcium/phosphorus ratio of 1.67 or more, salts are deposited in a crystalline form, while a low ratio results in amorphous deposits.

If the concentration of calcium and phosphorus ions increase homogeneally, large crystals are formed; small crystals are deposited if the ion concentrations increase inhomogeneally. In a situation that favours the precipitation of calcium salts of a crystalline nature, and in view of the pathophysiology of renal failure one can expect the deposition of predominantly microcrystalline deposits of hydroxyapatite, which was demonstrated.5

Particularly in the rather acute and pronounced diffuse episcleral and conjunctival inflammatory reactions of the patients of group C, the average serum calcium/phosphorus ratio was 2:00, the result of the rather marked increase of the serum calcium concentration. Here, one can expect massive shedding of crystals, which we observed clinically, one of the prerequisites of crystal induced inflammation.

Hydroxyapatite crystals, most of which are less than 0.5 μm long, cannot be seen by light microscopy and crystal phagocytosis could be difficult to demonstrate in a routine histopathological examination. Hydroxyapatite crystals have, however, a marked tendency to clump and to arrange themselves within a connective tissue matrix.10,11 These clumps of crystal and matrix can be seen by light microscopy, free or intra-leukocytic, as in periarteritis. In spite of the presence of subconjunctival extracellular hydroxyapatite crystals in an acutely inflamed episcleral and conjunctival tissue, we were not able to observe crystal phagocytosis. In group C and also in the majority of the patients in group B there is an increased polymorphonuclear leukocyte infiltration, but they are not in the vicinity of calcific deposits. We did not observe chemotaxis exerted by hydroxyapatite crystals in the conjunctiva.

The absence of crystal induced phagocytosis in the presence of hydroxyapatite crystals suggests a ‘switch-off’ phenomenon. Phagocytosis of crystals is expedited when certain proteins are adsorbed to the surface of the crystal12 and inhibited if crystals are coated by competing proteins. One can hypothesise that the absence of phlogistic activity of the hydroxyapatite crystals in the red eye of renal failure might be related to adsorbed molecules such as hyaluronate13 or glycoproteins.14 Adaption to crystals manifested clinically by their silent presence might be explained by such a mechanism.

Figure 2. Histopathology of a conjunctival biopsy of a patient from group C, clinically characterised by a toasty red episcleral hyperaemia extending beyond the palpebral aperture. Pavementing of the endothelium by polymorphonuclear leukocytes of the vascular wall is shown. Also visible are lymphocytes, eosinophilic cells, plasma cells, as well as perivascular infiltration of leucocytes. Haematoxylin and eosin, ×900.

Figure 3. Patient from group C: subepithelial calcific deposits as a large plaque adjacent to small calcific granules without any crystal phagocytosis. Giemsa stained, ×410.
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