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CASE REPORT

In vivo confocal microscopy in hydroxychloroquine-induced keratopathy

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Abstract
Background Vortex keratopathy, arising as a side effect of several medications, is characterized by golden-brown deposits in the cornea.
Methods A 41-year-old woman treated for sarcoidosis with hydroxychloroquine therapy and suffering from vortex keratopathy was examined by in vivo confocal microscopy. Scans of both corneas were performed.
Results By slit lamp examination, the left but not the right eye showed a golden-brown deposit throughout the cornea. In vivo confocal microscopy revealed the presence of highly reflective, dot-like intracellular inclusions concentrated in the basal epithelial layer. They were also detected within the anterior and posterior stroma, but not within the endothelium. In regions of the anterior stroma, devoid of inclusions, hyperreflective ramified keratocytes were observed, forming an extended interconnecting network.
Conclusion In addition to the granular deposits, in vivo confocal microscopy revealed hyperreflective, possibly phagocytic keratocytes.

Keywords Confocal microscopy · Cornea · Hydroxychloroquine

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Introduction

Vortex keratopathy (cornea verticillata) is characterized by a whorl-like pattern of greyish or golden-brown deposits in the corneal epithelium. It occurs in Fabry's disease and as a side effect of various systemic medications, namely amiodarone, aminoquinolones, indometacin, suramin, and tamoxin [6]. These drugs share cationic amphiphilic properties, allowing them to accumulate within lysosomes [3]. They give rise to undegradable inclusion bodies [9]. The whorl-like pattern may result from the centripetal migration of deposit-laden limbal epithelial cells [4]. Corneal deposits seldom affect visual acuity, and typically resolve once the medication is stopped [7].

By in vivo confocal microscopy, chloroquine [10] and amiodarone deposits in the cornea have recently been described to occur in the basal epithelial layer, stroma, and in the case of amiodarone, also in the endothelium [2]. Using the same technique, we describe corneal deposits in a patient with hydroxychloroquine keratopathy.

Case report

A 41-year-old woman with a 1-year history of sarcoidosis was referred to our clinic by her general physician for ophthalmologic control before introduction of hydroxychloroquine treatment (2×200 mg/day). Best-corrected visual acuity was 20/20 in both eyes. Intraocular pressure was normal and, by slit-lamp examination and funduscopy, neither the anterior segment nor the fundus showed visible pathologic traits. Visual field, multifocal ERG, and EOG were normal. Six months later, the patient returned to the clinic complaining of foreign body sensation, dryness and burning in both eyes. Best-corrected visual

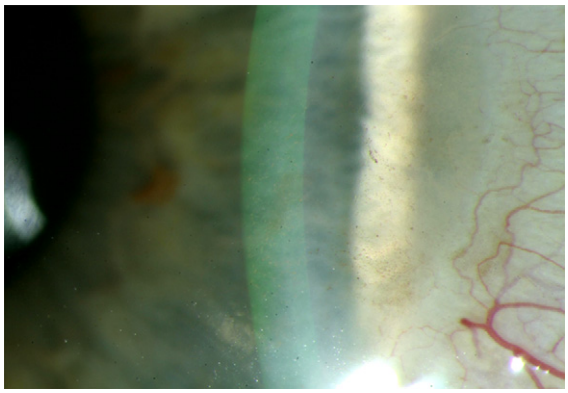

Fig. 1 Golden-brown deposits in the temporal limbus after hydroxychloroquine medication. Slit-lamp examination

acuity (20/20 in both eyes) was not affected. Slit-lamp examination revealed golden-brown deposits in the left cornea that were more pronounced near the limbus (Fig. 1). No deposits were visible in the right eye. Funduscopy was normal in both eyes. Diagnosis of dry eye was confirmed by a Schirmer test. Visual field, multifocal ERG, and EOG were repeated and showed no abnormalities.

In vivo confocal microscopy was performed using a Heidelberg Retina Tomograph II, Rostock Cornea Module (Heidelberg Engineering GmbH, Dossenheim, Germany). After topical anesthesia (0.4% oxybuprocaine, Novartis Pharma, Bern, Switzerland), Lacryvisc Gel (Alcon Labs, Zug, Switzerland) was applied before aligning the lens. Raw full-screen images were captured throughout the cornea and are presented without further image treatment.

Observations

In the cornea of the left eye, highly reflective intracellular inclusions, measuring between 2 and 15 μm in diameter, were observed in the basal epithelial cell layer (Fig. 2a). They were also detected throughout the anterior stroma (Fig. 2b,c), but decreased in number in the mid-stroma (Fig. 2d). No reflective inclusion bodies were observed in the endothelial cell layer. In the anterior stroma, however, highly reflective ramified cells were revealed (Fig. 2b,c). In several instances, these cells were interconnected and

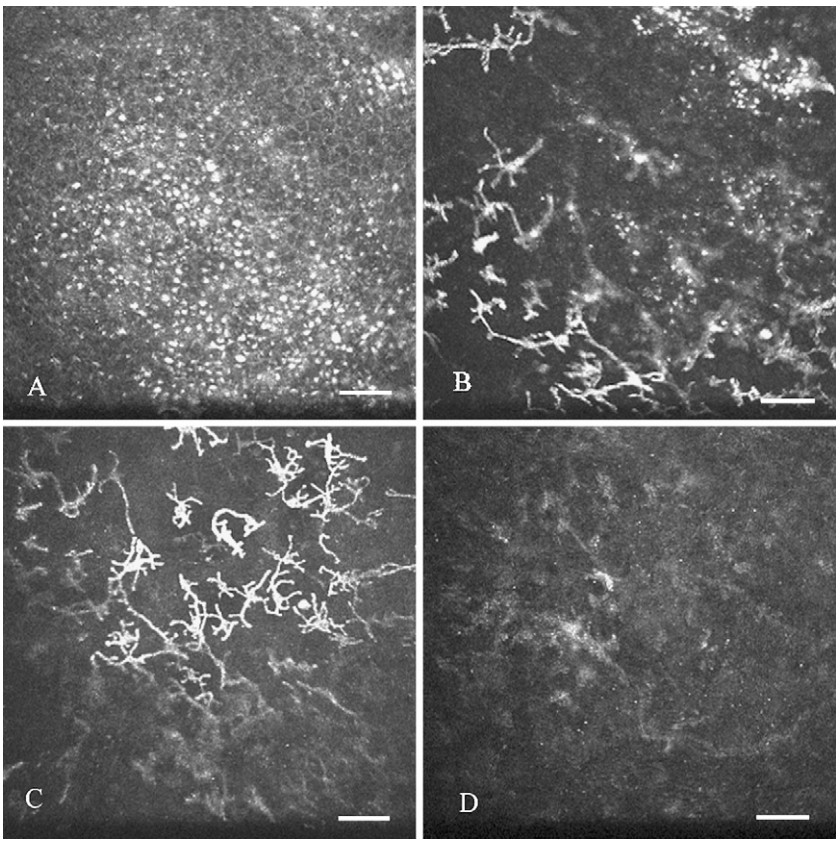

Fig. 2 In vivo confocal microscopy of the left cornea. Optical sections of basal epithelial layer (**a**), anterior (**b**, **c**), and mid-stroma (**d**). **a** Diffuse bright intracellular inclusion bodies in the basal epithelial layer, as recognized by its reticular structure. **b** Granular deposits (*upper right*) and highly reflective keratocytes (*lower left*) within the anterior stroma. **c** Anterior stroma with reflective keratocytes. Note absence of granular deposits in the focal plane of reflective keratocytes interconnected into a network. **d** Midstroma with few reflective dot-like deposits. *Bar*, 50 μm

formed an extended network, suggesting that they represent keratocytes. Their high reflectiveness, and the fact that no inclusion bodies were found in their surroundings, suggest that they had phagocytosed the deposits.

In the right eye, a few bright microdots were present exclusively in the basal epithelial cell layer (not shown).

Discussion

Medication with members of the chloroquine family (chloroquine, hydroxychlorique, amodiaquine) may produce ocular toxicity involving the cornea (vortex keratopathy), ciliary body, lens (posterior subcapsular cataracts), and retina (bull's eye maculopathy) [5]. Chloroquine is rapidly absorbed and becomes highly concentrated in several tissues throughout the body. Corneal deposits (verticillata) can be demonstrated in most patients taking chloroquine, but these changes very rarely impair vision. Such deposits form more frequently with chloroquine than hydroxychloroquine and often develop within 2–3 weeks following initiation [5]. They are located in the epithelium and anterior stroma and, in most cases, are reversible.

The present report demonstrates by in vivo confocal microscopy that the golden-brown deposit detected by slit lamp examination in the hydroxychloroquine-laden cornea corresponds to granular inclusions in the epithelium and stroma. A similar distribution was noted in chloroquine-induced keratopathy [10], whereas in advanced amiodarone-induced keratopathy, deposits were also found in the endothelium [2]. Nevertheless, the hydroxychloroquine inclusions strongly resemble those induced by chloroquine [10] and amiodarone [2], and may correspond to intracellular lysosome-like corpuscles containing phospholipid complexes [3, 9].

Most importantly, in vivo confocal microscopy revealed, in the anterior stroma, the presence of highly reflective ramified keratocytes with extended cytoplasmic processes forming an interconnecting network. Such hyperreflection is not detected in the normal cornea where, at best, the nucleus and perinuclear cytoplasm can be perceived. Interconnecting cellular processes were revealed, however, by vital staining of isolated cornea [8]. Remarkably, in the present case, no granular inclusions could be detected in the surroundings of reflective keratocytes, evoking the possibility that these keratocytes were actively phagocytizing and accumulating inclusion bodies. It has been shown that, upon corneal injury and infection, activated keratocytes indeed exert macrophage function [1].

Chloroquine-induced deposits are located intracellularly within lysosomes [9]. Intracellular accumulation of undegradable material might ultimately lead to cell death, the debris being phagocytized by neighboring keratocytes. Cell death, in turn, might account for the observed decrease in keratocyte density in amiodarone keratopathy [2], while phagocytosis might account for the disappearance of deposits when medication has stopped.

Conclusively, in vivo confocal microscopy revealed, upon hydroxychloroquine medication, the formation of corneal inclusion bodies that, in the anterior stroma, are likely to be phagocytosed by activated keratocytes, rendering these cells highly reflective.

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