Corneal surface changes in keratoconjunctivitis sicca. Part I: the surface proper. A non-contact photomicrographic *in vivo* study in the human cornea

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Abstract

Purpose To describe corneal surface changes in keratoconjunctivitis sicca (KCS).

Methods In all 23 patients with KCS were examined with the slit lamp and photographed by non-contact photomicrography.

Results Without staining, grey surface cells and small epithelial cysts were visible. Application of fluorescein sodium resulted in two types of micropunctate (15–30 μm) staining, a nonfluorescent (yellow/brown) and a fluorescent (green) one, in brilliantly green staining of cysts, and in enlarging fluorescent (green) flecks (70–1600 μm) with propensity to confluence. The flecks often developed in inconspicuous areas and showed abnormal subsurface cells. Application of rose bengal resulted in more or less intensively red-stained cells measuring about 30 μm in diameter, often less (15–20 μm), with or without a centrally located nucleus, individual or gathered in small groups or larger patches, and in confluent staining. Smaller and larger cells, with or without nuclei, weakly or strongly stained, were often located close to each other. The smaller green flecks showed a few centrally located red-stained cells, and the larger ones showed many almost uniform small red-stained nucleated ones.

Conclusions Corneal epithelial surface disease in the present KCS patients consisted of unspecific features—such as increased light-reflecting property (grey cells), disruptions of intercellular junctions (micropunctate green fluorescein staining),

pathological dye uptake (micropunctate yellow/brown fluorescein and red rose bengal stainings), and epithelial oedema (microcysts)—and also of features that seemed to be disease specific. Such features were, with fluorescein sodium, focal disruptions of the epithelial barrier function manifesting in enlarging green flecks, and, with rose bengal, abundant nucleated cells, particularly small ones organised in foci. The nature of the abnormal subsurface cells present in the areas of the green flecks, and the mechanisms behind the focal involvement are unclear.

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Introduction

Complaints of eye irritation, grittiness, burning, 'dry' feeling (or, more rarely, of excessive tearing), photophobia, and visual disturbances are common in 'dry eye' or keratoconjunctivitis sicca (KCS). Both terms have been used interchangeably, but at present KCS seems to be reserved for ocular surface disturbances attributed to aqueous tear deficiency.¹ Frequently used clinical diagnostic tests are the tear break-up time (BUT) (the time elapsed between the last blink and the appearance of dark 'holes' in the tear film stained green with fluorescein sodium), the Schirmer I test (the length of wetting in mm of a standardised filter paper strip placed for 5 min over the lower

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palpebral margin, without anaesthetic), and the van Bijsterveld score (the sum of rose bengal dye staining of the ocular surface within the interpalpebral area, divided into three parts—temporal and nasal conjunctiva and cornea—each estimated at a scale of 1–3). Less widespread are estimations of the extent of fluorescein sodium staining, and staining with lissamine green dye² instead of rose bengal. An evaluation of subjective assessments and diagnostic tests has been published recently.³ At present, there is no consensus on diagnostic criteria.

High-magnification *in vivo* observations of the corneal epithelium in KCS by the contact method of specular microscopy, without^{4–6} or with the aid of fluorescein sodium and rose bengal dyes,⁷ have concerned mainly the shapes and sizes of abnormal surface cells, and their respective representation.⁷ The purpose of the present study (Parts I and II) was to find out which corneal epithelial changes could be discerned in KCS, referable to *bona fide* aqueous tear deficiency, by a method requiring neither contact with the ocular surface nor the use of anaesthetics.⁸ It has to be stressed that there was no intention to correlate the findings with concurrent systemic diseases, to estimate the frequency of the various changes, or to grade the severity of the disease.

Patients and methods

The study included 23 patients (20 women, three men, mean age 60.3 years, range 34–81 years) with KCS as defined by two pathological results of three clinical tests: BUT ≤ 10 s, Schirmer I test ≤ 5 mm of wetting in 5 min, and van Bijsterveld score of ≥ 4 in one or both eyes. In all, 18 patients had primary Sjögren's syndrome (either previously diagnosed or later verified), three had other autoimmune diseases, and two had no known concurrent disease (patients no.1–23, Table 1). Excluded were patients with other surface diseases, known rosacea, psoriasis, atopy, diabetes, and contact lens wearers. The patients had either no topical treatment, or did not use any eye drops at least 1 h before examination. The test procedure consisted of application of fluorescein sodium, BUT, Schirmer I test, and rose bengal staining in the order mentioned. The patients were repeatedly examined with the slit lamp. Surface details were photographed in various illumination modes by non-contact photomicrography.⁸ Fluorescein sodium 1% and rose bengal 1% (both dyes without preservative) were used. The photographs were taken with Ektachrome 100 or 200 ASA film.

Table 1 Clinical data in 27 examined patients with KCS

Patient no.	Sex	Age	Systemic disease	Schirmer 1 test	Rose bengal score	Break-up time	Use of tear substitutes
1	M	81	None known	0/5	7/4	0/4	No
2	F	66	1'SS	10/5	8/3	1/1	No
3	M	62	1'SS	3/3	6/9	4/3	No
4	M	50	1'SS	0/0	7/7	0/1	No
5	F	40	1'SS	1/2	9/9	2/3	No
6	F	53	1'SS	0/0	8/8	1/1	No
7	F	64	1'SS	0/0	7/7	2/3	No
8	F	34	1'SS	1/0	8/8	0/0	No
9	F	58	None known	3/4	9/8	0/0	No
10	F	34	1'SS	0/0	5/4	0/0	Yes
11	F	63	1'SS	0/0	7/9	0/0	Yes
12	F	81	1'SS	4/3	5/9	2/1	Yes
13	F	68	1'SS	2/1	0/6	8/6	No
14	F	76	1'SS	0/0	9/9	0/0	Yes
15	F	70	Collagenosis	2/1	9/9	0/0	No
16	F	42	1'SS	1/0	3/7	1/1	Yes
17	F	68	Rheumat.arthritis	1/1	8/8	0/0	No
18	F	70	1'SS	1/1	6/7	1/2	No
19	F	39	1'SS	1/1	9/6	0/0	Yes
20	F	61	1'SS	0/0	5/5	0/0	No
21	F	75	Polymyalgia	1/1	5/5	3/4	Yes
22	F	68	1'SS	0/2	9/9	0/0	Yes
23	F	64	1'SS	0/1	5/7	0/0	Yes
24	F	74	1'SS	3/0	5/6	0/0	Yes
25	F	64	Rheumat.arthritis	1/2	6/6	2/3	Yes
26	F	40	1'SS	3/9	8/8	0/0	No
27	F	74	1'SS	2/3	8/8	3/2	No

1'SS, primary Sjögren's syndrome.
Part I: nos. 1–23, Part II: nos. 4–27.

Results

Surface changes were present anywhere on the cornea but they were usually more pronounced in the interpalpebral area, particularly so in its lower part. Test results are given in Table 1 and photographic findings in Figure 1.

Before staining

- Grey surface cells (20–30 μm), individual or in small groups or patches (Figure 1a).
- Small cysts (of about 10–65 μm in diameter). (Figure 1b).

With fluorescein sodium 1%

- Yellow/brown (nonfluorescent) stained surface cells of about 15–30 μm in diameter (Figure 1d), individual or in small groups or confluent into patches; the intensity of the staining varied between weak and strong.
- Green (fluorescent) staining:
 - green dots measuring about 15–30 μm in diameter (Figure 1c),
 - brilliantly green staining cysts (Figure 1c),
 - green flecks measuring about 70–1600 μm in diameter. As seen with the slit lamp, these flecks developed anywhere on the cornea, either immediately or after a short delay (seconds to minutes) after the application of fluorescein sodium, successively enlarged, and showed propensity to confluence (Figure 1e), sometimes resulting in large green-stained areas. Smaller flecks often appeared slowly in previously inconspicuous areas, created a brightly green background, and highlighted the yellow/brown (nonfluorescent) staining of a few surface cells otherwise easily missed (Figure 1e). Larger flecks developed more rapidly. The areas of the flecks showed fine granularity (Figure 1e, inset); in some flecks, closely packed small abnormal subsurface cells were clearly discernible (Figure 1f). Only occasionally, the development of larger flecks could be predicted by the presence of grouped grey surface cells.

No surface ulcerations in the sense of missing substance were observed.

With rose bengal 1%

More or less intensively red-stained surface cells of about 30 μm in diameter, often less (about 15–20 μm) and only exceptionally more (about 45 μm), individual or in small groups, alternatively in larger patches or confluent. Many of the individual cells were polygonal with sharp

edges. In areas of confluent staining, cell boundaries could not be distinguished. Small groups consisted of irregularly arranged adjacent cells, often in rows or forming a 'rosette'. Less intensively stained cells showed a more densely stained nucleus or appeared empty; no nucleus could be discerned in more densely stained ones. Areas between stained cells either showed a rose bengal haze or appeared unstained. More or less intensively red-stained cells, with or without nuclei, were often located close to each other (Figure 1g–k). Some areas showed adjacent rows of smaller and larger nucleated cells (Figure 1l). In some areas, the staining was confluent and no structures could be discerned. Small green flecks showed a few red-stained cells (not shown), and larger flecks showed many nucleated ones (Figure 1m and n).

Discussion

Both grey surface cells and epithelial cysts, visible without staining, are unspecific manifestations of epithelial suffering. In KCS, in the absence of other known causes, the grey cells might be an expression of abnormal cell maturation resulting in increasing light-reflecting property. Epithelial cysts pointed to an oedematous component.

With fluorescein sodium 1%, lacking known adverse effects on the epithelium,⁹ three types of staining were observed: (a) micropunctuate yellow/brown (nonfluorescent, adherent) staining revealing the same diseased cells as rose bengal,¹⁰ (b) micropunctuate green (fluorescent) staining revealing penetration and accumulation of green-stained fluid below diseased cells,¹¹ or in microcysts, and (c) green (fluorescent) flecks. The green flecks did not seem to represent epithelial erosions in the sense of surface depressions because of the loss of substance. With the slit lamp, erosions are discernible before staining, and with fluorescein sodium an immediate pooling of the green-stained fluid within them occurs, followed by diffusion into the tissues. In contrast, the green flecks often developed in inconspicuous areas, and the pooling of fluid was missing. The delay between the application of the dye and the appearance of the flecks, and their subsequent enlargement, indicated ongoing diffusion of the green-stained fluid into the tissues through disruptions in the epithelial barrier. The speed of their development and their final dimensions seemed to reflect the degree of the injury. The areas of the flecks showed closely packed abnormal cells apparently situated in the deeper epithelial layers. Their arrangement was strongly suggestive of epithelial cells showing either incipient swelling, or other membrane alterations resulting in changed refractive indices; however, a presence or contribution of similarly appearing invading

Figure 1 Examples of corneal epithelial changes captured in patients with keratoconjunctivitis sicca. (Fluorescein sodium 1%, rose bengal 1%, bars 200 μ m). Without staining, the epithelium shows grey surface cells (a) and small cysts (b). Green staining reveals penetration of tear fluid stained with fluorescein sodium into (semi) cystic spaces (fluorescent microdots (c) and the underlying tissues (diffuse green staining (d)). Abnormal surface cells stain yellow-brown with fluorescein sodium (non fluorescent microdots (d) cf. (g)). In this cornea (e and f), rounded green flecks, individual or partly confluent, reveal diffusion of green-stained tear fluid into the tissues. Fluorescein sodium-stained diseased surface cells appear as darker yellow-brown shadows against the brightly green background (e). The green flecks (enclosed in frame) show fine granularity (inset) suggestive of grouped abnormal subsurface cells. Such cells are clearly visible in (f) showing two additional areas of green flecks in the same cornea. Abnormal surface cells stain red with rose bengal (g). Individual cells are well delineated (g–k). Smaller or larger cells (i), nucleated or non-nucleated (j), more or less intensively stained ones (h and j, arrowheads) are often located close to each other. The cells are arranged in short rows (g), irregular small groups, sometimes appearing as a 'rosette' (h and k, arrows). Some areas show confluent staining (g). Areas between stained cells show rose bengal haze (g) or appear unstained. The area shown in (l) is diffusely stained green with fluorescein sodium. Against the green background, rose bengal-stained cell nuclei appear as dark dots. The less intensively stained cytoplasm slightly obscures the brilliantly green hue. The distances between the nuclei imply adjacent larger and smaller cells arranged in short rows. The green fleck (m and n), captured in different illumination modes, shows configuration suggestive of confluence of several rounded flecks. The surface shows small rose bengal-stained nucleated cells. The surroundings appear normal.

inflammatory cells¹² could not be excluded. Notable was the focal character: individual flecks were standing out against an apparently normal surrounding epithelium, larger flecks seemed to be the result of confluence of several smaller ones, and large green-stained areas the result of a rapid confluence of several green flecks.

Rose bengal, a tetrachloro-tetraiodo derivative of fluorescein sodium, has been found to have a toxic property both *in vitro*¹³ and *in vivo*; *in vivo*, its application in eyes preinstalled with fluorescein sodium resulted in myriads of newly formed green microdots.¹⁴ Rose bengal, since Sjögren's thesis¹⁵ the classical means in KCS diagnostics, has been traditionally believed to unspecifically stain damaged or dead surface cells. However, *in vitro*, also healthy epithelial cells have been found to take up the dye.¹³ Since this uptake could be blocked by tear components (such as mucin and albumin,¹³ and albumin, lactoferrin, transferrin, and lysozyme¹⁶) it has been proposed that the ability *in vivo* of rose bengal to stain cells is dictated by the status of tear film protection and not by the status of cell vitality.^{13,16}

In the present study, percentage calculations of the various cell dimensions visualised with rose bengal were not carried out, mainly because of difficulties to determine representative areas. The approximate cell diameters—about 20–30 μm , often less (15 μm), only exceptionally more (40–50 μm)—were about the same as found in normal superficial cell population by specular microscopy: 15 μm (in 32%), 25 μm (in 67.6%), and only occasionally larger.¹⁷ In KCS, by specular microscopy without staining, both large^{4,5} and small⁶ cells have been reported; with rose bengal staining, 51% of surface cells have been found to measure 15 μm , and this shift toward small cells has been interpreted as a premature senescence of surface cells.⁷

There were some other features inviting comparison with specular microscopic observations. Thus, the shapes of many rose bengal-stained cells, polygonal with straight edges, were similar to normal ones observed without staining.^{4,18,19} Nucleated superficial cells have been reported both in a variety of surface diseases including KCS⁶ and in normal corneae¹⁸ in which, however, they appeared less frequent.⁶ A later study has reported nuclei as present at least in some cells in 44% of normal corneae.¹⁹ A further feature apparently shared with normal epithelium, as judged from published specular microscopic photographs (for example as shown by Tsubota *et al*⁶), were adjacent smaller and larger superficial cells implying different stages of individual cell development/maturation during their vertical migration towards the surface. Yet, the picture of many small nucleated cells concentrated in a limited area did not seem to have any parallel either in normal

corneae or in any other surface disease (own observations). It has been suggested that an increased number of small superficial cells in KCS might either be because of excess exfoliation,⁷ or activated mitosis,⁶

The intensity of the red staining captured in the present study, ranging between not discernible and a very dense one, was a phenomenon largely dependent on illuminating conditions difficult to standardise. Despite that, since captured also in adjacent cells, it seemed to express various degrees of cell changes resulting in different affinity to the dye.

Many factors have been implicated, but the exact mechanisms behind KCS ocular surface changes are not clear (reviewed elsewhere²⁰). In the present 23 patients, of whom 21 (91.3%) had a systemic disease, static photographs did not reveal whether the various manifestations represented a sequence of events, or responses to different factors. The observed changes, whether unspecific (grey surface cells, microcysts, punctuate fluorescein, and rose bengal stainings) or apparently proper to KCS (many stainable small nucleated cells, green flecks), pointed to a profound disturbance concurrently resulting in abnormal surface cells, in epithelial oedema (microcysts), and in focal disruptions of the epithelial barrier function (green flecks) in areas showing cell changes in the deeper epithelial layers. The exact nature of the subsurface cell changes and the mechanisms behind the focal epithelial involvement in KCS are intriguing questions left open.

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