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The influence of rigid gas permeable lens wear on the concentrations of dinucleotides in tears and the effect on dry eye signs and symptoms in keratoconus

Gonzalo Carracedo[a,c,*], José Manuel González-Méijome[b], Alba Martín-Gil[c], Jesús Carballo[a], Jesús Pintor[c]

[a] Department of Optics II (Optometry and Vision), Faculty of Optic and Optometry, Universidad Complutense de Madrid, Madrid, Spain
[b] Clinical & Experimental Optometry Research Lab, Center of Physics (Optometry), School of Sciences, University of Minho, Braga, Portugal
[c] Department of Biochemistry and Molecular Biology IV, Faculty of Optic and Optometry, Universidad Complutense de Madrid, Madrid, Spain

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ABSTRACT

Purpose: To evaluate the signs and symptoms of dry eye and dinucleotide secretion in tears of keratoconus patients (KC) and the potential effect of rigid gas permeable (RGP) contact lens wear.
Methods: Twenty-three KC patients and forty control subjects were enrolled in this study. Signs of dry eye including tear volume, tear stability and corneal staining along with symptoms were assessed using the McMonnies questionnaire. Tears were collected using Schirmer strips, and dinucleotide concentrations in collected tears measured using high pressure liquid chromatography. Values obtained in KC and controls were compared. The effect of contact lens wear in KC was also assessed.
Results: KC eyes showed a significantly lower tear volume compared to controls, shorter tear break up time (TBUT), higher corneal staining and higher McMonnies dry eye questionnaire scores ($p < 0.05$). When compared with non-wearers, KC contact lens wearers showed significantly higher symptoms, lower Schirmer and TBUT values ($p < 0.05$). Concentration of Ap_4A ($0.695 \pm 0.304\ \mu M$ vs. $0.185 \pm 0.178\ \mu M$) and Ap_5A ($0.132 \pm 0.128\ \mu M$ vs. $0.045 \pm 0.036\ \mu M$) were higher in KC compared to controls ($p < 0.001$) and only Ap_4A was statistically higher in RGP wearers compared to non-wearers ($0.794 \pm 0.478\ \mu M$ vs. $0.417 \pm 0.313\ \mu M$) ($p < 0.05$).
Conclusion: Signs and symptoms of dry eye as well as concentrations of Ap_4A and Ap_5A were markedly increased in KC patients compared to controls. Moreover, Ap_4A and symptoms of dry eye were statistically higher in RGP wearers compared to non-wearers. This seems to indicate that factors such as RGP contact lens wear might exacerbate the clinical condition of dry eye.

1. Introduction

Keratoconus (KC) is an ocular pathology causing a protrusion of the cornea. This pathology is bilateral, asymmetric and progressive [1,2] and affects about 2% of the population worldwide [2]. The cause of KC is not well understood, but it seems to be multifactorial. Factors such as an exposure to ultraviolet light, allergy and genetic mechanisms are the predominant reasons triggering the disease [3–5]. Other factors described in the literature as risk factors for the progression of KC include eye rubbing, with half of the patients reporting intense eye rubbing [6]. The need these patients feel to rub their eyes is due to ocular discomfort symptoms including itching, which might be closely related to eye dryness [7].

Only a very small number of studies have investigated the link between KC and dry eye [8–10]. In these studies, the results show a lower tear break time (BUT) in KC patients and a marked presence of corneal staining. In addition, patients with KC have a higher concentration of pro-inflammatory molecules such as interleukins and metalloproteinases compared to healthy subjects, this being even more elevated in patients using RGP contact lenses [11–13]. Inflammation is one of the most important signs described in the definition of dry eye given by the DEWS 2007 [14]. All these findings suggest a possible interaction between KC and dry eye.

In recent years, the presence of diadenosine polyphosphates in human tears and in aqueous humor has been shown [15,16]. These molecules can increase tear secretion when instilled topically [17].

* Corresponding author at: Department of Optics II (Optometry and Vision), Faculty of Optic and Optometry, Universidad Complutense de Madrid, Madrid, Spain.
E-mail address: jgcarrac@ucm.es (G. Carracedo).

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Moreover, these substances can increase the production of lysozyme in tears, contributing to ocular surface defense [18]. Additionally, some of these molecules accelerate the rate of corneal wound healing (diadenosine tetraphosphate, Ap_4A) while others inhibit this process (diadenosine pentaphosphate, Ap_5A) [19]. These molecules have been proposed as objective biomarkers of dry eye, since their presence is significantly increased in patients with this disease [20,21]. In a recent study, it has been found that Ap_4A tear concentrations were higher in KC patients than healthy subjects. All patients with KC were RGP wearers [10]. A higher Ap_4A concentration in healthy RGP wearers has also been shown to be related [22].

The release of diadenosine polyphosphates to the tears is caused by shear stress. Corneal epithelial cells release these molecules as a consequence of the lid contact during the blinking process [23]. The CLEK study found an increase of about 25% of corneal scarring in KC patients wearing RGP contact lenses compared to non-users, possibly partly due to the abrasion caused by the contact lens to the corneal surface [24]. We hypothesized that patients with KC who wear RGP contact lenses have higher tear concentrations of diadenosine polyphosphates.

To the best of our knowledge, there is currently no study that evaluates the influence of RGP lenses on tears and ocular surface physiology in patients suffering from KC. Therefore, the aim of this study is to examine the signs, symptoms and adenosine polyphosphate tear concentrations in patients with and without KC as well as the influence of RGP contact lens wear on these parameters in KC.

2. Methods

2.1. Patients

Twenty-three patients (13 males and 10 females) with ages ranging from 20 to 59 (average 34.75 ± 6.12 years) with KC and forty healthy subjects (24 males and 16 females) with ages ranging from 20 to 61 (29.02 ± 6.92 years) were recruited at the Clinical & Experimental Optometry Research Lab (University of Minho, Braga, Portugal) and at the Optometric Clinic of the Faculty of Optic and Optometry (University of Complutense, Madrid, Spain). All patients with KC were diagnosed by an external ophthalmologist and recommended to the research. The study was conducted in compliance with good clinical practice guidelines, institutional review board regulations, informed consent regulations, and the tenets of the Declaration of Helsinki (WMA, 2008) [25]. The protocol of the study was approved by the institutional review board of the University Complutense of Madrid. All the subjects enrolled in the study were adults over 18 years of age, who were able to give informed consent. After the purpose of the study was explained and all doubts clarified to the participants, a consent form was presented and signed by both the patient and the researcher.

KC patients should have been wearing spectacles or aspheric RGP corneal design at least during the last year to be part of the study. Patients with soft contact lenses, hybrid or scleral or previous surgery were excluded. In order to study the influence of RGP lens wearing on the ocular surface integrity, the KC group was divided into users and non-users of RGP lenses. Control group subjects had all worn soft contact lenses on a monthly wear daily basis during the previous 2 years, except eight subjects who had never worn contact lenses at all. Additional demographic characteristics of the population are shown in Table 1.

2.2. Trials

Tests of tear volume, tear stability, corneal staining and recording of subjective symptoms including dryness and discomfort using the McMonnies questionnaire were carried out at the beginning of the day before contact lens wear [26,27].

Tear secretion was measured using the Schirmer I test (without anaesthesia). The tear collection was always performed following the Van Bijsterveld criteria [28]. The Schirmer strip (Tear Flo, HUB pharmaceuticals, USA) was placed on the temporal tarsal conjunctiva of the lower lid for 5 min with the eyes closed. The volume of tears, as millimetres of moistened strip, were recorded and the Schirmer strips were placed in Eppendorf tubes containing 500 μL of Ultrapure water, then the samples were frozen at −80 °C until the high pressure liquid chromatography (HPLC) analysis was performed [20].

To avoid contaminating the strip with fluorescein, fluorescein was applied five minutes after the Schirmer I test to evaluate tear break up time (TBUT) and corneal staining. In order to warrant repeatability of the staining procedure, a solution was prepared using a 10% concentration of sodium fluorescein diluted in saline (NaCl 0.9%). For each application, a micropipette with 5 μl of diluted fluorescein solution was applied in the inferior conjunctival sac and 20 s later TBUT was analyzed through a slit lamp at 16x, using a chronograph to record the time to break after the patient was asked to blink twice and then keep their eyes open. The cornea was divided into five areas to record the grade of staining and, as proposed by the Report of the National Eye Institute and Industry-Sponsored Dry Eye Workshop [29], Cornea and Contact Lens Research Unit (CCLRU) grading scales were used [30].

2.3. Tear preparation and HPLC analysis

After thawing, the samples were strongly vortexed for 5 min. The strips were carefully rinsed and the liquid in the Eppendorf tube was heated in a 100 °C bath for 20 min to precipitate proteins. In order to pellet the proteins, the tubes were centrifuged at 4000 rpm for 30 min. Diadenosine polyphosphates are not degraded by this treatment as previously demonstrated [31]. Supernatants were chromatographed through SEP-PAK Accell QMA cartridges [32]. Briefly, 250 μL of the supernatant was passed through the cartridges that were previously mixed with 3 mL of ultrapure water. The elution of the nucleotides and dinucleotides was performed by applying 1 mL of a solution containing 0.2 M KCl, 0.1 M HCl. Prior to injection into the HPLC, samples were neutralised with KOH. These eluents were injected at a volume of 10–100 μL.

Determination and quantification of diadenosine polyphosphates were performed by HPLC. The chromatographic system consisted of a Waters 1515 isocratic HPLC pump, a 2487 dual absorbance detector and a Reodyne injector, all managed by the Breeze software from Waters. The column was a Novapack C18 (15 cm length, 0.4 cm diameter) from Waters. The system was equilibrated overnight with the following mobile phase: 0.1 M KH_2PO_4, 2 mM tetrabutyl ammonium, 17% acetonitrile, pH 7.5 [19].

Table 1
Demographic characteristics of participants in the study.

Parameter	Control	Keratoconus		
		Total	No RGP wear	RGP wear
Patients	40	23	11	12
Mean age (years) ± SD	29.02 ± 6.92	34.75 ± 6.12	35.96 ± 4.82	33.11 ± 3.16
Age range (years)	[20,61]	[18,59]	[23,59]	[18,44]
Gender (male/female)	[24,16]	[13,10]	[7,4]	[6,6]
Mean keratometry (D)				
Flat	43.87 ± 2.53	49.46 ± 6.32	46.97 ± 3.35	51.97 ± 5.62
Steep	45.31 ± 1.39	56.35 ± 8.76	53.25 ± 6.53	60.81 ± 6.79

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