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Ocular surface epithelial thickness changes with SD-OCT in patients treated with oral isotretinoin

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SD-OCT 检测服用异维 A 酸患者的眼表上皮厚度变化

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摘要

目的:使用频域光学相干断层扫描(SD-OCT)检测口服异维 A 酸患者角膜及结膜上皮厚度。

方法:共 64 例 64 眼寻常痤疮患者纳入研究。32 例患者为 A 组,每日口服异维 A 酸 0.5mg/kg。另外 32 例患者为 B 组,每日口服异维 A 酸 0.8mg/kg。使用 SD-OCT 于服药前、治疗 45d、4mo 以及结束治疗 1mo 后,检测中央角膜厚度(CCT)、中央角膜上皮厚度(CCET)、中央角膜上皮基底膜厚度(CCEBMT)、非上皮中央角膜厚度(NECCT)和球结膜上皮厚度(BCET)。

结果:共有 44 例女性(68.75%)和 20 例男性(31.25%),平均年龄 21.68±3.75 岁。在两组中,CCET、CCT 和 BCET 于服药 45d、4mo 以及治疗结束 1mo 后较治疗前变薄。CCEBMT 于治疗 45d 以及 4mo 时降低,但最后一次随访时与治疗前比较差异无统计学意义。治疗中和治疗后 NECCT 与治疗前相比无明显差异。

结论:口服异维 A 酸治疗的患者,眼表上皮厚度降低,而 NECCT 未受影响。这些患者角膜上皮厚度降低主要是因为上皮变薄。结束治疗 1mo 后,CCEBMT 恢复到治疗前水平,眼表上皮厚度有所增加。

关键词:异维 A 酸;眼表;上皮厚度;光学相干断层扫描

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Abstract

• AIM: To use *in vivo* spectral-domain optical coherence tomography (SD-OCT) to investigate ocular surface epithelial thickness changes in patients treated with oral isotretinoin.

• METHODS: A total of 64 eyes of 64 acne vulgaris patients were enrolled into two group: thirty-two patients received 0.5 mg/kg isotretinoin daily (Group A), and the other 32 patients received 0.8 mg/kg daily (Group B). The central corneal thickness (CCT), central corneal epithelium thickness (CCET), central corneal epithelium basal membrane thickness (CCEBMT), non-epithelial central corneal thickness (NECCT) and bulbar conjunctival epithelium thickness (BCET) were evaluated using SD-OCT at baseline, at the 45th day, at the fourth month of treatment and at the first month after the end of treatment.

• RESULTS: There were 44 females (68.75%) and 20 males (31.25%) with a mean age of 21.68±3.75y. In both groups, CCET, CCT and BCET were significantly thinner at the 45th day, at the fourth month of the treatment and at the first month after the end of treatment as compared with baseline. CCEBMT decreased significantly at the 45th day and at the fourth month of treatment, but there was no difference between the baseline and the last visit. There were no significant difference in NECCT during and after treatment as compared with the baseline.

• CONCLUSION: Ocular surface epithelial thickness decreased in patients treated with oral isotretinoin, whereas NECCT was not affected. The decreasing corneal thickness in patients treated with isotretinon is mainly due to epithelial thinning. After a one-month cessation of isotretinoin treatment, CCEBMT returned to the baseline value, and ocular surface epithelial thickness increased.

• KEYWORDS: isotretinoin; ocular surface; epithelium thickness; optical coherence tomography
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INTRODUCTION

Isotretinoin is a synthetic vitamin A derivate that has been used to treat acne vulgaris and several other skin disorders[1]. Despite its therapeutic effect, the systemic use of isotretinoin can result in various systemic and ocular side effects, as previously described in various studies. The reported ocular side effects are abnormal meibomian gland secretion, blepharoconjunctivitis, corneal opacities, decreased dark adaptation, decreased tolerance to contact lenses, decreased vision, increased tear osmolarity, meibomian gland atrophy, myopia, ocular discomfort, ocular sicca, photophobia, pseudotumor cerebri and keratitis. All reported adverse ocular effects were found to be reversible upon the discontinuation of isotretinoin therapy[2-4].

To the best of our knowledge, *in vivo* alterations of bulbar conjunctival and corneal epithelium thickness after using oral isotretinoin have not yet been studied. *In vivo* imaging of the ocular surface can be performed *via in vivo* confocal microscopy (IVCM), which is a minimally invasive technique[5]. Optical coherence tomography (OCT) is an *in vivo*, non-invasive and non-contact technique that has been used recently to measure corneal and conjunctival epithelial thickness[6-8]. Previous studies have evaluated epithelial thickness using the OCT intensity profile, with the computer-software-controlled cursors being manually placed at the reflectivity peak corresponding to the tissue interfaces[6,9]. The tear film layer and ocular surface epithelium can be separated manually in the images obtained with spectral-domain optical coherence tomography (SD-OCT)[6]. In this study, we aimed to investigate *in vivo* ocular surface epithelial thickness changes in patients treated with oral isotretinoin *via* SD-OCT.

SUBJECTS AND METHODS

This prospective study was approved by the Medical Ethics Committee. The study was carried out in adherence to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants. Sixty-four eyes of 64 acne vulgaris patients treated with oral isotretinoin were recruited from March 2015 to January 2016. Thirty-two participants received 0.5 mg/kg isotretinoin daily (Group A), and the other 32 participants received 0.8 mg/kg daily (Group B). Only the right eye of each patient was enrolled in the study.

The medical histories and demographic data of all participants were noted. All subjects underwent ophthalmic examination, including best-corrected visual acuity, slit-lamp biomicroscopy, fundus examination and anterior segment SD-OCT scanning (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) at baseline, at the 45th day and fourth month of treatment and at the first month after the end of treatment.

Patients above 18y were included in the study. The exclusion criteria included allergic or inflammatory ocular disease, history of ocular surface disorder, using contact lenses, history of ocular surgery, chronic use of topical eye medications and the use of other systemic medications.

OCT Examination and Image Analysis Spectral-domain optic coherence tomography with an anterior segment module was used to measure corneal thickness, corneal epithelium thickness, corneal epithelium basal membrane thickness and conjunctival epithelium thickness. For corneal imaging, a horizontal scan was used at the central location (Figure 1, A-C). For each variable, three measurements were obtained in the 1mm zone at the center of the images. For both corneal and conjunctival images, the first and second hyper-reflective layers were defined as the tear film layer and basal membrane, respectively. Central corneal epithelium thickness (CCET) was determined as the distance between the inferior margin of the tear film layer and the inferior margin of the basal membrane. Central corneal thickness (CCT) was assessed as the whole corneal thickness under the tear film layer. Conjunctival images were taken at the center of the temporal bulbar conjunctiva *via* horizontal scanning (Figure 1, D-F). Bulbar conjunctival epithelium thickness (BCET) was identified as the distance between the inferior margin of the tear film layer and the inferior margin of the basal membrane.

Three measurements were performed between 2 and 3 mm from the limbus, as Francoz *et al*[10] described. The mean of three measurements was used for the statistical analyses of each corneal and conjunctival variable.

The Statistical Package for the Social Sciences (SPSS) Windows 15.0 program was used for the statistical analyses. The normality of the data was evaluated with a Kolmogorov-Smirnov test, and the data were seen to be normally distributed ($P>0.05$). Thickness changes between the follow-up periods were compared using an ANOVA test for repeated measures. To compare the groups, an independent t-test was used for the continuous variables, and a chi-square test was used for the categorical data. A $P<0.05$ was accepted as statistically significant within a 95% confidence interval.

RESULTS

The data of 64 participants were analyzed. There were 44 females (68.75%) and 20 males (31.25%) with a mean age of 21.68±3.75y. There was no statistically significant differences between Group A and Group B with regard to age, gender and baseline anterior segment OCT parameters (Tables 1 and 2).

The statistical significance of the thickness changes between the follow-up periods were same in Groups A and B. CCT and CCET were significantly thinner at the 45th day and fourth month of the treatment and at the first month after the end of treatment as compared with the baseline. However non-epithelial central corneal thickness (NECCT) did not differ during the follow-up as compared to the baseline. Central corneal epithelium basal membrane thickness (CCEBMT) decreased significantly at the 45th day and fourth month of treatment, but there was no difference between the baseline and the last visit. BCET decreased significantly at the 45th day

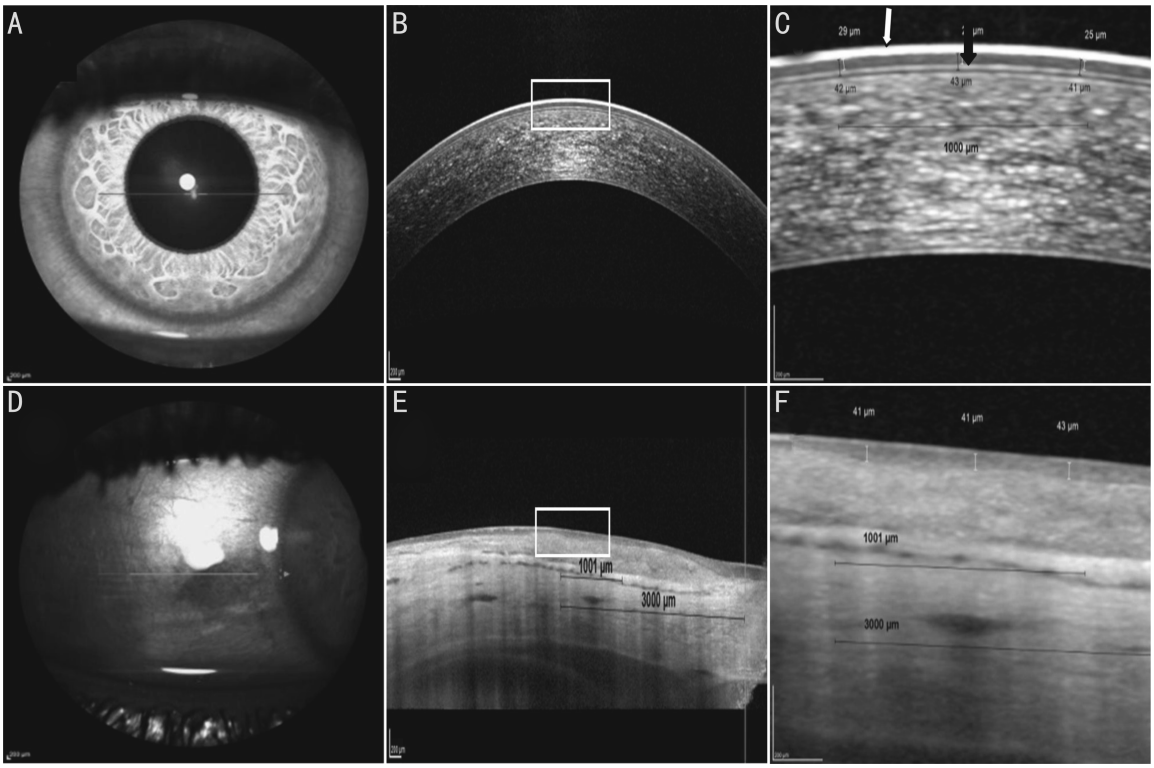

Figure 1 The SD OCT images of ocular surface epithelia in randomly selected subjects A-C: Corneal epithelium (CE) analysis; A: Live confocal scanning laser ophthalmoscope (cSLO) image of the central cornea with the horizontal scan mark; B: corresponding SD-OCT (B-scan), the white rectangle shows the area analyzed; C: CE thickness measurement with software cursors, the tear film appears hyperreflective (white arrow) and the basal epithelial membrane appeared hyperreflective (black arrow); D-F: bulbar conjunctival epithelium (BCE) analysis; D: Live cSLO image; E: corresponding SD-OCT image; the white rectangle shows the bulbar conjunctival zone between 2 and 3mm from limbus (horizontal line); F: BCE thickness measures.

Table 1 Demographic features of participants

Types	Group A (0.5mg/kg daily, $n=32$)	Group B (0.8mg/kg daily, $n=32$)	P
Age	21.75±4.5	21.62±2.89	0.895[a]
Gender(F/M)	20/12	24/8	0.281[b]

[a]By indepedent sample t test; [b]By chi-square test.

Table 2 Alterations of anterior segment OCT parameters during the follow up

Indexes	Group A ($n=32$)				P^a	Group B($n=32$)				P^b
	baseline	45d	4mo	1mo after the treatment		baseline	45d	4mo	1mo after the treatment	
CCET	47.09±4.02	44.03±4.11	42.93±4.07	46.1±4.16	<0.001	47.5±2.78	45.31±3.46	43.96±3.68	46.43±3.32	<0.001
P	0.64[c]	<0.001[d]	<0.001[d]	0.001[d]	–	–	<0.001[d]	<0.001[d]	<0.001[d]	–
	–	<0.001[e]	0.002[e]	–	–	–	<0.001[e]	<0.001[e]	–	–
CCEBMT	11.03±0.86	10.46±0.94	10.18±0.85	10.75±0.84	<0.001	10.87±1.01	10.5±0.8	10.18±0.82	10.68±0.85	<0.001
P	0.512[c]	<0.001[d]	<0.001[d]	0.058[d]	–	–	0.009[d]	<0.001[d]	0.071[d]	–
	–	0.025[e]	<0.001[e]	–	–	–	0.001[e]	<0.001[e]	–	–
CCT	538.56±35.14	535.31±35.7	532.96±37.69	537.4±35.81	<0.001	521.31±36.26	518.93±36.04	517.5±36.82	520.56±36.46	<0.001
P	0.058[c]	<0.001[d]	<0.001[d]	<0.001[d]	–	–	<0.001[d]	<0.001[d]	<0.001[d]	–
	–	<0.001[e]	<0.001[e]	–	–	–	<0.001[e]	<0.001[e]	–	–
NECCT	491.46±35.1	491.28±34.99	490.03±37.34	491.41±35.01	0.515	473.81±35.63	473.62±35.01	473.53±35.4	473.91±35.49	0.124
P	0.052[c]	–	–	–	–	–	–	–	–	–
BCET	44.34±5.36	40.87±5.07	39.15±4.64	43.31±5.38	<0.001	45.43±4.44	41.93±4.97	41.31±4.44	44.06±4.36	<0.001
P	0.382[c]	<0.001[d]	<0.001[d]	<0.001[d]	–	–	<0.001[d]	<0.001[d]	<0.001[d]	–
	–	<0.001[e]	<0.001[e]	–	–	–	<0.001[e]	<0.001[e]	–	–

[a]:ANOVA test for repeated measures (within Group A); [b]:ANOVA test for repeated measures (within Group B); [c]=indepedent sample t test (comparison between Group A and B); [d]:Bonferroni post-hoc analyses(comparison of ocular surface thicknesses with baseline); [e]:Bonferroni post-hoc analyses (comparison of ocular surface thicknesses with last visit); CCET: Central corneal epithelium thickness; CCEBMT: Central corneal epithelium basal membrane thickness; CCT: Central corneal thickness; NECCT: Non-epithelial central corneal thickness; BCET: Bulbar conjunctival epithelium thickness.

and fourth month of treatment and at the first month after the end of treatment as compared with the baseline. Therefore, CCT, CCET, CCEBMT, and BCET were significantly thicker at the last visit than at the 45th day and fourth month. Alterations in the anterior segment OCT parameters during the follow-up are shown in Table 2.

Table 3 Comparison of ocular surface epithelium changes from baseline between groups

Parameters	Group A (0.5 mg/kg daily, $n=32$)	Group B (0.8mg/kg daily, $n=32$)	P
CCET baseline-45thd	2.76±1.74	2.48±1.53	0.325
CCET baseline-4thmo	4.15±1.62	3.53±2.29	0.214
CCET baseline- 1stmo after treatment	1.09±1.71	1.06±1.16	0.932
BCET baseline-45thd	3.46±2.01	3.50±1.93	0.950
BCET baseline-4thmo	5.08±2.71	4.22±2.03	0.071
BCET baseline- 1stmo after treatment	1.03±1.40	1.37±1.51	0.351

Ocular surface epithelium changes from baseline were compared between Group A and Group B. No significant difference was found between two groups with regards to ocular surface epithelium alterations (Table 3).

DISCUSSION

Isotretinoin therapy may result in dry eyes, and the effects of oral isotretinoin on the ocular surface and tear film are well-defined[11-12]. Previous studies have revealed that meibomian gland function depression due to isotretinoin therapy induces increased evaporation and tear film instability, causing the drying and secondary irritation of the conjunctiva and cornea[11-14]. In addition, the presence of isotretinoin and its metabolites in the tear film may directly irritate the ocular surface[11,13].

The corneal epithelium, which forms the anterior protective surface, consists of the stratified squamous epithelium and its underlying intact basement membrane (BM). The effects of isotretinoin on the corneal epithelium and corneal thickness have been evaluated in various studies using many different methods[12,15-18]. Dry eye caused by isotretinoin is one of the mechanisms responsible for corneal thinning. In patients with dry eye, some authors have used IVCM to observe decreased epithelial cell density, with a tendency toward epithelial thinning[19-21]. Studies have shown that dry eye can cause the thinning of the central and midperiphery cornea *via* increased osmolarity, chronic desiccation and immune activation[16,22-23].

Yildirim *et al*[16]. evaluated the effect of isotretinoin on corneal thickness using Sirius corneal pachymetries. The corneal thickness was significantly thinner at first, third and sixth months of isotretinoin therapy as compared to the baseline. Cumurcu *et al*[17] and Yüksel *et al*[18] evaluated CCT using Pentacam Scheimpflug topography in patients who used isotretinoin. Significantly decreased CCT values were detected at the sixth month of treatment as compared to the baseline. Previous studies did not define which corneal layer caused decreased corneal thickness. In our study, we take advantage of SD-OCT, which is a completely noninvasive imaging method that allows high-resolution analysis and measurements. We report that central corneal thickness and CCET were significantly thinner at the 45th day and the fourth month of treatment and at the first month after the end of treatment as compared with baseline. However, non-epithelial central corneal thickness (NECCT) did not differ during follow-up. These findings reveal that decreasing corneal thickness in patients treated with isotretinon is mainly due to epithelial thinning. We also found that central corneal epithelium basal membrane thickness (CCEBMT) decreased significantly at the 45th day and fourth month of treatment, but there was no difference between baseline and the last visit. To the best of our knowledge, this is the first report of the thickness profile of the epithelial basement membrane layer using ultra-high-resolution OCT. Central corneal thickness, CCET and CCEBMT were significantly thicker at the last visit than at the 45th day and fourth month of treatment.

Francoz *et al*[10] used SD-OCT and reported the absence of differences in CET between patients with dry eye syndrome, patients on intraocular pressure (IOP)-lowering medication and middle-aged control groups, despite increased BCET in the dry eye group and the IOP-lowering treatment group. This discordance may have occured due to the relative lack of inflammatory cells and immune mediators in the cornea as compared with the conjunctiva, rendering the cornea less sensitive to inflammatory changes[10]. We consider dry eye and direct toxic effects to be potential reasons for corneal thinning. In our study, we revealed that decreased corneal thickness in patients treated with isotretinon is mainly due to epithelial thinning.

The bulbar conjunctiva epithelium is well-recognized as consisting of multiple layers of cylindrical epithelial cells. The superficial and intermediate epithelial cell layers contain round or oval mucus-secreting goblet cells[24].

In patients with dry eye, Wakamatsu *et al*[25] detected a decrease in the density of superficial, intermediate and deeper conjunctival epithelial cells using IVCM. They suggest that this reduction may be due to the elevation of the ocular surface inflammatory status and disturbances in the overall turnover of epithelial cells.

Liang *et al*[26] reported significantly increased mean BCET in dry eye patients, as measured by using SD-OCT, as compared to normal eyes. They suggested that the infiltration of inflammatory cells and tissue edema observed within the conjunctiva in dry eye patients may explain, at least in part, the thickening of the conjunctival epithelium. However, Queiroga *et al*[27] observed that inflammatory cells remain unchanged in patients treated with isotretinoin and suggested that local toxicity may explain conjuntival epithelium thinning. Queiroga *et al*[27] found alterations in both the exposed conjunctiva (temporal bulbar region) and the unexposed conjunctiva (superior bulbar region) in patients treated with oral isotretionin. This hypothesis regarding local toxicity is in agreement with reports published by Rismondo *et al*[11]. Karalezli *et al*[15] observed conjunctival epitelial changes and significantly higher impression cytology scores for

eyes, including decreased goblet cell density and a reduction in the nucleus-to-cytoplasm ratio and cell cohesiveness. Impression cytology findings may explain conjunctival ephitelial layer thinning in patients treated with isotretinoin, which is consistent with our study.

Although IVCM and impression cytology have provided many advances in the exploration of ocular surface diseases, these techniques cannot provide precise *in vivo* measurements of epitelial thickness[5,28-29].

We detected that BCET decreased significantly at the 45th day and fourth month of treatment and at the first month after the end of treatment as compared with the baseline. However, BCET was significantly thicker at the last visit than at the 45th day and fourth month. Our results revealed that the effect of isotretinoin on the conjunctiva may be directly related to a toxic effect. This effect is not permanent.

The statistical significance of thickness changes between follow up periods were the same in Group A (0.5 mg/kg isotretinoin) and Group B (0.8 mg/kg isotretinoin).

One limitation of this study is that in terms of measurement technique, the ruler we used for thickness measurement is calibrated for measuring corneal tissue based on the refractive index of the cornea. The lack of consistency with dry eye tests may be another limitation. Previous studies have revealed that 6mo after cessation, corneal thickness began to return to baseline values[16]. Unfortunately, our patients underwent only a one-month follow-up after the discontination of the drug.

Consequently, systemic isotretinoin treatment causes the thinning of the ocular surface epithelium, and dose seems to have no effect on this thinning. Decreasing corneal thickness in patients treated with isotretinon is mainly due to epithelial thinning. Our study results reveal that after the one-month cessation of oral isotretinoin treatment, CCEBMT began to return to the baseline value, and ocular surface epithelial thickness was increasing. We require large-series studies with SD-OCT for the precise evaluation of epithelial thickness over time, including its eventual normalisation.

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