·Letter to the Editor·

Evaluation of corneal endothelium after UVA/riboflavin cross-linking in thin keratoconic corneas

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Dear Sir,

UVA/riboflavin cross-linking (CXL) has been used clinically applied for the treatment of keratoconus and corneal edema *via* enhancement of corneal stiffness [1-2]. The safety of the corneal endothelium is of prime importance during CXL treatment. In clinical practice, a corneal thickness (CT) of 400 μm has traditionally been regarded as the minimum treatable thickness, thereby avoiding damage to the corneal endothelium [3]. Although CXL has been applied to thinner corneas, using a hypoosmotic solution onto cornea and inducing edema [4]. CXL safety still needs further evaluation because of lower relative concentration of collagen in the hydrated stroma [5]. This study aims to evaluate the changes of corneal endothelial density (ECD) in cases where the CT is <400 μm before iatrogenic corneal swelling and CXL treatment.

The current study is a prospective comparative case series of patients treated for progressive keratoconus in Shandong Provincial Hospital. After abrading the epithelium, CTs (at the thinnest point) were assigned to one of two groups: thin group (CT<400 μm) and normal group (CT>400 μm). In the thin group, distilled water was applied topically every 2min after 8.5 mm diameter epithelium removal, before cornea thickness reached 400 μm. In both groups, an isosmotic solution (0.1%) of riboflavin was instilled every 2min throughout the 30min soaking time, then the CTs were evaluated again. Subsequently, the cornea was illuminated with an ultraviolet (UV) light for 30min using a UVA lamp (UVX 1000 system, IROC Innocross AG Co. Ltd., Switzerland) at a wavelength of 365 nm, irradiance of 3.0 mW/cm^2, and total dosage of 5.4 J/cm^2. Hypo-osmolar riboflavin administration was continued every 2min during UV illumination. CT and ECD were checked *via* anterior optical coherence tomography (OCT) [6] and non-contact specular microscopy [7], respectively, before treatment and at weeks 1, 2 and 3; months 1, 3, and 6; and year 1 after CXL. The study followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Results were analyzed using the Mann-Whitney U test, unpaired t-test. All statistical analyses were performed using version 17.0 SPSS software. A P value of <0.05 was considered significant in all cases.

The thin group comprised 10 patients (6 men, 4 women; n=12 eyes). The control group comprised 18 patients (10 men, 8 women; n=30 eyes). Mean baseline CTs (with epithelium) ranged from 320 to 373 μm (average, 345±21 μm) in thin group and from 481 to 598 μm (average, 512±46 μm) in normal group. CTs after epithelial removal ranged from 288 to 328 μm (average, 302±19 μm) and after pre-CXL inducement of corneal edema, CTs ranged from 407 to 469 μm (average, 426±21 μm) in thin group. CTs were significantly increased on week-1 (P<0.05 to baseline for two groups), decreased by week-3 (P>0.05 to baseline) in thin group and week-2 (P>0.05 to baseline) in normal group then remained stable for 1y (P>0.05 to baseline for two groups; Table 1).

ECD were significantly decreased at week 1 (P<0.05 to baseline), recovering by week 3, then remained stable for 1y in thin group. But in normal group, there was no significant difference in ECD values observed between each time after CXL and baseline (all P>0.05; Table 1). As revealed by anterior OCT, the mean CXL demarcation line depths were 246±12 and 294±58 μm in the thin and normal groups, respectively, at month-1. No corneal infections, stromal scar or significant haze were observed during follow-up. No statistically significant difference were noted between preoperative and postoperative (1y) best spectacle corrected visual acuity (BSCVA) values (P=0.586), K-values

Table 1 Corneal thickness and corneal endothelial density following CXL

Post-CXL time	Thin cornea group		Normal cornea group	
	CT (μm)	ECD (/mm^2)	CT (μm)	ECD (/mm^2)
Baseline	345±21	3161±280	512±46	2817±236
Week-1	448±46[a]	2566±547[a]	585±58[a]	2893±332[b]
Week-2	415±41[a]	2656±411[a]	527±54[b]	2873±263[b]
Week-3	340±37[b]	3002±324[b]	515±56[b]	2905±247[b]
Month-1	335±27[b]	3166±279[b]	537±57[b]	2775±260[b]
Month-3	333±35[b]	2968±275[b]	496±47[b]	2760±227[b]
Month-6	327±27[b]	3035±311[b]	504±44[b]	2642±255[b]
Year-1	331±28[b]	3066±274[b]	506±42[b]	2704±238[b]

[a]P<0.05 versus baseline; [b]P>0.05 versus baseline. CXL: UVA/riboflavin cross-linking; CT: Corneal thickness; ECD: Corneal endothelial density.

(P=0.271), or spherical equivalent refraction (P=0.136).

Using the currently recommended CXL protocol, the amount of UV radiation reach the corneal endothelium is about 0.18 mW/cm^2, less than the theoretical endothelial damage threshold of 0.35 mW/cm^2 [4]. Kymionis *et al* [8] found a significant decrease in ECD in thin corneas (<400 μm) after CXL. But Hafezi *et al* [9] found no clinical signs of endothelial damage or other side effects in corneas with thicknesses of 320-400 μm after CXL when thin corneas were preoperatively swollen to thicknesses of at least 400 μm. In our study, we found that the ECD in thin group had not decreased significantly after 2wk. The minimal value of the ECD on week-1 may be caused by the corneal edema that occurred soon after CXL, which, in turn, reduces the recognition rates of intact endothelial cells. Using the treatment protocol described herein, our results substantiate post-CXL endothelial safety of CTs of 288-328 μm (without the epithelium). Considering the potential reduction of corneal thickness and ECD as revealed in this study, a large scale study with long time follow-up is needed in thin corneas.

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