Original Article

Clinical Observation of Transepithelial Corneal Collagen Cross-linking by Iontophoresis of Riboflavin in Treatment of Keratoconus

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Abstract

Purpose: To evaluate the efficacy and safety of transepithelial collagen cross-linking by iontophoretic delivery of riboflavin in treatment of progressive keratoconus.

Methods: Eleven patients (15 eyes) with progressive keratoconus were enrolled. After 0.1% riboflavin-distilled water solution was deliveried via transepithelial iontophpresis for 5 min with 1 mA current, and ultraviolet radiation (370 nm, 3 mW/cm^2) was performed at a 1.5 cm distance for 30 min. The follow up were 6 months in all eyes. The uncorrected visual acuity, corrected visual acuity, endothelial cell counting, corneal thickness, intraocular pressure, corneal curvature, corneal topography, OCT and corneal opacity before and 6-month after surgery were analyzed.

Results: At 6 month postoperatively, mean uncorrected visual acuity and corrected visual acuity changed from 0.36 to 0.30 and from 0.42 to 0.57 without statistical significance. The mean value of each index of corneal curvature declined without statistical significance.Kmax value dereased from 60.91 to 59.91, and the astigmatism declined from 3.86 to 3.19. Central corneal thickness decreased from 460.93 μm to 455.40 μm, and thinnest corneal thickness declined from 450.87 μm to 440.60 μm with no statistical significance. Intraocular pressure was significantly elevated from 10.85 mmHg to 12.62 mmHg. Endothelial cell count did not change significantly. No corneal haze occurred. Mean depth of corneal demarcation line was 288.46 μm at 1 month postoperatively.

Conclusion: Transepithelial corneal collagen cross-linking by iontophoresis is effective and safe in the treatment of progressive keratoconus, and yields stable clinical outcomes during 6-month follow up. However, long-term follow up is urgently required. (***Eye Science 2014; 29:160-164***)

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Keywords: keratoconus; corneal collagen cross-linking; iontophoresis; treatment outcome

Introduction

Keratoconus is a degenerative disorder of the eye characterized as structural changes within the cornea cause it to thin and change to a more conical shape than the normal gradual curve, which may progress into visual loss and require corneal transplantation. How to control the development of keratoconus determines the success of treatment. At present, riboflavin ultraviolet corneal collagen cross-linking is a novel pathological therapy of preventing the progress of keratoconus[1,2]. The underlying mechanism is that riboflavin, as a photosensitizer, enters corneal stroma and then is activated by ultraviolet radiation to release reactive oxygen species to cause amino cross-linking among collagen fibers and increase mechanical strength of corneal stroma[3,4].

Riboflavin, as a water-soluble macromolecule, is difficult to pass through corneal epithelial barrier. Thus, the administration method of de-epithelization has been adopted in conventional corneal collagen cross-linking[5-7]. Corneal epithelial removal yields se vere pain, long recovery time and potential risk of infection. Currently, transepithelial corneal cross-linking is increasingly diversifying into various ap proaches[8-11], such as use of different penetration enhancers[12-14], ultrasonic introduction[15], needle injection[16-18] and iontophoresis. Use of penetration enhancers and ultrasonic introduction yield low efficacy and the procedures of needle injection are complicated. Thus, iontophoresis is becoming the focus of recent studies.

In 2014, we conducted preliminary animal experiment and demonstrated that iontophoresis using 0.1% riboflavin solution could achieve the yellow staining effect similar to de-epithelized administration[19]. On the basis of previous studies, we reported the outcomes of 6-month follow up as below.

Materials and methods

Study subjects

In total, 11 patients (15 eyes) diagnosed with progressive keratoconus in our hospital between July and December 2012 were enrolled in this study, including 9 males (11 eyes) and 2 females (4 eyes). Subsequent follow up endured for at least 6 months.

Diagnosis and exclusion criteria

In this study, patients with progressive keratoconus were included. Corneal topography revealed that keratoconus was aggravated. The maximum corneal curvature increased > 1.00 D within one year. Patients with corneal scars or corneal thickness < 400 μm were excluded. This study was approved by the ethnic committee of our hospital. Informed consents were obtained from all patients.

Surgical procedures

The conjunctival sac was irrigated and disinfected, local anaesthesia by one drop of oxybuprocaine and the forehead skin was disinfected by ethanol. Patients lay on the bed in a supine position, the eyelids were opened using eye speculum, the iontophoresis instrument was connected and 0.1% of riboflavin solution was delivered by iontophoresis for 5 min at a current of 1 mA. Then, the cornea and aqueous humor were observed under slit-lamp examination. If the yellow staining was not obvious, another 5 min iontophoresis was performed. If desirable yellow staining was obtained, ultraviolet radiation was conducted at a wavelength of 370 nm for 30 min (3 mW/cm^2). The riboflavin solution was administered on the corneal surface every 3 min during the period of irradiation. The pain intensity was closely observed and oxybuprocaine was delivered for local anaesthesia as necessary. After treatment, corneal reactions and pain intensity were monitored.

Preoperative examination and postoperative follow up

All patients underwent uncorrected/correct visual acuity test, endothelial cell counting, corneal thickness, intraocular pressure measurement, corneal curvature, corneal topography, OCT and corneal haze examinations. The test results were recorded.

Statistical analysis

SPSS 16.0 software was adopted for data analysis. Comparison of different indexes before and after surgery was conducted by t-test. If normal distribution and variance homogeneity were not met, rank sum test was utilized. $P<0.05$ was considered as statistical significance.

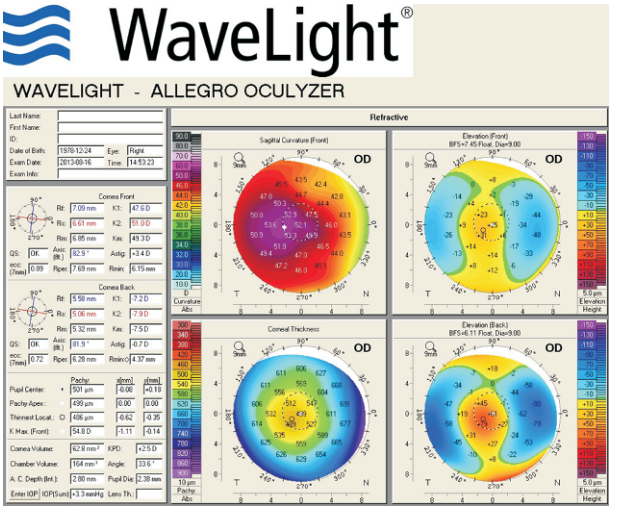

Figure 1 Corneal tonography before surgery

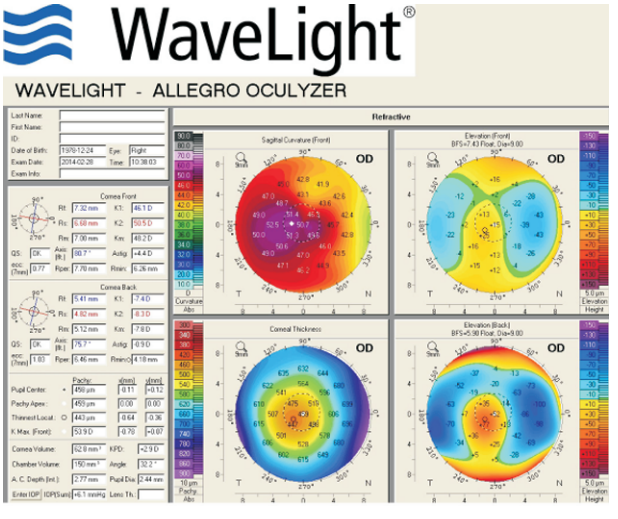

Figure 2 Corneal tonography at postoperative 6 months

Results

Time of electric current

In most patients, the yellow staining of corneal

Table 1 Clinical observation of transepithelial collagen cross-linking by iontophoretic delivery of riboflavin (means±s)

Parameters	Before cross-linking	At 6 months after cross-linking	*t* or u value	*P*
Uncorrected visual acuity	0.36±0.34	0.30±0.28	0.528	0.6019
Corrected visual acuity	0.42±0.25	0.57±0.32	1.357	0.1876
Corneal curvature				
K1	49.43±6.42	49.31±6.94	0.373	0.709
K2	53.30±6.27	52.46±7.37	1.078	0.281
K_{mean}	50.89±6.19	50.50±6.89	0.565	0.572
K_{max}	60.91±10.73	59.91±13.54	1.037	0.300
Astigmatism	3.86±2.70	3.19±2.54	0.696	0.4922
Corneal thickness				
Central corneal thickness	460.93±49.93	455.40±46.73	0.313	0.7563
Thinnest corneal thickness	450.87±48.32	440.60±43.88	0.609	0.5473
Intraocular pressure	10.85±1.35	12.62±2.47	2.27	0.0325*
Corneal endothelial count	2972.14±461.30	3176.00±307.13	1.376	0.1804

*$P<0.05$

aqueous humor similar to de-epithelized administration could be observed under slit-lamp examination after 5-min electric current and 10-min electric current was needed in one case.

Comparison of clinical indications before and after transepithelial collagen cross-linking by iontophoretic delivery of riboflavin

At 6 months after treatment, corrected visual acuity was increased from 0.42 to 0.57 and the corneal endothelial count was equally elevated with no statistical significance. The corneal curvature, uncorrected visual acuity and corneal thickness had a declining tendency with no statistical significance. Kmax value declined from 60.91 to 59.91 and from 3.86 to 3.19 for astigmatism. The central corneal thickness declined from 460.93 μm to 455.40 μm, and from 450.87 μm to 440.60 μm for the thinnest corneal thickness. Only intraocular pressure was significantly elevated from 10.85 mmHg up to 12.62 mmHg, probably correlated with the increases in corneal intensity. Albeit corneal thickness became thinner, it may be the manifestations of corneal cross-linking and remain to be further observed.

Pain intensity and corneal reactions

Mild reactions were observed intraoperatively in most cases. Approximately 1/3 of patients presented with slight pain and was mitigated by 2 to 3 drops of oxybuprocaine. The pain was alleviated after postoperative 3 d. No oral intake of analgesics was given. During early stage, corneal epithelial edema was observed and almost recovered 3 d later or 5 d in several cases.No corneal haze was seen after surgery.

Postoperative demarcation line depth

Postoperative demarcation line depth was measured by OCT and the mean demarcation line depth was (288.46±30.00)μm at postoperative 1 month.

Discussion

Iontophoresis treatment adheres to the principle of electric charge repulsion and attraction. The medication ion was placed in the same electrode under direct current, introduced into the skin, mucosa or wound[20,21]. Iontophoresis has been applied in the field of ophthalmology for over 100 years, which is a minimally invasive administration approach[22,23].

Arboleda et al[24]. measured the concentration of riboflavin in aqueous humor of mice. Mastropasqua et al[25]. detected the levels of riboflavin within corneal stroma via iontophoresis by autopsy. Cassagne et al[26]. found that the concentration of riboflavin in the cornea via iontophoresis was lower compared with that of conventional de-epithelized approach. Vinciguerra et al[27]. analyzed the biomechanical changes in human cadaveric eyes following ultraviolet radiation cross-linking via iontophoresis, whereas the clinical efficacy was lower than that of conventional de-epithelization. Bikbova et al[28]. added penetration enhancers into the riboflavin solution and gradually increased the current intensity from 0.2 mA to 1 mA for 10 min. Compared with conventional de-epithe-

lization, iontophoresis caused smaller depth of corneal cell apoptosis and demarcation line. The depth of demarcation line was closely correlated with the effect of cross-linking[29]. Therefore, albeit the severity of diseases was properly controlled, the hardening effect was not satisfactory.

In our previous animal experiment, 0.1% of riboflavin solution was delivered into corneal stroma via iontophoresis at 1 mA for 10 min and the concentration was similar to that of conventional de-epithelized administration[19]. This approach was adopted in this clinical study and yielded good effect after 6-month follow up.

In this study, yellow staining of corneal aqueous humor was observed in most cases after 5 min electric current and 10 min in merely one eye, which significantly reduced the treatment time compared with de-epithelized administration (30 min). Additionally, the treatment time was shorter than that of our previous animal experiment (10 min), probably due to the structural difference between rabbit and human eyes and the lower corneal epithelial barrier function. Bikbova et al[28]. gradually increased the current intensity from 0.2 mA to 1 mA, whereas we utilized 1 mA throughout the experiment. Moreover, smaller optic cup contained less riboflavin solution and it was convenient to operate.

This 6-month follow up demonstrated that desirable yellow staining of the cornea and aqueous humor could be obtained via iontophoresis. Postoperative visual acuity and corneal curvature were stable. The demarcation line depth was even slightly deeper than that of conventional de-epithelized administration. Postoperative intraocular pressure was significantly elevated, suggesting the hardening effect of the cornea. Patients presented with mild pain intraoperatively and rapidly healed postoperatively. No corneal endothelial injury or corneal haze was observed. All the evidence confirmed the safety of this treatment. However, the study with a larger sample size and longer follow up is urgently required.

Even the penetration enhancer was not utilized, while the efficacy of iontophoresis was equally satisfactory probably due to the following reasons. First, the quantity of parasitic ion in the distilled water was the smallest and the resistance force of riboflavin ion was the minimal. The quantity of medication via iontophoresis is correlated with a variety of factors, such as solution concentration, parasitic ion, current intensity, time of electric current, *etc*. The quantity of parasitic ion in complex solvents reduces the amount of medication via iontophoresis. Second, the osmotic pressure of the solution was low. In this study, the corneal epithelial edema endured for 1-3 d, which affected the corneal epithelial barrier function and accelerated the transportation of riboflavin. However, the results remain to be further elucidated by pathological examination.

In addition, conventional de-epithelized administration is likely to induce corneal dehydration. It requires high standards of corneal thickness, which should be >400 μm before ultraviolet radiation. However, transepithelial cornea is protected by corneal epithelia and thus the dehydration is not obvious. Moreover, corneal epithelia partially block the ultraviolet radiation and enhance the safety. Therefore, it is hypothesized that this technique is efficacious and safe for patients with corneal thickness < 400 μm, which remains to be elucidated.

Study limitations: In this study, the biomechanical effect, long-term efficacy and the underlying mechanism of iontophoresis are lacking and remain to be clarified. Taken together, transepithelial corneal collagen cross-linking via iontophoresis of 0.1% riboflavin is a promising treatment of keratoconus.

References

1 Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye Res, 1998, 66: 97–103.

2 Wollensak G. Cross linking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol, 2006, 17: 356–360.

3 McCall AS, Kraft S, Edelhauser HF, et al. Mechanisms of corneal tissue cross-linking in response to treatment with topical riboflavin and long-wavelength ultraviolet radiation (UVA). Invest Ophthalmol Vis Sci, 2010, 51: 129–138.

4 Kamaev P, Friedman MD, Sherr E, et al. Photochemical kinetics of corneal cross-linking with riboflavin. Invest Ophthalmol Vis Sci, 2012, 53: 2360–2367.

5 Raiskup F, Spoerl E. Corneal crosslinking with riboflavin and ultraviolet A. Part II. Clinical indications and results. Ocul Surf, 2013, 11: 93–108.

6 Hayes S, O'Brart DP, Lamdin LS, et al. Effect of com-

plete epithelial debridement before riboflavin-ultraviolet-A corneal collagen crosslinking therapy. J Cataract Refract Surg,2008,34:657-661.
7 Samaras K, O'brart DP, Doutch J, et al. Effect of epithelial retention and removal on riboflavin absorption in porcine corneas. J Refract Surg,2009,25:771-775.
8 Baiocchi S, Mazzotta C, Cerretani D, et al. Corneal crosslinking:riboflavin concentration in corneal stroma exposed with and without epithelium. J Cataract Refract Surg,2009,35:893-899.
9 Bottós KM, Schor P, Dreyfuss JL, et al. Effect of corneal epithelium on ultraviolet-A and riboflavin absorption.Arq Bras Oftalmol,2011,74:348-351.
10 Zhang ZY, Zhang XR. Efficacy and safety of transepithelial corneal collagen crosslinking. J Cataract Refract Surg,2012,38:1304-1305.
11 Raiskup F,Spoerl E.Corneal crosslinking with riboflavin and ultraviolet A.I. Principles.Ocul Surf,2013,11:65-74.
12 Kissner A,Spoerl E,Jung R,et al.Pharmacological modification of the epithelial permeability by benzalkonium chloride in UVA/Riboflavin corneal collagen cross-linking. Curr Eye Res,2010,35:715-721.
13 Filippello M, Stagni E, O'Brart D.Transepithelial corneal collagen crosslinking: bilateral study. J Cataract Refract Surg,2012,38:283-291.
14 Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. J Refract Surg, 2010, 26: 942-948.
15 Lamy R, Chan E, Zhang H, et al. Ultrasound-enhanced penetration of topical riboflavin into the corneal stroma. Invest Ophthalmol Vis Sci,2013,5908-5912.
16 Krueger RR, Ramos-Esteban JC, Kanellopoulos AJ. Staged intrastromal delivery of riboflavin with UVA cross-linking in advanced bullous keratopathy:laboratory investigation and first clinical case.J Refract Surg,2008, 24:730-736.
17 Kanellopoulos AJ. Collagen cross-linking in early keratoconus with riboflavin in a femtosecond laser-created pocket:initial clinical results.J Refract Surg,2009,25:1034-1037.
18 Dong Z, Zhou X. Collagen cross-linking with riboflavin in a femtosecond laser-created pocket in rabbit corneas:6-month results.Am J Ophthalmol,2011,152:22-27.
19 Li N, Peng X, Fan Z, et al. Iontophoretic delivery of riboflavin into the rabbit cornea:a primary study. Eye Science,2014,29:30-35.
20 Li H, Yu Y, Faraji Dana S, et al. Novel engineered systems for oral, mucosal and transdermal drug delivery. J Drug Target,2013,21:611-629.
21 Kalia YN, Naik A, Garrison J, et al. Iontophoretic drug delivery. Adv Drug Deliv Rev,2004,56:619-658.
22 Gaudana R, Ananthula HK, Parenky A, et al. Ocular drug delivery. AAPS J,2010,12:348-360.
23 Eljarrat-Binstock E, Domb AJ. Iontophoresis: a non-invasive ocular drug delivery.J Control Release,2006,110: 479-489.
24 Arboleda A, Kowalczuk L, Savoldelli M, et al. Evaluating in vivo delivery of riboflavin with coulomb-controlled iontophoresis for corneal collagen cross-linking:a pilot study. Invest Ophthalmol Vis Sci,2014,55:2731-2738.
25 Mastropasqua L, Nubile M, Calienno R, et al. Corneal cross-linking:intrastromal riboflavin concentration in iontophoresis-assisted imbibition versus traditional and transepithelial techniques. Am J Ophthalmol,2014,157: 623-630.
26 Cassagne M, Laurent C, Rodrigues M, et al. Iontophoresis transcorneal delivery technique for transepithelial corneal collagen crosslinking with riboflavin in a rabbit model. Invest Ophthalmol Vis Sci,2014 Mar 18.pii: iovs.13-12595v1.doi:10.1167/iovs.13-12595 [Epub ahead of print].
27 Vinciguerra R, Spoerl E, Romano MR, et al. Comparative stress strain measurements of human corneas after transepithelial UV-induced cross-linking:impregnation with iontophoresis,different riboflavin solutions and irradiance power. Invest Ophthalmol Vis Sci,2012,53.E-abstract #1518.
28 Bikbova G, Bikbov M. Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin.Acta Ophthalmol,2014,92:e30-34.
29 Kymionis GD, Tsoulnaras KI, Grentzelos MA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. J Cataract Refract Surg,2014,40:736-740.