Original Article

Transforming growth factor-β₁ level in tears and corneal haze formation following flap-on or flap-off Epi-LASIK

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Abstract: Objective To observe the influence of flap-on or flap-off Epipolis laser in situ keratomileusis (epi-LASIK) on the release of transforming growth factor-β₁ (TGF-β₁) in tear fluid and corneal haze formation. Methods Thirty patients (60 eyes) with myopia underwent epi-LASIK surgery with epithelial flap repositioning (flap-on) in the right eyes and epithelial flap removal (flap-off) in the left eyes. The level of TGF-β₁ in tears was measured preoperatively and on days 1, 3, and 7 postoperatively. Concomitant haze was graded at 1, 3, and 6 months after surgery. Results The mean preoperative spherical equivalent refraction was -4.98±2.28 D (-2.50 to -7.25 D) in flap-on group and -5.20±4.02 D (-1.75 to -7.00 D) in flap-off group, showing no significant difference between the two groups (P=0.80). TGF-β₁ levels in the tear fluid were similar in the two groups preoperatively (P=0.11) and at 1, 3, and 7 days postoperatively (P=0.55, 0.45, 0.19, respectively). TGF-β₁ levels in tears gradually decreased after the first postoperative day in both groups, but were still higher than the preoperative value till the 7th postoperative day. Corneal haze scores in the two groups were similar at 1 month (P=0.98), 3 months (P=0.52), and 6 months (P=0.72) after the operation. Conclusion Flap-on and flap-off epi-LASIK surgeries do not differ significantly in postoperative TGF-β₁ levels in the tear fluid or in the postoperative haze scores. TGF-β₁ may play a role in corneal wound healing.

Key words: Epipolis laser in situ keratomileusis; epithelial flap; transforming growth factor-β₁; haze

INTRODUCTION

The number of patients receiving corneal refractive surgery on healthy corneas has grown exponentially in the past two decades. Surface ablation is the current trend to stage a comeback [1]. Epipolis laser in situ keratomileusis (epi-LASIK), as an alternative surface ablation procedure, was first developed for correction of refractive errors such as myopia and astigmatism by Pallikaris [2] in 2003. Theoretically, the preserved epithelial sheet may represent a barrier that protects the photoablated corneal stroma from inflammation mediators [3,4]. However, reports remain inconclusive about whether maintaining a viable epithelial flap contributes to less postoperative pain, faster visual rehabilitation, and better visual outcomes [5,8]. Some refractive surgeons believe that epithelium repositioning decreases pain and protects the bare anterior corneal surface from cytokines that promote scarring [9], while other surgeons believe that replacing the epithelium hinders the formation of a new epithelial sheet and thereby prolongs surface healing [6].

Haze formation is a complication that occurs after surface ablation techniques and can not be completely prevented after epi-LASIK. Transforming growth factor-β₁ (TGF-β₁) has emerged as a key regulator of haze and scarring in the cornea and other tissues [10-11]. The purpose of this study was to observe the influence of epi-LASIK with the epithelial flap repositioned (“flap-on”) or removed (“flap-off”) on the release of TGF-β₁ in tear fluid and postoperative corneal haze formation.

PATIENTS AND METHODS

Patients

Thirty patients (60 eyes) including 12 men and 18 women aged 25.62±5.86 years (ranging from 20 to 34 years), who underwent refractive surgery with flap-on or flap-off epi-LASIK between July 2008 and July 2011, were included in this prospective study. All the patients received a full explanation of the surgical procedure and informed consent was obtained before surgery. The mean preoperative spherical equivalent refraction was -4.98±2.28 D (-2.50 to -7.25 D) in the right eyes undergoing flap-on technique, and -5.20±4.02 D (-1.75 to -7.00 D) in the left eyes with flap-off technique. There was no statistically significant difference in the baseline manifest refraction between the flap-on and flap-off groups (P=0.80). Before operation the patients were carefully examined to exclude the presence of ocular inflammation, allergy or other ocular or systemic diseases. The patients were advised not to wear their contact lenses two weeks before the operation.
Surgical techniques

All the patients had bilateral epi-LASIK in the same session performed by the same surgeon. The right eye was treated first with the epithelial flap repositioned, followed by operation on the left eye with the epithelial flap removed. Epithelial separation was performed using an instrument designed by Kangning Company (KM-5000 d, Wuxi, China). An epithelial flap with a superior hinge was created, and the flap was retracted using a spatula. In eyes for flap-off epi-LASIK, the epithelial sheet was separated using the same epikeratome and removing a beaver on the hinge except for the total or free epithelial sheets, which were removed without additional manipulations. The laser treatment was performed using an excimer laser (VIST Star S4, Santa Clara, CA, USA). After ablation, the stroma was washed gently, and in the eyes for flap-on epi-LASIK, the flap was repositioned over the bed. A PureVision bandage contact lens (diameter 14.0 mm, base curve 8.6 mm, power -0.50 D; Bausch & Lomb) was placed onto the eyes at the end of the procedure.

Tear fluid TGF-β1 measurements

Tears were collected preoperatively and at 1, 3, and 7 days postoperatively from the lower conjunctival sac without topical anesthesia using a scaled 20 μl microcapillary tube. Special attention was paid not to irritate the cornea or conjunctiva. An attempt was made to collect the tear fluid samples without trauma, and the collection time for 20 μl sample was recorded. All the tear samples were collected by one doctor at the same time of the days.

TGF-β1 level in the tear sample was determined by quantitative sandwich enzyme immunoassay technique (Quantikine human TGF-β1, R & D system Inc, Minneapolis, MN, USA). The microtiter plates were coated with recombinant human TGF-β1 receptor. To activate latent TGF-β1, to immunoreactive TGF-β1, 0.1 ml of 1 N HCl was added to the tear samples (10-fold) and incubated for 10 min. The acidified samples were neutralized by adding 0.1 ml of 1.2 N NaOH/0.5 mol/L HEPES. Subsequently, 200 μl of activated samples were added to the wells and incubated for 3 h. The unbound material was removed with washing buffer, and 200 μl of 1000-fold diluted antibodies to human TGF-β1 conjugate were added and incubated at room temperature for 1.5 h. After washing the plates for 3 times, 200 μl of the substrate solution was added to each well and incubated at room temperature for 20 min, followed by addition of 50 μl stop solution to each well. The optical density of each well was measured with an EIA reader (LP 400, Sanofi, Diagnostics Pasteur, France) at 450 nm within 30 min.

Postoperative follow-up

Postoperative medications included pranoprofen eye drops 4 times daily (Senju Pharmaceutical Co., Ltd, Japan) and combined eye drops of tobramycin and dexamethasone 4 times daily (S.A. Alcon Couvreur, N. V, Belgium) until the removal of the therapeutic lens. After the removal of the lens, 0.1% fluorometholone eye drops (Senten Pharmaceutical Co., Ltd, Japan) were applied to the treated eyes 4 times daily in a tapered dose for 5 weeks. Artificial tears (Allergan, America) were prescribed for use at the patient’s discretion.

Follow-up visits were scheduled at 1, 3 and 5 to 7 days postoperatively, and then at 1 month, 3 months and 6 months postoperatively. The contact lens was removed when epithelialization was complete (usually between postoperative days 5 and 7). The grade of corneal haze at 1, 3 and 6 months after surgery was considered for statistical analysis.

Haze levels were graded based on Fantes classification method (0=normal; 0.5=possible to observe opacity under indirect light; 1=possible to observe opacity under direct light; 2=possible to observe iris in detail; 3=difficult to observe iris in detail; 4=not possible to observe iris in detail).

Statistical analysis

Statistical analysis was performed with SPSS software version 11.0 (SPSS, Inc., Chicago, IL). Group differences for continuous variables were tested using paired Student’s t test. The measurement results of TGF-β1 levels are presented as Mean±SD. Haze scores were evaluated using Pearson Chi-square test with tables of contingency. A P value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Tear fluid TGF-β1 levels

Before and at 1, 3, and 7 days after the operation, the mean tear fluid TGF-β1 levels showed no significant difference between the eyes receiving flap-on and flap-off epi-LASIK(P>0.05, Tab.1).

Tab.1 Pre- and postoperative TGF-β1 levels in tears (pg/min) in patients receiving flap-on and flap-off epi-LASIK

<table>
<thead>
<tr>
<th></th>
<th>Flap-on group</th>
<th>Flap-off group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>53.12±10.83</td>
<td>48.24±12.66</td>
<td>0.11</td>
</tr>
<tr>
<td>Day 1</td>
<td>82.34±12.04</td>
<td>78.78±30.32</td>
<td>0.55</td>
</tr>
<tr>
<td>Day 3</td>
<td>74.25±21.66</td>
<td>70.39±17.83</td>
<td>0.45</td>
</tr>
<tr>
<td>Day 7</td>
<td>67.24±10.87</td>
<td>60.32±26.45</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Although TGF-β1 levels in the tear fluid gradually decreased after the first postoperative day in both flap-on and flap-off groups, the levels on postoperative day 7 were still higher than the preoperative level, suggesting a role of TGF-β1 in corneal wound healing.
Corneal haze

In general, corneal haze decreased with time, and none of eyes in this study developed corneal haze worse than score 1 during the follow-up. There was no significant difference in corneal haze between the two groups at any of the postoperative time points (P>0.05, Tab.2). In the first postoperative month, haze score of 1 was found only in 3 eyes in flap-on group and 2 eyes in flap-off group. These 5 eyes all had high myopia preoperatively.

Tab.2 Number of eyes with haze scores of 0 to 1 during follow-up

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Haze score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>Flap-on</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Flap-off</td>
<td>17</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>3 months</td>
<td>Flap-on</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Flap-off</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>6 months</td>
<td>Flap-on</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Flap-off</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

As the major complication affecting the stability and predictability of surface corneal refractive surgery [13-14], postoperative haze formation is an important consideration when evaluating surface ablation techniques. Epi-LASIK, which is the latest developed surface ablation, can also be associated with haze formation. Stromal myofibroblasts were reported to be a significant contributor to corneal haze. Corneal myofibroblasts, which produce atypical extracellular substances such as fibronectin, laminin, and tenasin but less crystallins, have been shown to cause greater light scatter and contribute to haze [15-17]. Previous studies have demonstrated conclusively that TGF-β1 is the key cytokine regulator of myofibroblast differentiation [18-20]. Structural and functional defects in the epithelial basement membrane are probably essential for epithelium-derived TGF-β to enter the stroma at a sufficient concentration to modulate the development of stromal myofibroblasts from their precursor cells [21-22]. TGF-β has 3 known mammalian isoforms: β1, β2, and β3 [23]. The human embryonic primarily expresses TGF-β1, and heals without scarring after injury, while in adult tissues, TGF-β1 and TGF-β2 show increased expressions and cause scarring [24]. In the cornea, TGF-β1 has been isolated in the epithelium, stroma, and tear fluid [25-26]. We therefore attempted to compare corneal haze after flap-on and flap-off epi-LASIK by measuring TGF-β1 levels in tears and by direct observation with slit lamp.

In our patients, we found no obvious differences in TGF-β1 levels in tears or haze scores at any postoperative time points between the eyes receiving flap-on and flap-off epi-LASIK. We hypothesize that this was due to the following reasons: First, the bandage contact lens placed on the eyes until epithelium recovery may serve as an effective barrier that protect the wound surface from lid action, diminished corneo-lacrimal reflex, and reduced the influx of tear fluid growth factors such as TGF-β1. Second, the bandage contact lens covered the epithelial cutting edge, which may prevent the release of TGF-β1 and other cytokines from the stroma and the damaged or regenerating epithelium; Third, the separation of the epithelial sheet using an epikeratome may leave a smoother stromal surface with more regular borders for photoablation in flap-on and flap-off group, which decreases postoperative corneal haze.

Our data showed that both flap-on and flap-off epi-LASIK were associated with a low incidence and mild condition of corneal haze. At the first postoperative month, haze score of 1 was found only in 3 eyes of the flap-on group and 2 eyes of the flap-off group. These five eyes were all with myopia more than -6.0 D preoperatively. Therefore, the development of corneal haze may not be affected by the presence of the flap but by the ablation depth. This was similar with the results of previous studies [6-7].

The presence of high levels of TGF-β1 in the tear fluid till the 7th postoperative day suggests a role of TGF-β1 in corneal wound healing. After corneal epithelial injury, cytokines and such growth factors as TGF-β1 are released into the tear film by the lacrimal gland [27]. We hypothesize that during epithelium regeneration after epi-LASIK, some cytokines and growth factors from the tear fluid may still enter the corneal stroma and cause activation of stromal keratocytes. The activated keratocytes at the borders of the ablation zone then transform into myofibroblasts, which may migrate into the subepithelial space to result in corneal haze formation [28].

In conclusion, we found that flap-on and flap-off epi-LASIK resulted in comparable TGF-β1 level in tears and haze scores and caused similar incidences of corneal haze. Still, further investigations involving a larger number of subjects and a longer follow-up period are needed for further confirmation of these results.
Epi-LASIK术中上皮瓣去留对术后角膜上皮下雾状混浊及泪液中TGF-β1的影响

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摘要:目的 观察机械法准分子激光上皮瓣下角膜磨镶术(Epi-LASIK)术中上皮瓣去留对术后角膜上皮下雾状混浊(haze)及泪液中haze形成的主要调控因子转化生长因子β1(TGF-β1)的影响。方法将30例(60眼)接受Epi-LASIK的患者分为两组,右眼(30眼)中保留上皮瓣为实验组,左眼(30眼)中弃上皮瓣为对照组,采用酶联免疫吸附实验双抗体夹心法测定两组术后1,3,7,14天泪液中TGF-β1水平,并与两组术后1,3,6个月haze的情况。结果术前等效镜片屈光度(±4.98±2.28 D)与去瓣组 (-5.20±4.02 D)比较无统计学意义(P=0.80)。两组术后1,3,6个月haze程度的比较无统计学意义(P分别为0.98,0.52,0.72)。两组术前及术后1,3,7天泪液中TGF-β1水平比较亦无统计学意义(P分别为0.11,0.05,0.45,0.19)。观察期间两组术后泪液中TGF-β1水平下降,但始终高于术前水平。结论 上皮瓣去留对Epi-LASIK术后haze及泪液中TGF-β1水平无明显影响,术后泪液中TGF-β1可能在角膜伤口愈合反应中起一定作用。关键词:Epi-LASIK;上皮瓣;转化生长因子β1;上皮下雾状混浊

REFERENCES

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