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Q9 治療用コンタクトレンズの適応と管理の注意点について教えてください

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1．治療用コンタクトレンズ装用の基本的な作用機序は角膜上皮の機械的保護作用にある．
2．上皮びらんの予防や上皮の脱落の抑制効果が期待でき，疼痛の軽減や視力改善が図れる場合もある．
3．通常の点眼治療に反応しない難治例を適応とすべきで，治療への理解や協力が得られない患者には用いない．
4．合併症の発生予防には，ドライアイと感染に対する対応が重要となる．

はじめに

コンタクトレンズ装用がドライアイ，アレルギー性結膜炎，感染性角膜炎などさまざまな角結膜合併症の原因となることはよく知られている．しかし，逆説的なようだがコンタクトレンズを角膜疾患の治療に用いる場合も少なくなく，治療用コンタクトレンズとよばれている．ここではその適応と管理の注意点について述べる．

治療用コンタクトレンズのメリット

治療用コンタクトレンズの適応となる角膜疾患にはさまざまなものがあるが，通常の点眼治療などに反応しない症例が適応となる（表1）．治療用としてのコンタクトレンズの基本的な作用機序は，角膜上皮の機械的保護作用であり，bandage lensという別名にその特徴がよく表現されている[1]．

表1 治療用コンタクトレンズの適応

上眼瞼とのスペーサーとして
再発性上皮びらん，水疱性角膜症，上輪部角結膜炎など
上皮の脱落の抑制，創傷治癒や分化の促進
糸状角膜炎，遷延性上皮欠損，薬剤起因性上皮障害など
創傷部の保護
角膜移植術後，phototherapeutic keratectomy（PRK）術後など
前房の保持
角膜潰瘍穿孔，穿孔切迫など

再発性上皮びらんや水疱性角膜症では，上眼瞼と角膜の間にスペーサーとして入ることで接着の弱い上皮を保護し，上皮びらん発作を抑制することができる（図1）．上皮の脱落を抑制することで，創傷治癒や正常な上皮への分化を促す作用も期待できる（図2）．糸状角膜炎や上輪部角結膜炎，遷延性上皮欠損，薬剤起因性上皮障害などではこの効果を期待して用いられることが多い[1]．

やや副次的だが臨床上重要な効果としては，レンズ装用によって疼痛が軽減され，視力の改善も得られる場合があることがあげられる．図3に緑内障点眼薬による薬剤起因性上皮障害の症例を示す．密度の濃い点状表層角膜症がみられ，矯正視力は0.1まで低下していた．この症例では治療用レンズを用いた直後に（0.6）まで視力が改善し，異物感も軽減した．薬剤起因性上皮障害であるために角膜上皮障害の消失までには3カ月を要したが，角膜所見の改善の前にとりあえず，視力回復と鎮痛効果が得られることは治療用レンズの大きなメリットと考え

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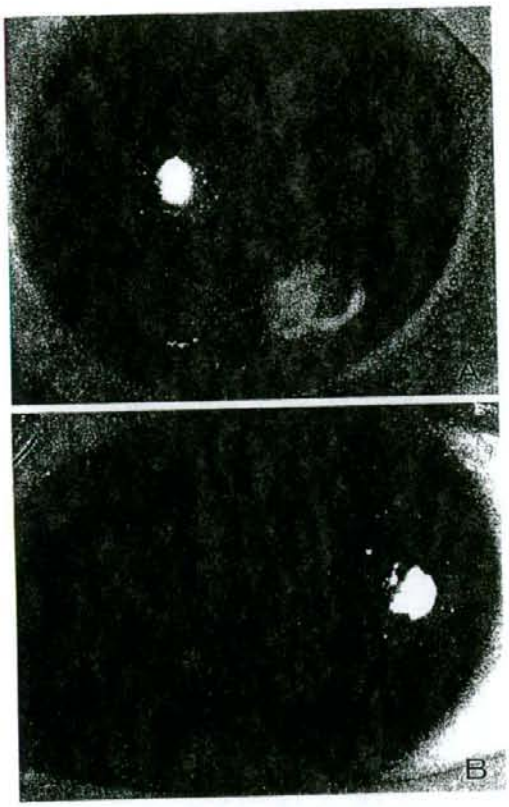

図1　脆弱な上皮の機械的保護作用
再発性上皮びらんで点眼や軟膏による治療に反応しない例（A）や格子状角膜ジストロフィで上皮びらんを繰り返す例（B）では，びらん発作の予防として治療用コンタクトレンズが適応となる．

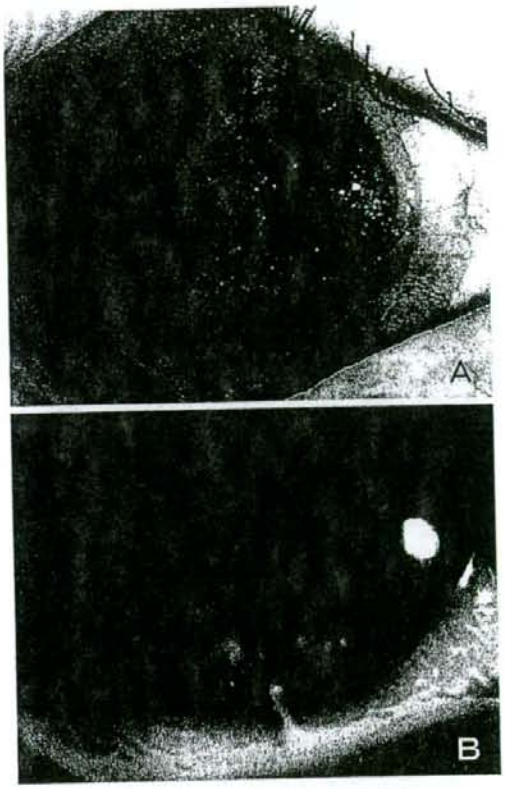

図2　上皮化や正常な分化の促進作用
遷延性上皮欠損（A）や糸状角膜炎（B）では，治療用コンタクトレンズにより上皮の脱落抑制による上皮化の促進，正常な分化が期待できる．

られる．

角膜移植術後やphototherapeutic keratectomy（PRK）術後では，創傷部の保護とともに鎮痛効果を期待して用いられることが多い．また，角膜潰瘍穿孔例や穿孔切迫例には前房保持を期待して用いられることもある．この場合には，治療用レンズで前房を保持している間に原疾患の治療が奏効すれば，外科的治療を回避できることもある．

治療用コンタクトレンズのリスク管理

急性の単純性上皮びらんの治療にコンタクトレンズを用いた論文が欧米では散見されるが，通常はあまり用いられない[2]．これは単純性上皮びらんが数日以内に治癒する予後良好な疾患であり，レンズ装用に伴うリスクがメリットを上回らないと判断されるからである．レンズのずれや脱落による原疾患の悪化の可能性に加えて，治療用レンズは連続装用で用いられるのが通例であり，就寝時の角膜酸素分圧の低下やレンズの固着による新たな角膜障害発生も懸念される[3]．また，コンタクトレンズは連続装用で用いると感染性角膜炎の発症リスクが増大することが知られている[4]．治療用コンタクトレンズを用いる場合には，これらの合併症のリスクについて常に注意を払う必要がある．

用いるコンタクトレンズとして問題となるのは，治療用として認可されているレンズは数種類しかなく，必ずしも酸素透過性が良好とはいえないことである．このために使い捨てコンタクトレンズや定期交換型レンズが使

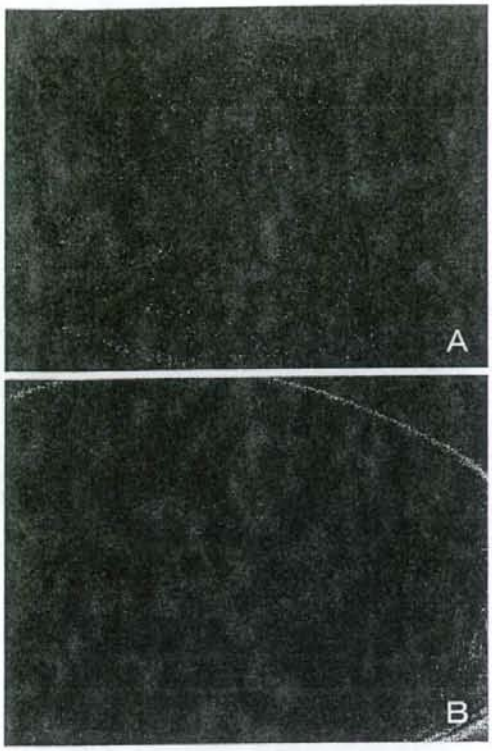

図 3　視力や異物感の改善作用
密度の濃い点状表層角膜症のために矯正視力が 0.1 まで低下していた薬剤起因性上皮障害の例（A）では治療用レンズを用いた直後に（0.6）まで視力が改善し，異物感も軽減した．ただし，角膜上皮障害の消失までには 3 カ月の装用期間を要した（B）．

用されることが多いが[1,5]，認可されている用法ではないことを認識しておく必要がある．筆者はワンデーアキュビュー®または O2 オプティクス®を用いることが多い．ワンデーアキュビュー®は装用感が良いのが利点であり，使いやすいレンズであるが，乾燥感が出やすく，ドライアイ合併例には使いにくい．O2 オプティクス®は酸素透過性が非常に高く，汚れにくい点など治療用レンズとして用いるのに有利な特性を持つが，固着に注意する必要がある．

合併症の発生を予防するには，ドライアイと感染に対する対応が重要と思われる．人工涙液やヒアルロン酸の点眼は，乾燥や固着の防止のために有用と思われる．ドライアイで涙液量が少なく，装用困難な場合には涙点プラグを用いて涙液量を増やしたうえで装用させるのも一つの手段である．また，感染予防として抗菌剤を処方する場合もあるが，薬剤起因性上皮障害や遷延性上皮欠損などでは点眼薬の薬剤毒性も考慮すべきであり，必ずしも必須ではないと考える．また，連続装用の期間が長くなると合併症への対応が遅れがちになるので，装用期間は原則的に 1 週間，長くても 2 週間程度にとどめるのが良いようである．

おわりに

以上，述べたように治療用コンタクトレンズは適応を間違えなければ，良好な治療効果を得ることができ，難治性角膜疾患治療のためにぜひ身につけておきたい治療手段の一つである．ただし，合併症のリスクもあるので，その対策を怠らないこと，治療への理解や協力が得られない患者には用いないことも重要と思われる．

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1. 細隙灯顕微鏡

Slit-lamp biomicroscope

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はじめに

細隙灯顕微鏡による観察は，眼科診療の基本となる検査であり，眼科を受診した患者に細隙灯顕微鏡検査を行わないことは稀なくらいである。細隙灯顕微鏡は基本的には前眼部・中間透光体の病変や異常所見を検出し，その程度や範囲，性状を把握するための生体顕微鏡であるが，その応用範囲は広い。フルオレセインなどの色素を用いて生体染色を施せば詳細に眼表面の状態を知ることができ，アプラネーショントノメータを用いれば眼圧を測定できるし，前置レンズや検査用コンタクトレンズを用いると網膜硝子体の病変の観察に用いることもできる。スペキュラーマイクロスコープやフレアセルメータなど細隙灯顕微鏡の原理や観察方法を用いて開発された検査機器も少なくない。

細隙灯顕微鏡検査はすべての眼科医が習熟すべき検査であり，さまざまな観察法や生体染色などテクニックを駆使すると，細隙灯顕微鏡だけでも非常に多くの生体情報を得ることができる。細隙灯顕微鏡検査には「習うより慣れよ」の側面もあるが，知らないとできない観察法も少なくない。ここでは，細隙灯顕微鏡の観察方法について前眼部の病変を中心にして示すとともに，フルオレセインによる生体染色についても述べる。

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Key words：細隙灯顕微鏡，角膜，フルオレセイン，slit-lamp biomicroscope, cornea, fluorescein

I. 細隙灯顕微鏡の観察法

細隙灯顕微鏡での基本的な観察法を図1に示す。このなかには特に意識しなくても自然にできる観察法と意識しないと絶対にできない観察法がある。細隙灯顕微鏡を用いる際には，どの方法を用いると最も病変をよく把握できるか実際に試しながら観察するのが，上達のこつである。

1. 直接照明法

角膜や水晶体などの透光体を光学切片として観察する方法で，細隙灯顕微鏡の最も基本的な観察法のひとつである。観察する組織の厚みや奥行き，形状がわかるのが最大の特徴である。角膜の厚みや前房の深さなど奥行きの情報を与えてくれる(図2)。また角膜混濁や白内障などでは混濁の位置や深さを知ることもできる。

直接照明法は，眼内炎症のパラメータである細胞やflareを観察する際に必須の方法であり，この場合には，前房内のスリット光の幅を細くし，できるだけ明るい光(overloadをかけられる機種ではかけたほうが観察しやすい)で観察を行う(図3)。

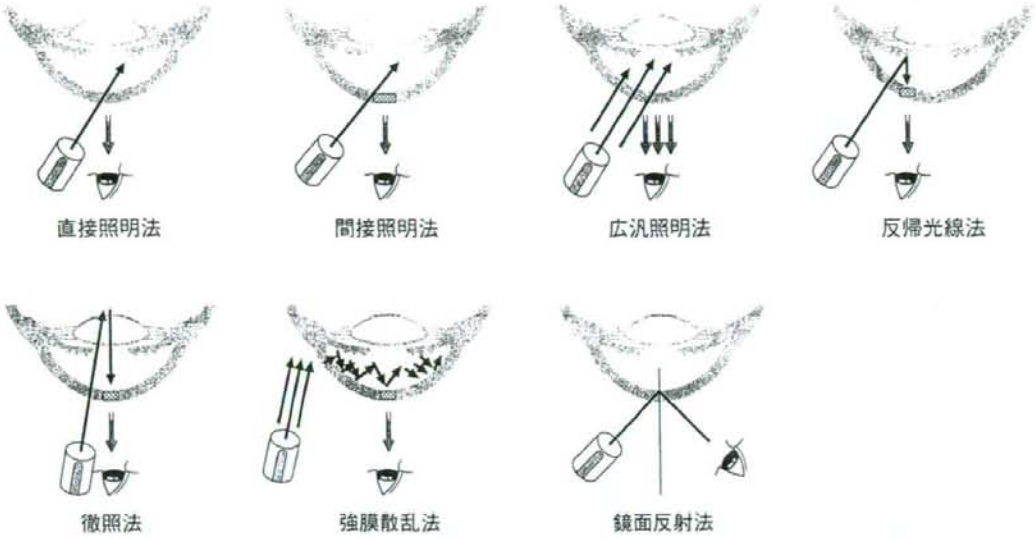

図1　細隙灯顕微鏡の観察法

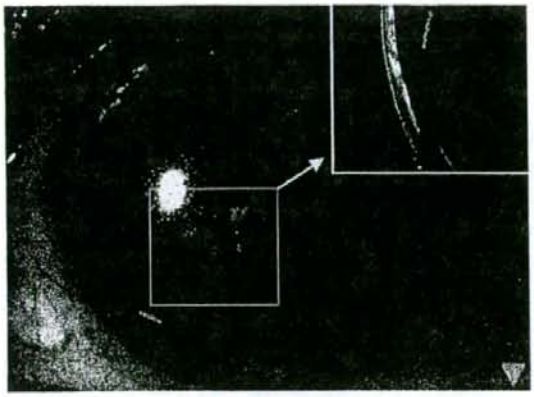

図2　直接照明法
症例は陳旧性の角膜白斑で点墨術を施されている。直接照明法では，虹彩前癒着が明らかとなる。奥行きや深さの情報を得るには直接照明法が必須である。

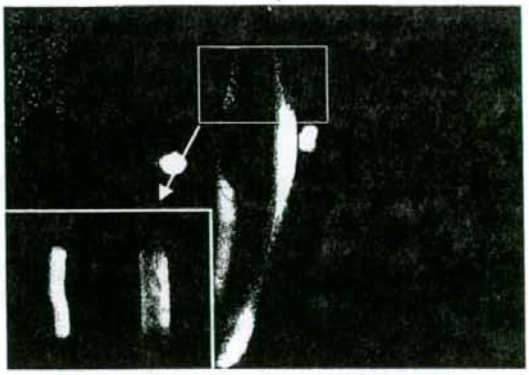

図3　直接照明法による前房内細胞の観察
前房内の細胞や flare を観察するには光束を明るく細くして用いると良い。

2.　間接照明法

スリット光が組織に当たった散乱光で，周囲の組織を観察する方法である。直接照明法のスリット光の幅を少しだけ拡げて観察すると間接照明が背景光代わりになって全体の位置関係が把握しやすくなる。実際には，こうした直接照明法と間接照明法が組み合わさったような状態で観察していることが多いと思われる(図4)。

瘢痕性の角膜混濁，実質や上皮の浮腫，細胞浸潤，角膜後面沈着物などは，直接照明法よりも間接照明法のほうが観察しやすくなる場合がある。

3.　広汎照明法

スリット光ではなく，幅の広い光を当てて観察対象を面で捉える方法である。ランプの電圧を下げるかフィルターをかけて光量を落とした状態でスリット光の幅を広くする，もしくはディフューザーを用いると良い。ペンライトで

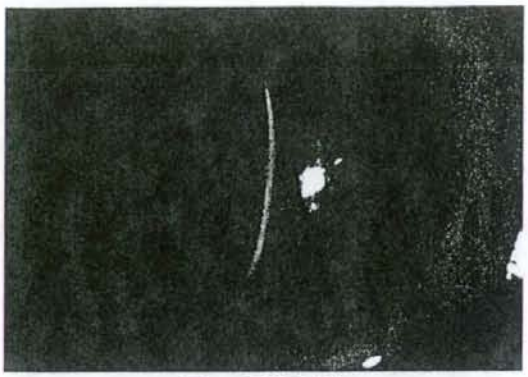

図4 間接照明法
水疱性角膜症の症例である。スリット光だけで撮影しているのに角膜や虹彩の全体像がわかるのは散乱光によるものである。

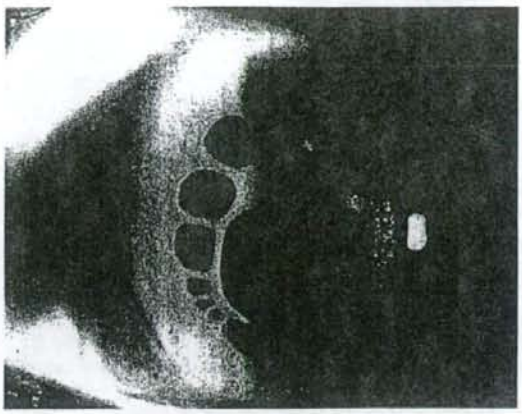

図5 広汎照明法
タイゲソン表層角膜炎の症例。光量を落とした幅の広い光で全体を観察する方法。面で観察できるため，病変の全体像を把握するのに良い方法である。

照明して拡大鏡で観察するのと同じ条件であり，病変全体の形がわかるのが大きなメリットとなる。淡い角膜混濁や角膜新生血管などでは広汎照明法でよく描出される場合もある(図5)。

広汎照明法は透光体ではない組織，結膜や強膜，眼瞼，虹彩を観察する場合には基本となる観察法である。結膜充血は直接照明法で観察すると反射光が強すぎて正確な所見を取りにくくなるからである。これ以外にも乳頭，濾胞やマイボーム腺開口部，虹彩紋理の所見はディフューザーを用いて低倍で観察を行うと最も良い結果が得られる。

4. 反帰光線法

反帰光線法はやや意識をしないとできない観察法のひとつである。スリット光を前眼部に当てると角膜の半透明の弓状の線とオレンジ色の虹彩の線が見える。スリット光が当たった部分に注目して観察すると直接照明法になってしまうが，オレンジ色の虹彩からの反射光が角膜に当たる部分に観察系のフォーカスを合わせるようにする(観察系と照明系を意識のなかでずらす)方法が虹彩からの反帰光線法である。

この方法を用いると，ほとんど透明に近い微細な角膜病変を映し出すことができる。淡い上皮や実質の混濁，細い新生血管，マイクロシスト，角膜後面沈着物などがよい適応になる(図6)。

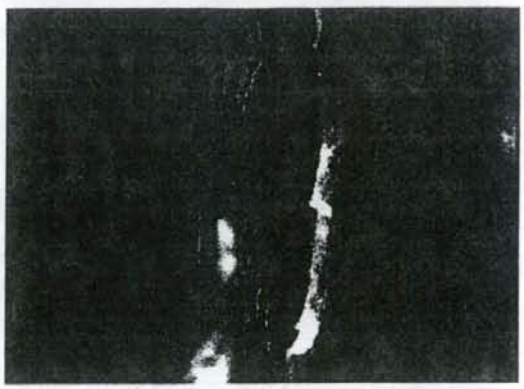

図6 反帰光線法
虹彩からの反射光が角膜に帰ってくる部分に観察系のフォーカスを合わせて観察する方法。写真は格子状角膜ジストロフィの症例で，線状の混濁が描出されている。

5. 徹照法

虹彩からの反射光を用いるのが反帰光線法で，散瞳した状態で眼底からの反射光を利用して角膜や水晶体の混濁病変を浮かびあがらせる観察法が徹照法である。観察系との角度をつけずに中等度の幅のスリット光を瞳孔縁より入れ，眼

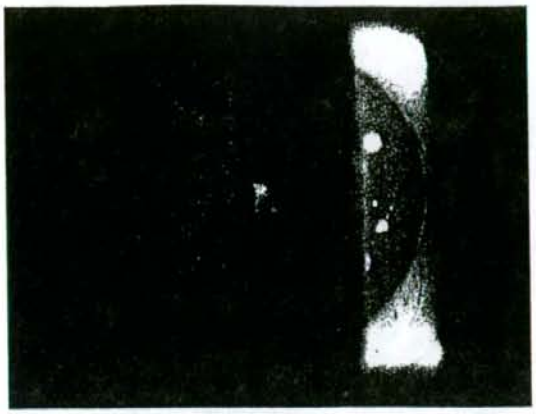

図7　徹照法
眼底からの反射光を利用し，角膜や水晶体の混濁病変を描出する方法。写真はアベリノ角膜ジストロフィ。

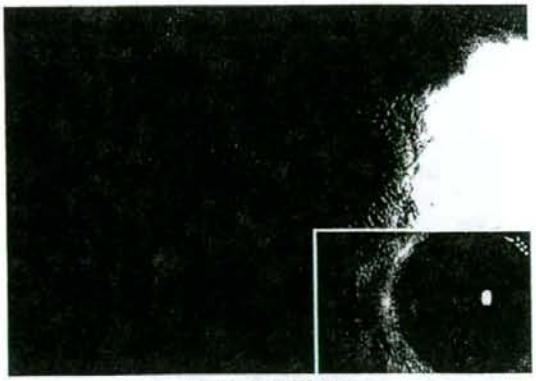

図8　強膜散乱法
強膜に幅広の明るい光を当て，その散乱光で角膜を観察する方法。写真は上皮内癌の症例で，淡く混濁した病変が拡がっている様子が描出されている。

底からのオレンジ色の明るい反射光が帰ってくるポイントを探す(図7)。病変の全体像をレリーフのように浮かびあがらせて観察できる点で非常に優れた方法であり，角膜や水晶体の混濁の形態を把握するのに有用である。

6.　強膜散乱法

強膜に幅広の明るい光を当て，その散乱光で角膜を観察する方法である。細隙灯顕微鏡では通常は照明系と観察系が同軸になっているので，これを解除してから観察する必要がある。観察系と照明系をわざとずらして観察する方法であり，意識的に行わないとできない観察法である。

角膜の微細な病変を浮かびあがらせて，病変の形や範囲を把握したいときに有用な方法なので，ぜひ覚えておきたい方法である(図8)。

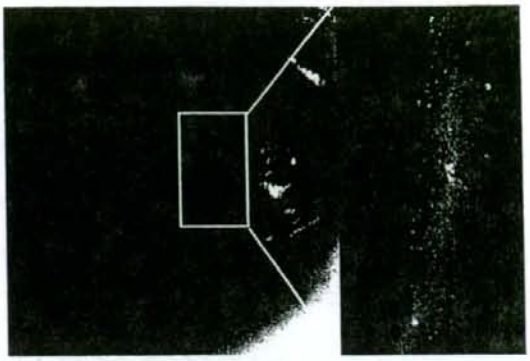

図9　鏡面反射法
角膜内皮細胞の定性的評価に有用な方法。写真は放射状角膜後面切開術後のもので，内皮細胞が粒状に観察され，細胞密度が減少していることがわかる。

7.　鏡面反射法

角膜内皮の評価にはスペキュラーマイクロスコープが用いられるが，その原理となっているのが細隙灯顕微鏡の鏡面反射法である。鏡面反射像を得るためには，観察眼に頭の中で垂線を引き，スリット光の方向と観察系の方向を垂線に対して線対称となるように置く。実際上は，この角度(垂線に対して30度くらい)を大体セットしておいて，角膜内皮に焦点を合わせ，入射光の角度を少しずつ変えていくことで鏡面反射(specular reflex)が得られるポイントを探すことになる(図9)。スリット光はやや広めにとり，角膜表面からの明るい反射の近辺の角膜後面を探すと良い。観察系の倍率は最初は16倍程度にしておき，鏡面反射像が確認できたら倍率を上げるようにする。

個々の内皮細胞の形態までは通常わからないが，内皮細胞ひとつひとつが小さな粒状に見え，定性的に内皮機能を評価することができる。

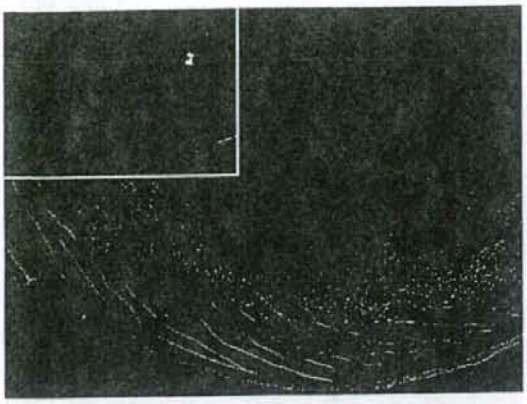

図10　ブルーフリーフィルターを用いた
点状表層角膜症の観察
通常のブルーフィルターに比べて検出感度が大幅に向上する。

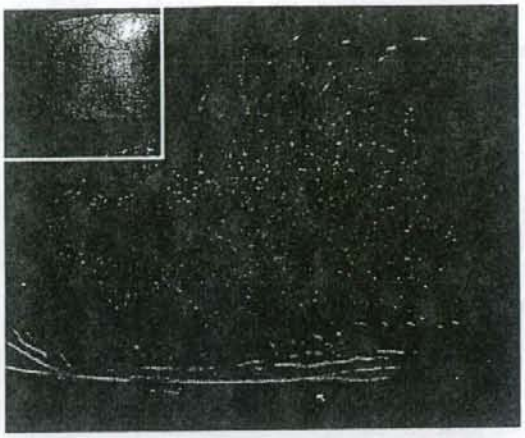

図11　ブルーフリーフィルターを用いた
結膜上皮障害の観察
臨床的にはローズベンガル染色とほぼ同一の染色性を示す。

II. フルオレセインの活用

生体染色色素にはフルオレセイン，ローズベンガル，リサミングリーンなど種々のものがあるが，臨床上最も利用され，かつ応用範囲が広いのはフルオレセインである。フルオレセインは蛍光色素であり，励起光として今まではブルーフィルターが用いられてきた。しかし，励起光の波長を変え観察系にブルーフリーフィルターを入れる(後者だけでもかなりの効果がある)と蛍光の観察効率がかなり向上する。蛍光眼底撮影と原理は同じであるがぜひ試してみていただきたい(図10)。

フルオレセインには大きく分けると2つの役割がある。ひとつは角結膜上皮障害を検出する本来の意味の生体染色色素としての役割で，もうひとつは透明な涙液を可視化する役割である。

狭義の生体染色陽性とは，フルオレセインが角結膜上皮の欠落した部分を染色するという意味であり，点状表層角膜症や上皮びらんが相当する。結膜上皮障害も同じようにフルオレセインで染色されるが，結膜の皺の部分での pooing や結膜上皮の stainning(結膜上皮はバリア機能が弱いために時間が経つとフルオレセインが透過して全体が染色されてしまう)が問題になり，見えかたに慣れるのに多少の経験が必要となる。ローズベンガルはムチンで被覆されない上皮細胞を染色するとされており，厳密な意味でフルオレセインの結膜染色と同一かどうかは議論があるが，少なくとも臨床的には同一部位が同程度に染色される(図11)。

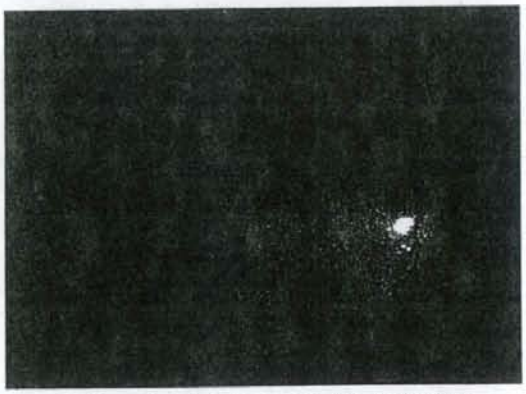

図12　フルオレセインによる上皮の質の評価
角膜移植後の遷延性上皮欠損が修復された直後の症例。フルオレセインで染色して数分経つと全体が淡く染色される。角膜上皮の透過性亢進を示す所見である。

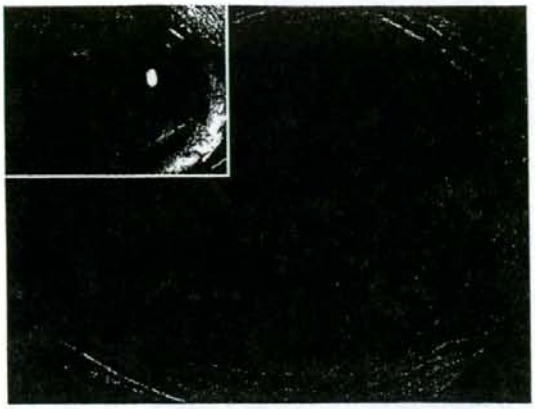

図13　フルオレセインによる異常上皮の検出
陳旧性の角膜混濁の症例だが，かなりの部分が結膜上皮に覆われていることがわかる。

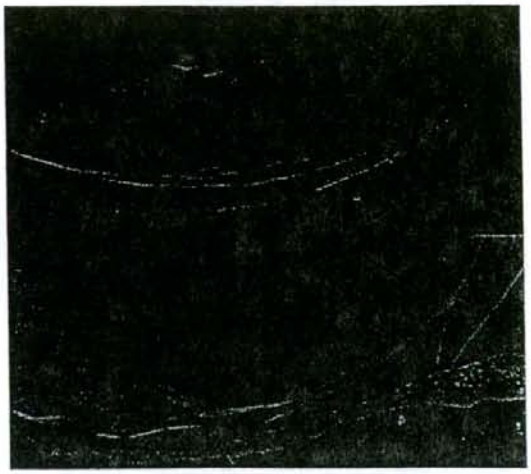

図14　フルオレセインによる粘膜皮膚移行部の観察(マイボライン)
上の正常者では粘膜皮膚移行部が1本の線として観察されるが，下のマイボーム腺機能不全では凹凸が生じ，診断の根拠となる。

正常の角膜上皮は細胞間の接着装置が発達しており，時間が経ってもフルオレセインを透過しない。しかし質の悪い角膜上皮は色素を透過するし，結膜上皮も前述したようにフルオレセインを透過する。この性質を用いると，フルオレセインで上皮の質の評価が可能になる。図12は修復されたばかりの角膜上皮欠損であり，フルオレセインを点眼してから数分が経過したところである。角膜上皮が淡く染色され，バリア機能の障害があることがわかる。図13は陳旧性の角膜混濁で，一見すると正常角膜上皮に被覆されているように見えるが，フルオレセイン染色を施すと角膜の大半は実は結膜上皮で覆われていることがわかる。上皮の種類による染色性の違いは，眼瞼縁の粘膜皮膚移行部(結膜と眼瞼皮膚の境界)の観察にも応用できる。結膜は染色性が高く，皮膚は染色されないため，粘膜皮膚移行部は正常では1本の線として描出される(マイボライン，図14)。マイボーム腺機能不全では結膜の前方移動のためにこの線に凹凸が生じ，診断の根拠となる。

フルオレセインのもうひとつの大きなメリットは透明な涙液を可視化できることである。角膜上での涙液膜破綻の状態を観察すれば涙液の安定性を評価することができ，定量的には涙液層破砕時間として評価される。涙液の貯留部位であるメニスカスを観察するとその高さから涙液量が推定できる。またメニスカスの占拠病変である結膜弛緩の存在もわかりやすくなる。ハードレンズのフィッティング検査も涙液を可視化することで，レンズ後面と角膜前面の距離を評価していることに他ならない。

おわりに

以上，細隙灯顕微鏡の観察法について生体染色を含めて概説した。病変を直接，自分の目で見ることができるのは眼科診療の楽しみのひとつであり，細隙灯顕微鏡を使いこなすことでこの楽しみはより大きくなるはずである。診断や病態の理解のためには，細隙灯顕微鏡で得られた情報を問診や病歴などと総合することが重要であることを強調してこの稿の結びとしたい。

Corneal penetration of simultaneously applied topical levofloxacin, norfloxacin and lomefloxacin in human eyes

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ABSTRACT.

Purpose: **This study was performed to assess the corneal penetration of three topically applied fluoroquinolones (levofloxacin, norfloxacin and lomefloxacin) in corneal buttons obtained from patients undergoing penetrating keratoplasty.**

Methods: **Fourteen patients received three drops each of 0.5% levofloxacin, 0.3% norfloxacin and 0.3% lomefloxacin (the standard clinically available preparations) over a 30-min interval beginning 90 mins before their scheduled keratoplasty. Corneal samples obtained from excised buttons at the time of surgery were stored at − 80 ° until analysis. The concentration of the administered fluoroquinolones was measured using high-performance liquid chromatography.**

Results: **The mean corneal concentration of levofloxacin (4.6 ± 3.5 μg/g, mean ± standard deviation) was significantly higher than that of lomefloxacin (2.7 ± 1.8 μg/g, p = 0.0018) and norfloxacin (1.3 ± 1.2 μg/g, p = 0.00012).**

Conclusion: **Levofloxacin achieves a higher mean corneal concentration than norfloxacin and lomefloxacin in the human cornea.**

Key words: cornea – fluoroquinolones – high-performance liquid chromatography – levofloxacin

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Introduction

Bacterial keratitis is a common, potentially sight-threatening ocular infection caused by various species of bacteria (O'Brien 1997). The causative organism is rarely identified in time for the initial antibiotic regimen to be organism-specific, and is sometimes not identified despite extensive efforts. Thus, successful treatment of presumed bacterial keratitis often requires empirical selection of an effective broad-spectrum antibiotic regimen.

The fluoroquinolones are the newest family of antibacterial agents used in the treatment of bacterial keratitis (Leibowitz 1991; Neu 1991; O'Brien et al. 1995; Hyndiuk et al. 1996; O'Brien 1997). Double-blind, randomized clinical trials have shown that single-agent fluoroquinolone therapy with ofloxacin (O'Brien et al. 1995) or ciprofloxacin (Hyndiuk et al. 1996) is comparable in efficacy to therapy combining fortified beta-lactam agents with aminoglycosides. Their bactericidal activity against the most frequently observed gram-positive and gram-negative ocular pathogens is generally excellent and their high potency has made fluoroquinolones a common choice for the topical therapy of bacterial keratitis. Currently, four topical fluoroquinolones (ofloxacin, norfloxacin, lomefloxacin and levofloxacin) have been approved for clinical use in Japan. In addition to these compounds, ciprofloxacin has been used clinically in the USA and Europe. While fluoroquinolones have similar spectra of activity in general, they are not equally potent against a number of bacteria and their pharmacokinetic properties differ (Neu 1991; Ogawa & Hyndiuk 1993; Ernst et al. 1997; Wimer et al. 1998). The clinical usefulness of fluoroquinolones is thought to depend on several factors, including their *in vitro* bactericidal activity, their ability to penetrate the site of infection and their relative toxicities.

Levofloxacin, the L-isomer of the racemate ofloxacin, is significantly more potent than the D-isomer and represents the active component of ofloxacin (Ernst et al. 1997; Wimer

et al. 1998). As the purified potent isomer of ofloxacin, levofloxacin is roughly twice as biologically active as ofloxacin (Ernst et al. 1997; Wimer et al. 1998). Another important benefit of levofloxacin is its high solubility in water; the concentration of the clinically available topical levofloxacin preparation is 0.5%, while that of other fluoroquinolones is 0.3% (Ross & Riley 1990). It might be expected that the higher concentration of topical levofloxacin would aid in achieving higher corneal and aqueous humour penetration. Kawashima et al. (1995) reported that 0.5% levofloxacin achieved a significantly higher maximum concentration in aqueous humour than 0.3% ofloxacin after topical application of three drops of each agent at 15-min intervals in a rabbit model. We also recently reported that topical levofloxacin achieves better penetration in human aqueous humour than lomefloxacin and norfloxacin (Yamada et al. 2003). However, there are currently no data in the literature that assess the corneal concentration of levofloxacin after topical administration. The present study was designed to assess the relative topical penetration of three fluoroquinolones (levofloxacin, norfloxacin and lomefloxacin) in the human cornea.

Methods

Fourteen patients (10 women and four men; mean age 64 ± 17 years) who underwent penetrating keratoplasty at Keio University Hospital were enrolled (Table 1). Indications for penetrating keratoplasty included leukoma ($n = 5$), graft failure ($n = 5$), bullous keratopathy ($n = 3$) and keratoconus ($n = 1$). Exclusion criteria included the presence of apparently non-intact corneal epithelium, active corneal inflammation, and topical antibiotic use within 1 week of enrolment. Each subject received thorough written and oral explanations of the study and provided written informed consent prior to enrolment. The study was approved by the Committee for the Protection of Human Subjects at Keio University School of Medicine.

Topical preparations of 0.5% levofloxacin (Santen Pharmaceutical Co. Ltd, Osaka, Japan), 0.3% norfloxacin (Banyu Pharmaceutical Co. Ltd, Tokyo, Japan), and 0.3% lomefloxacin (Senju Pharmaceutical Co. Ltd, Osaka, Japan) were obtained from each manufacturer. All patients received three drops of each of the three study drugs over a 30-min period, with one drop of each administered at 0, 15 and 30 mins. At each dosing interval, patients first received one drop of one drug, followed by a 2-min delay, then one drop of the second drug, and finally one drop of the third drug after another 2-min delay. The order of drug administration varied across patients in a crossover fashion, with each patient randomly assigned to one of three regimens (Table 1).

Drops were administrated by a nurse to ensure strict compliance with the administration regimen.

Table 1. Patients were randomly assigned to one of three treatment regimens. At 0, 15- and 30-mins, all patients received one drop each of 0.5% levofloxacin, 0.3% norfloxacin and 0.3% lomefloxacin in the sequence listed.

Patient no.	Age (years)	Gender	Diagnosis	Treatment sequence
1	73	Female	Bullous keratopathy	LVFX/NFLX/LFLX
2	79	Male	Regraft	NFLX/LFLX/LVFX
3	51	Male	Keratoconus	LFLX/LVFX/NFLX
4	56	Female	Regraft	LVFX/NFLX/LFLX
5	71	Female	Leukoma	NFLX/LFLX/LVFX
6	70	Female	Regraft	LFLX/LVFX/NFLX
7	62	Female	Leukoma	LVFX/NFLX/LFLX
8	76	Female	Leukoma	NFLX/LFLX/LVFX
9	66	Female	Bullous keratopathy	LFLX/LVFX/NFLX
10	85	Male	Regraft	LVFX/NFLX/LFLX
11	44	Female	Leukoma	NFLX/LFLX/LVFX
12	54	Male	Regraft	LFLX/LVFX/NFLX
13	21	Female	Leukoma	LVFX/NFLX/LFLX
14	82	Female	Bullous keratopathy	NFLX/LFLX/LVFX

LVFX = levofloxacin, NFLX = norfloxacin, LFLX = lomefloxacin.

Patients received concurrent surgical preparation with miotic/mydriatic drops, and surgery commenced approximately 1 hour after application of the last antibiotic drop. After excision, the host corneal button was divided into two halves using a razor blade and the halves were blotted to dry using a cellulose sponge. The tissue was stored at − 80 ° until analysis. At the time of analysis, corneal specimens were weighed, pulverized and homogenized in 0.8 ml of 0.1 M phosphate buffer. The drugs were eluted by adding 6 ml of chloroform. After brief centrifugation, the supernatant layer was removed. The elute was concentrated by evaporation under nitrogen gas, and reconstituted to 0.5 ml of an 85 : 15 mixture of 0.05 M phosphoric acid (pH 3.0) and acetonitrile. Preliminary experiments using rat corneas showed > 90% recovery of calibrator solution processed in the presence of drug-free corneas (data not shown).

Drug levels in the corneal buttons were determined using high-performance liquid chromatography (HPLC) (Yamada et al. 2002). An HPLC system consisting of an L-7100 solvent delivery system (Hitachi, Tokyo, Japan), an L-7480 fluorescence detector (Hitachi), and a C-21 chromatography workstation (System Instrument, Tokyo, Japan) was used. Elution was performed using an ODS-80 column (Tosoh Inc., Tokyo, Japan) at 50 ° with a mobile phase consisting of an 85 : 15 mixture of 0.05 M phosphoric acid (pH 3.0) and acetonitrile. The flow rate was 0.7 ml/min and detection was performed by fluorescence (excitation 290 nm, emission 470 nm). Drug concentrations were determined using a calibration line constructed using preparations of known concentration ranging from 0.025 μg/ml to 1.25 μg/ml. Drug concentrations were calculated from the peak height on the chromatogram and are expressed as micrograms of drug per gram of corneal tissue.

Results are presented as the mean ± standard deviation (SD). Statistical significance was calculated by comparing results by Wilcoxon signed rank test. A level of $p < 0.05$ was considered statistically significant.

Results

A typical chromatogram is shown in Fig. 1. The retention times of norfloxacin,

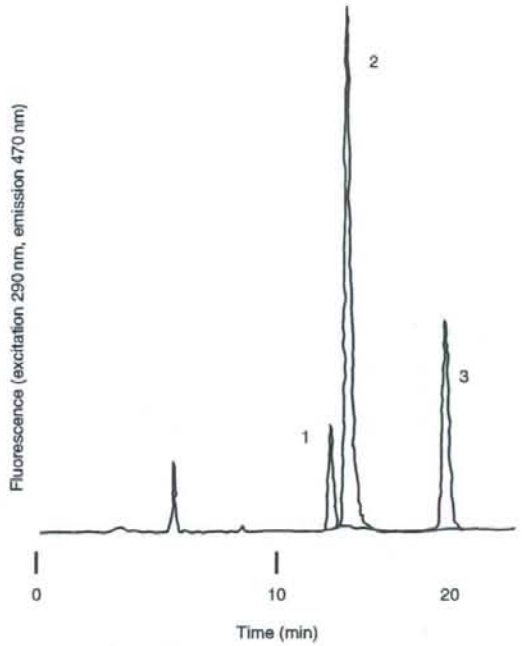

Fig. 1. High performance liquid chromatogram of fluoroquinolones. The retention times of (1) norfloxacin, (2) levofloxacin and (3) lomefloxacin were 14.1 mins, 15.0 mins and 19.6 mins, respectively.

levofloxacin and lomefloxacin were 14.1, 15.0 and 19.6 mins, respectively.

The fluoroquinolone concentrations measured in the corneal buttons are shown in Table 2.

The mean concentration ± SD of norfloxacin, levofloxacin and lomefloxacin were 1.3 ± 1.2 μg/g, 4.6 ± 3.5 μg/g, and 2.7 ± 1.8 μg/g, respectively. The difference in corneal concentration was significant between norfloxacin and levofloxacin ($p = 0.00012$, Wilcoxon signed rank test), and lomefloxacin and levofloxacin ($p = 0.0018$).

Table 2. Intracorneal concentrations of norfloxacin, levofloxacin and lomefloxacin.

Patient no.	Drug concentration (μg/g)		
	Norfloxacin	Levofloxacin	Lomefloxacin
1	0.7	4.0	3.3
2	0.9	1.5	0.8
3	0.3	1.4	0.4
4	4.9	11.9	6.4
5	1.1	4.2	3.1
6	1.6	2.8	1.5
7	0.2	0.6	0.4
8	0.6	4.2	3.0
9	0.8	12.1	5.9
10	1.4	1.9	0.9
11	2.6	4.4	3.2
12	1.9	8.5	3.1
13	0.5	2.8	3.0
14	1.3	4.6	3.5
Average	1.3	4.6	2.7
SD	1.2	3.5	1.8

Discussion

In this study, corneal fluoroquinolone concentration demonstrated great (approximately 20-fold) variability, although all the patients were given identical amounts of each drug. Similar variability has been observed in previous studies of fluoroquinolone penetration into the cornea and aqueous humour (McDermott et al. 1993; Diamond et al. 1995; Donnenfeld et al. 1997; Price et al. 1997; Yamada et al. 2003). Multiple factors including tear turnover rate, blinking frequency and completeness, timing of sampling, and epithelial continuity are thought to contribute to this large interpatient variability. In the present study, the condition of the corneal epithelium may have been the most important factor, given that all subjects were patients undergoing penetrating keratoplasty (McDermott et al. 1993; Diamond et al. 1995; Donnenfeld et al. 1997; Price et al. 1997).

The presence of significant interpatient variability required that a large sample size be sought to attempt to detect significant differences among the three fluoroquinolones. For this purpose, we used the analytical method reported by Diamond et al. (1995). Any factors that promote or inhibit the penetration of one drug would be expected to have an essentially identical effect on each agent, because all three fluoroquinolones were administered to each eye virtually simultaneously. All three fluoroquinolones were assayed simultaneously in each corneal sample in order to increase the effective sample size. However, the instillation of multiple eyedrops at one time has potential drawbacks (DeSantis 1994). When a drop of one medication is followed closely by drops of other medications, substantial washout (decreasing drug penetration) and change in pH (which may increase or decrease drug penetration) may occur. We set 2-min intervals between the administration of each eyedrop to minimize the above effects (DeSantis 1994). However, in a previous study, we found that the mean concentration of levofloxacin in the aqueous humour was 0.6 ± 0.3 μg/ml when three drops each of 0.5% levofloxacin, 0.3% norfloxacin and 0.3% lomefloxacin were administered (Yamada et al. 2003). This value was lower than the concentration of levofloxacin achieved in the aqueous humour (1.0 ± 0.5 μg/ml) when levofloxacin alone was administered (Yamada et al. 2002). These

results suggest that the instillation of multiple eyedrops, even using 2-min intervals between drops, results in reduced drug penetration. Therefore, these data cannot be directly compared with the results of other studies in which only a single drug was administered.

This study demonstrated distinct differences in corneal drug concentrations achieved among the three fluoroquinolones. Levofloxacin had the highest corneal concentration, followed by lomefloxacin, with norfloxacin showing the lowest mean concentration. The concentration of each fluoroquinolone administered in this study was not uniform, given that the levofloxacin preparation was a 0.5% solution, while lomefloxacin and norfloxacin were administered as 0.3% solutions. The high solubility of levofloxacin in water at neutral pH, which permits the higher 0.5% concentration of the levofloxacin preparation, has been noted to represent an advantage of this drug over other fluoroquinolones (Ross & Riley 1990).

Antibiotics used for the treatment of bacterial keratitis should demonstrate excellent corneal penetration and sufficient *in vitro* activity against a broad spectrum of bacteria (O'Brien 1997). The mean intracorneal concentration of levofloxacin achieved in the present study was greater than the MIC_{90} of levofloxacin against most common pathogens of bacterial keratitis, although the mean concentration was lower than the MIC_{90} of some *Pseudomonas* strains (Table 3) (Une et al. 1988; Ernst et al. 1997; Wimer et al. 1998). The mean intracorneal concentration of lomefloxacin exceeded the MIC_{90} of the above-mentioned pathogens, excluding *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Une et al. 1988; Neu 1991). The mean intracorneal concentration of norfloxacin, however, did not exceed the MIC_{90} of most pathogens (Chin et al. 1988).

Some caution should be exercised when interpreting the data presented. The success or failure of therapy is not necessarily predicted by the mean intracorneal concentration exceeding the MIC_{90} value or not. Resistance or susceptibility as determined through MIC90 assays might not correlate with clinical response because these *in vitro* assays are based on clinical response to systemic infections in media that differ from the corneal stroma. The large interpatient variability observed in this study and in previous studies (McDermott et al. 1993; Diamond et al. 1995; Donnenfeld et al. 1997; Price et al. 1997) indicates that drug penetration may occasionally be quite low even if the average achieved concentration is satisfactory. Penetration may be improved in patients with inflamed corneas (such as in the setting of bacterial keratitis) and/or non-intact corneal epithelium (Ozturk et al. 1999). More frequent drug application and/or longer duration of drug application may increase intracorneal drug concentrations (McDermott et al. 1993; Price et al. 1997). Notably, some *Staphylococcus* and *Pseudomonas* strains are highly resistant to all fluoroquinolones (Kowalski et al. 2001). The data in the present study, however, may be appropriately used to attempt to predict relative *in vivo* potency among the fluoroquinolones.

In this study, topically applied levofloxacin achieved higher corneal concentrations than lomefloxacin or norfloxacin. These data may favour the selection of levofloxacin, rather than norfloxacin or lomefloxacin, for the initial treatment of bacterial keratitis. However, the precise role of levofloxacin in clinical practice will need to be determined by investigations assessing clinical outcomes.

Table 3. Norfloxacin, levofloxacin and lomefloxacin MIC_{90} values for bacteria associated with stromal keratitis.

Organism	Norfloxacin MIC_{90} (μg/ml)*	Levofloxacin MIC_{90} (μg/ml)†‡	Lomefloxacin MIC_{90} (μg/ml)‡
Staphylococcus aureus	2	0.25–0.5	0.78
Staphylococcus epidermidis	1	0.19–0.41	1.56
Streptococcus pneumoniae	16	0.06–3.13	12.5
Enterococcus faecalis	32	1–3.13	12.5
Proteus mirabilis	0.5	0.06–0.25	0.39
Klebsiella pneumoniae	0.5	0.1–3.13	1.56
Pseudomonas aeruginosa	2–8	1–8	50

* * Chin et al. (1988)
† Une et al. (1988)
‡ Wimer et al. (1998)

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Phospholipids and Their Degrading Enzyme in the Tears of Soft Contact Lens Wearers

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Purpose: Low tear phospholipids levels are associated with tear film instability in soft contact lens wearers. We assayed levels of phospholipids and their degrading enzyme secretory phospholipase A_2 ($sPLA_2$) both in tears and deposited on contact lenses composed of 2 hydrophilic materials after 1 day of routine use.

Methods: Polymacon (Medalist; FDA group 1, low water/nonionic) and Etafilcon A (One Day Acuvue; group 4, high water/ionic) contact lenses were worn for 12 hours by 16 experienced contact lens wearers. Phospholipids in tear fluids and deposited on contact lenses were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. Double-antibody sandwich ELISA was used to determine group IIa $sPLA_2$ concentrations, and $sPLA_2$ activity was assayed using 1,2-diheptanoyl thiophosphatidylcholine as substrate.

Results: Phospholipids concentrations in tears with Polymacon and Etafilcon A were 186 ± 39 and 162 ± 33 μg/mL, respectively. The latter concentration was significantly lower than that observed in the same subjects when not wearing contact lenses ($P = 0.0023$). In tears, both group IIa $sPLA_2$ concentrations and enzymatic activity remained unchanged, regardless of lens wearing. However, Etafilcon A (0.57 ± 0.09 μg/lens) showed more group IIa $sPLA_2$ deposition than Polymacon (0.01 ± 0.01 μg/lens; $P < 0.001$). Furthermore, group IIa $sPLA_2$ deposited on Etafilcon A but not on Polymacon lenses retained its enzymatic activity.

Conclusion: Significant differences of group IIa $sPLA_2$ deposition were found in the 2 lenses tested. Such deposition might induce phospholipid hydrolysis in tears and thereby promote tear film instability in hydrophilic contact lens wearers.

Key Words: contact lens, dry eye, phospholipids, secretory phospholipase A_2, tears

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Contact lens–induced dry eye is a major cause of contact lens intolerance.[1–3] According to 1 survey conducted in Japan, more than 80% of soft contact lens wearers occasionally experience a dry sensation in the eyes. This feeling of dryness can cause many patients to reduce their contact lens wearing time or may render them intolerant of contact lens wear.[3] Tear film stability, clinically estimated by tear film break-up time (BUT), is especially compromised in soft contact lens wearers.[2,3] Contact lens intolerance is best described by tear film stability and tear volume. Thus, although an association between contact lens wear and tear film instability is well recognized, the mechanism is not fully understood.

Normal tears are characterized by low surface tension that is intimately associated with tear film stability.[4] Recent studies have shown that phospholipids play crucial roles in maintaining low surface tension of tears.[4–9] In the most widely accepted model of tear film structure, the aqueous-mucin layer is coated by 2 thin lipid layers: polar lipids such as phospholipids lie adjacent to the aqueous layer, whereas nonpolar lipids are present at the tear–air interface.[5,6] Phospholipids are important for maintaining stable tear film by linking the nonpolar hydrophobic outer lipid layer and the aqueous layer of tear film.

It has been shown that low levels of phosphatidylcholine and phosphatidylethanolamine in meibum are linked to the severity of meibomian gland dysfunction.[10] We recently reported that concentrations of these phospholipids in the tears of patients with marginal and moderate dry eye were significantly lower than those in subjects without dry eye.[11] Guillon et al[12] reported that low levels of tear phospholipids are associated with short tear film BUT in soft contact lens wearers. Recent studies have also shown that low surface tension of tears is not caused by phospholipids alone but to a complex of phospholipids and tear proteins.[4] Moreover, some proteins such as tear lipocalin and secretory phospholipase A_2 ($sPLA_2$) may regulate phospholipids levels in tear fluids. Tear lipocalin, which comprises 15% to 33% of total protein mass in tears,[13,14] binds various lipids including phospholipids.[15–17] Although the physiological roles of lipocalin are not fully understood, tear lipocalin may adjust the lipid composition of tears by binding lipids from tear fluid and/or releasing lipids into tear fluid. We recently reported that concentrations of tear lipocalin in patients with meibomian gland dysfunction are significantly lower than in normal controls.[18] Tear lipocalin concentration correlated positively with tear film BUT and negatively with fluorescein staining scores in these patients. On the other hand, Grus et al[19]

reported that lipocalin concentrations are somewhat elevated in the tears of contact lens wearers versus in normal controls, and Glasson et al[20] reported that contact lens–intolerant subjects had significantly higher amounts of lipocalin in tears compared with subjects who were able to tolerate contact lens wear.

$sPLA_2$ is a lipolytic enzyme that catalyzes hydrolysis of phospholipids at the *sn*-2 position, yielding free fatty acid and lysophospholipid.[21] Group IIa $sPLA_2$ is the most abundant form of $sPLA_2$ in tears. It has been found in tears in concentrations averaging from 1.45 to 54.5 μg/mL, levels that exceed normal serum group IIa $sPLA_2$ concentrations by 4 orders of magnitude.[22,23] Group IIa $sPLA_2$ in tears is purported to serve a bacteriocidal function.[24] However, as suggested by Song et al,[25] excess amounts of this enzyme may compromise tear film stability through hydrolysis of tear phospholipids. Glasson et al[20] reported that contact lens–intolerant subjects had slightly but significantly higher tear concentrations of group IIa $sPLA_2$ than contact lens–tolerant individuals and hypothesized that decreased tear phospholipids and tear film instability might be caused by the action of group IIa $sPLA_2$ in tear fluids.

As described above, phospholipids levels in the tears of hydrophilic contact lens wearers are lower than in normal controls, which might contribute to tear film instability. We posited 2 hypotheses to explain this phenomenon: (1) phospholipids deposition occurs on contact lenses and (2) tear phospholipids are degraded by group IIa $sPLA_2$ deposition on contact lenses. To test these 2 hypotheses, we assayed phospholipid, protein, and $sPLA_2$ content in tears and on contact lenses composed of 2 frequently replaced hydrophilic materials after 1 day of routine use.

MATERIALS AND METHODS

Subjects and Contact Lenses

Sixteen experienced contact lens wearers (4 men and 12 women) ranging in age from 26 to 44 years, with no history of eye disease except for refractive errors, participated in this study. Each subject wore Polymacon (Medalist; Bausch & Lomb Japan, Tokyo, Japan; FDA group 1, low water/non-ionic) and Etafilcon A (One Day Acuvue; Johnson & Johnson Japan, Tokyo, Japan; group 4, high water/ionic) contact lenses for 12 hours (from 7:00 AM to 7:00 PM) on different days. At the end of daily wear, tears were collected using a Schirmer test strip from the right eye of each subject. The length of wet portion of each strip was recorded. Then, Schirmer test strips and worn contact lenses from all subjects' right eyes were stored at –80°C until analysis. On a different day, subjects were instructed not to wear contact lenses for 1 day; in the evening, tears in the right eye of all subjects were collected using a Schirmer test strip. The protocol was approved by our institutional review board, and all subjects provided written informed consent before participation.

Phospholipids Analysis

Schirmer test strips and contact lenses were cut in half and used for lipids analysis and protein analysis. Lipids were extracted by a modified Bligh and Dyer procedure.[26] In short, samples were placed in a test tube with 1.0 mL of 2:1 chloroform:methanol extraction solvent (Wako, Osaka, Japan) for 16 hours. After adding 0.2 mL of water, the tubes were vortexed for 30 seconds. The aqueous layer was discarded, and the lipid layer was subjected to analysis.

Phospholipids levels were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion. After lipid extracts were evaporated to dryness under nitrogen gas, 50 μL of 10 mmol/L TRIS hydrochloride (Sigma Chemical, St. Louis, MO) buffer (pH 7.8) containing 2.0 U/mL phospholipase C (from *Bacillus cereus*; Sigma Chemical) was added, and samples were incubated at 37°C for 20 minutes.[27] Samples were incubated at 37°C for an additional 30 minutes after adding 50 μL of 175 mmol/L diethanolamine hydrochloride (Sigma) buffer (pH 9.6) containing 2.0 U/mL alkaline phosphatase (human placental origin; Sigma). Then, 50-μL aliquots of each sample were placed in a 96-well microplate and mixed with molybdate-malachite green reagent (BIOMOL, Plymouth Meeting, PA). Absorbance of solutions at 620 nm was measured by spectrophotometer.

Total Protein and $sPLA_2$ Analysis

Solvent consisting of a 50:50 mixture of 0.2% trifluoroacetic acid and acetonitrile (Wako) was used to extract proteins. Strips and lenses were placed in extraction solution for 16 hours, and extraction solution was subsequently analyzed.[28] The total protein content of extraction solution was measured by bicinchoninic acid (BCA) analysis. Sample solution (10 μL) was mixed with 300 μL of protein assay regent (Cytoskeleton, Denver, CO), which was comprised of bicinchoninic acid and cupric sulfate, in a 96-well microplate. Absorbance of the solution at 595 nm was measured by spectrophotometer. A standard curve established with bovine serum (Sigma) was used to quantify protein contents of the lens extract.

Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine group IIa $sPLA_2$ concentrations in protein samples. For this purpose, a commercial ELISA kit (Cayman Chemicals, Ann Arbor, MI) was used according to the manufacturer's instructions. Samples were diluted to 1/500 or 1/5000 concentrations, and absorbance was measured at 420 nm by spectrophotometer.

Measurement of $sPLA_2$ activity was performed with a commercial $sPLA_2$ activity assay kit (Cayman Chemicals). This assay uses a 1,2-dithio analog of heptanal phosphatidylcholine, which serves as substrate for most PLA_2, with the exception of cytosolic PLA_2. On hydrolysis of the thio ester bond at the *sn*-2 position by PLA, free thiols were detected using 5,5-dithio-*bis*-(2-nitorobenzonic acid). Absorbance was measured at 420 nm by spectrophotometer.

RESULTS

Phospholipids

Results of the determination of phospholipids in tears and deposited on contact lenses are detailed in Table 1 and Figure 1. Phospholipids concentrations in tears with Polymacon were 186 ± 39 μg/mL, which is lower but not

TABLE 1. Phospholipids and their Degrading Enzyme $sPLA_2$ in Tears and from Worn Contact Lenses (CL)

	Tears			Worn CL	
	Without CL	Polymacon	Etafilcon A	Polymacon	Etafilcon A
Phospholipids (tears: μg/mL; CL: μg/lens)	220 ± 35	186 ± 39	162 ± 33*	2.1 ± 0.4	1.8 ± 0.4
Total protein (tears: mg/mL; CL: mg/lens)	5.93 ± 1.49	6.59 ± 2.11	5.64 ± 2.69	0.04 ± 0.04	0.48 ± 0.06†
Group IIa $sPLA_2$ (tears: μg/mL; CL: μg/lens)	5.02 ± 0.68	5.13 ± 0.33	5.02 ± 0.51	0.01 ± 0.01	0.57 ± 0.09†
$sPLA_2$ activity (tears: mmol/minute/mL; CL: mmol/minute/lens)	0.47 ± 0.18	0.52 ± 0.18	0.47 ± 0.18	ND	0.13 ± 0.04

*P = 0.0023, †P < 0.0001; Mann-Whitney test.
ND, not detected.

significantly different versus concentrations observed without contact lens wear (P = 0.059; Mann-Whitney U test). On the other hand, phospholipids concentrations in tears with Etafilcon A were 162 ± 33 μg/mL, which is significantly lower than concentrations without contact lenses (P = 0.0023; Mann-Whitney U test). Phospholipid content recovered from contact lenses was 2.1 ± 0.4 and 1.8 ± 0.4 μg/lens in the Polymacon and Etafilcon A groups, respectively, which is not significantly different.

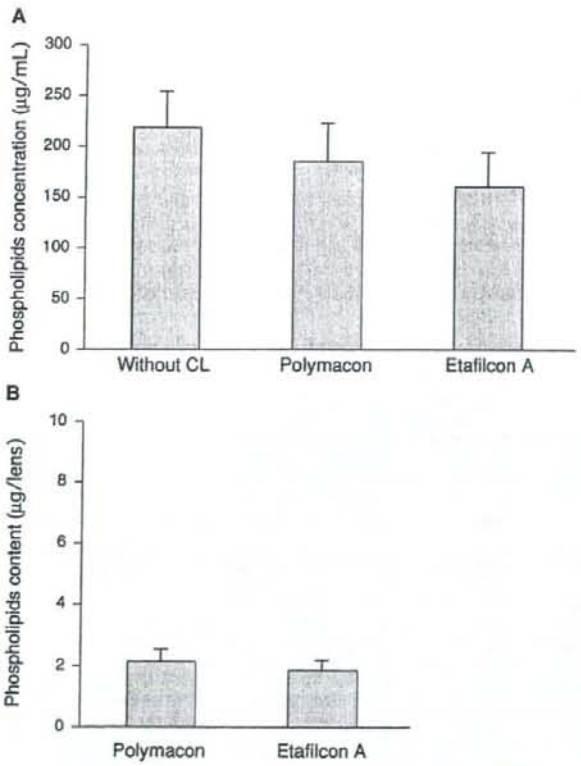

FIGURE 1. Phospholipids in tears (A) and deposited on hydrophilic contact lenses (B). Phospholipids concentrations in tears with Polymacon and Etafilcon A were 186 ± 39 and 162 ± 33 μg/mL, respectively. The latter concentration was significantly lower than that observed in the same individuals without contact lens wear (P = 0.0023; Mann-Whitney U test).

Total Protein

Total protein concentrations in tears are listed in Table 1. Total protein concentrations in tears were not significantly different among the 3 groups. In contrast, tears in the Etafilcon A group (0.48 ± 0.06 mg/lens) revealed significantly (P < 0.0001; Mann-Whitney U test) higher total protein contents compared with the Polymacon group (0.04 ± 0.04 mg/lens).

Group IIa $sPLA_2$ and $sPLA_2$ Activity

Group IIa $sPLA_2$ concentrations and enzymatic activities in tears and deposited on contact lenses are detailed in Figures 2 and 3. In tears, both group IIa $sPLA_2$ concentrations and enzymatic activities were not significantly different among the 3 groups. However, lens deposition in the Etafilcon A group (0.57 ± 0.09 μg/lens) showed significantly (P < 0.0001; Mann-Whitney U test) higher group IIa $sPLA_2$ content compared with the Polymacon group (0.01 ± 0.01 μg/lens). Group IIa $sPLA_2$ deposited on Etafilcon A lenses retained enzymatic activity: $sPLA_2$ activity in this group was 0.13 ± 0.04 mmol/min/lens. $sPLA_2$ activity was not detected in the Polymacon group.

No significant quantity of proteins and lipids was recovered from unworn Etafilcon A and Polymacon contact lenses.

DISCUSSION

In this study, phospholipid concentrations in tears were estimated by phosphorus determination with ammonium molybdate through enzymatic digestion.[27] Although this method is not suitable for analyzing the class of phospholipids, it enabled us to determine the quantity of phospholipids in small volumes of tears, because phospholipids of all classes contain a single inorganic phosphate. Phospholipids concentrations in tears with Etafilcon A wear were significantly lower than in those without contact lenses. Although it has been reported that phospholipids levels in the tears of soft contact lens wearers are lower than in normal controls, our result shows for the first time that lipids composition of tears is altered by contact lens wear.[12] To explore the mechanism of this phenomenon, we also analyzed the amounts of phospholipids deposited on contact lenses. However, these amounts of phospholipids recovered from worn lenses were small and considered insignificant. Lipid deposition patterns observed in this study are consistent with previously published work.[29–31] Therefore, it is not likely that decreased phospholipids levels

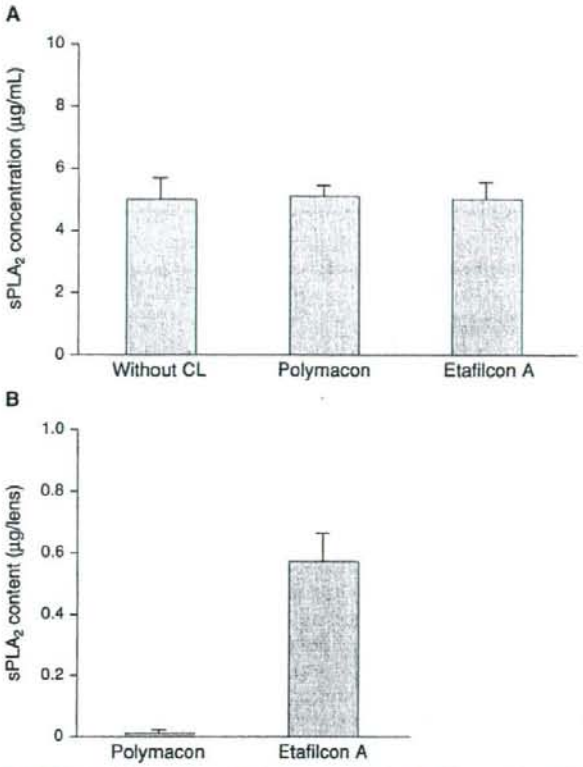

FIGURE 2. Group IIa $sPLA_2$ concentration in tears (A) and content deposited on contact lenses (B). Group IIa $sPLA_2$ concentrations in tears were not significantly different among 3 groups. Etafilcon A (0.57 ± 0.09 μg/lens) showed significantly ($P < 0.0001$; Mann-Whitney U test) higher group IIa $sPLA_2$ deposition than Polymacon (0.01 ± 0.01 μg/lens).

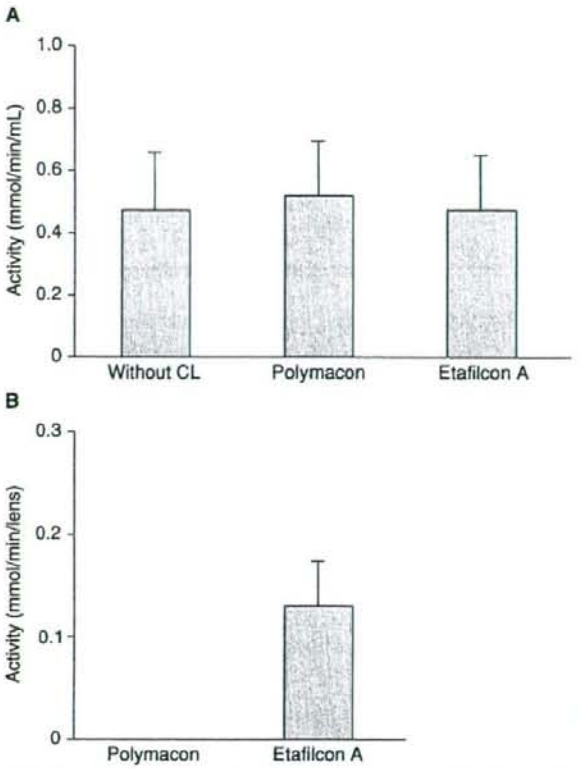

FIGURE 3. $sPLA_2$ activities in tears (A) and deposited on contact lenses. $sPLA_2$ activity in tears was not significantly different among 3 groups. B, $sPLA_2$ activity in the Etafilcon A group was 0.13 ± 0.04 mmol/min/lens, whereas no significant $sPLA_2$ activity was detected in the Polymacon group.

in tears of hydrophilic contact lens wearers result from deposition of phospholipids on the lenses.

It is widely recognized that contact lens adsorption of proteins is a complex process influenced by many variables such as water content and surface charge on lenses.[29–34] Significant differences were found in protein deposition profiles of the 2 lens groups tested in this study. Etafilcon A lenses accumulated significantly more protein including group IIa $sPLA_2$ than Polymacon lenses. Protein deposition is thought to be predominantly influenced by the ionic charge of the lens material.[30] Ionic materials such as methacrylic impart a negative charge to the lens and thus favor deposition of positively charged species such as lysozymes. Group IIa $sPLA_2$ is also highly cationic in tears,[35] resulting in electrostatic attraction to negatively charged lens material.

Glasson et al[20] reported that contact lens–intolerant wearers had significantly higher tear concentrations of group IIa $sPLA_2$ than subjects who were able to tolerate contact lens wear. These researchers suggested that decreased tear phospholipids and tear film instability might be caused by enzymatic action of group IIa $sPLA_2$ in tears. In this study, however, there was no significant difference of group IIa $sPLA_2$ concentration in the tears of subjects regardless of contact lens wear. Instead, we found significant deposition of group IIa $sPLA_2$ on Etafilcon A contact lenses. Aho et al[36] reported that contact lens wearers had statistically lower group IIa $sPLA_2$ content in tears at noon and at 4:00 PM versus healthy controls. They proposed that transient low levels of group IIa $sPLA_2$ observed in contact lens wearers might be caused by contact lens absorption of group IIa $sPLA_2$. Our results seem to support this hypothesis. Assuming that total tear volume of normal subjects is 10 μL and concentration of group IIa $sPLA_2$ in normal tears is 54.5 μg/mL,[23] tears of normal subjects would be expected to contain a total of 0.55 μg of group IIa $sPLA_2$. Therefore, the amount of group IIa $sPLA_2$ deposited on Etafilcon A contact lenses in this study is as high as the total amount expected in normal tears. We also showed that group IIa $sPLA_2$ deposited on contact lenses retained its activity as a lipolytic enzyme. Retention of enzymatic activity by group IIa $sPLA_2$ seems to be of functional significance.

Our results suggest a novel additional mechanism for contact lens–induced dry eye: the activity of group IIa $sPLA_2$ deposited on contact lenses may play a role in the development of tear film instability. It should be noted, however, that

concentrations of phospholipids in the tears of subjects in this study were lower than those of normal control subjects in our previous study, even when they had not worn contact lenses for at least 1 day.[11] In that study, we reported that phospholipid concentration in tears from patients with moderate dry eye was 182.3 ± 89.2 μg/mL, which is significantly lower than those in normal subjects (379.0 ± 97.8 μg/mL). There might be other changes in the ocular surface of contact lens wearers to decrease phospholipid levels in their tears.

In summary, we found significant group IIa $sPLA_2$ deposition on Etafilcon A contact lenses. Contact lens enzyme deposition may promote phospholipid hydrolysis in tears, resulting in decreases of tear phospholipids and increases of free fatty acids. These biochemical alterations may lead to tear film instability and thereby contribute to contact lens intolerance.

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Lattice Corneal Dystrophy Type III in Patients with a Homozygous L527R Mutation in the *TGFBI* Gene

Late-onset lattice corneal dystrophy (LCD) is associated with decreasing vision, minor recurrent epithelial erosions or no erosions at all, and lattice lines much thicker than those usually observed in LCD types I and II. A patient with this type of LCD is classified as LCD type III.[1] Most LDC type III cases have been reported in Japanese patients, and the inheritance pattern is proposed to be autosomal recessive. However, Stock et al.[2] reported that although LCD type IIIA resembles type III clinically, it differs in that type IIIA has an autosomal dominant inheritance pattern. A later study reported that LCD type IIIA is caused by mutations in the transforming growth factor beta-induced (*TGFBI*) gene.

More recently, a heterozygous L527R mutation in the *TGFBI* gene has been reported to be the cause of late-onset LCD in six Japanese patients.[3] Interestingly, only two of these had a family history of LCD. Hirano et al.[4] reported that two Japanese patients with late-onset LCD also had a heterozygous L527R mutation and no family history. They clinically diagnosed LCD type III in these two patients.

We present the characteristics of two patients with late-onset LCD who were homozygous for the L527R mutation.

Case Reports

Patient 1 was a 78-year-old man who presented with decreased vision in both eyes. His corrected visual acuity was 0.3 OD and 0.1 OS. He is the younger brother of patients 2 and 3 in the family with LCD type III reported by Hida et al.[1] (their Fig. 2). His corneal opacities were bilateral and observed as grayish nodular deposits in the midstroma with relatively thick lattice lines that extended from limbus to limbus (Figs. 1A, B). He had no history of corneal erosions. Penetrating keratoplasty was performed on his left eye in March 1996 and on his right eye in December 2001. Histologic findings of the excised corneas showed large deposits of amyloid in the stroma, predominantly midway between the epithelium and the endothelium.

Patient 2 was a 78-year-old man who presented with decreased vision in both eyes. His corrected visual acuity was 0.3 OD and 0.01 OS. The corneal opacities consisted of grayish nodular deposits in the central midstroma and relatively thick lattice lines that extended from limbus to limbus in both eyes (Fig. 1C). The appearance of the corneal opacities was similar to those of patient 1. His sister had undergone keratoplasty at the age of 60 years, but the details of the condition of her cornea could not be obtained. There was no history of corneal erosions in patient 2. Penetrating keratoplasty was performed on the left eye in January 1993. Histologic findings of the excised cornea showed large stromal deposits of amyloid (Fig. 1D).

A genetic investigation was performed according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained. Direct sequencing[5] of the *TGFBI* gene revealed homozygous L527R mutations (Fig. 2).

Comments

Recently, we reported on five Japanese patients with late-onset LCD who also carried a heterozygous L527R mutation.[5] One (case 3 in reference 5) of the five patients had been previously reported as having LCD type III by Hida et al.[1] (case 5 in reference 1), and only one (case 4 in reference 5) with the L527R mutation had a family history of LCD, an affected sibling. Thus, most patients with late-onset LCD reported in Japan have relatively thick lattice lines and/or tiny, discrete nodular deposits, but no family history or affected siblings. They are heterozygous[3–5] or homozygous for the L527R mutation. These results indicate that the heterozygous L527R mutation in the *TGFBI* gene for LCD has low penetrance in the Japanese population, but the homozygous L527R mutation might have increased penetrance. Interestingly, the corneas in those patients with a homozygous L527*R* mutation appeared very similar to the corneas of the heterozygous patients. The reason for this is unknown.

Mutations in the *TGFBI* gene can cause LCD type IIIA as well as LCD type III, both late-onset LCD. Because the corneal appearance in patients with type III is very similar to that in patients with type IIIA, the two types are diagnosed by the inheritance pattern. However, the difference most likely results from the degree of penetrance of the *TGFBI* gene mutation.