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Treatments for Severe Type of Retinopathy of Prematurity

Noriyuki Azuma

Department of Ophthalmology, National Center for Child Health and Development

Patients with severe type of retinopathy of prematurity (ROP) that often results in blindness, when progresses to retinal detachment, recently increase, because extremely-low-birth-weight infants are able to survive. When ROP progresses to stage 3, retinal photocoagulation is performed as an initial treatment. Scleral buckling or vitreous surgery may treat retinal detachment, but often fails to provide useful vision. Type II ROP designated by the Japanese Diagnostic and Therapeutic Criteria for ROP or aggressive posterior ROP by the International Classification of ROP rapidly progresses to a total retinal detachment within 1 to 2 weeks if left untreated. However, recent advance of early intervention with photocoagulation and vitreous surgery successfully treat retinal detachment in such severe forms of ROP.

重症未熟児網膜症に対する早期硝子体手術

東 範行

〔要 約〕

重症未熟児網膜症（Ⅱ型/aggressive posterior retinopathy of prematurity）は，光凝固が奏功せずに網膜剝離へ進行すれば，予後がきわめて不良である。これに対して早期硝子体手術を行った。国際分類 stage 4 の比較的早期に，水晶体を除去して硝子体を広汎に切除すると，高率に網膜が復位し，黄斑が形成され，良好な視反応が得られた。重症未熟児網膜症は，前もって光凝固が十分に行われており，時宜を得れば，早期硝子体手術によって予後を顕著に改善することができる。

はじめに

未熟児網膜症には劇症型とも言える重症型が存在する。わが国では以前より厚生省分類[1,2]でⅡ型として注意を喚起してきたが，2005年に改定された国際分類[3]はこの概念を全面的に受け入れ，aggressive posterior retinopathy of prematurity（ROP）と規定した。近年，体重が極端に少ない超低出生体重児が救えるようになり，わが国でもⅡ型/aggressive posterior ROPや従来とは異なる非定型重症例が増加している[4]。このⅡ型/aggressive posterior ROPはしばしば光凝固では進行が阻止できず，網膜全剝離にいたることも多く，予後がきわめて悪い。国立成育医療センターでは，この網膜症に早期硝子体手術を行い，1年余の短期間ではあるが，予想をはるかに超えた良好な成果を得ている[5]。本手術の導入によって，重症未熟児網膜症の治療適応が大きく変わると思われるので，その概略と考えをここに述べる。

Ⅰ．未熟児網膜症治療のこれまでの変遷

未熟児網膜症の治療は，血管増殖が網膜内から硝子体内へある程度進行すると，まず光凝固が行われる。この光凝固治療はわが国で1968年に世界に先駆けて行われ[6]，以後厚生省分類3期網膜症に対して広く行われるようになったが，米国ではずっと遅れて1988年にまず冷凍凝固に対するmulticenter trial（CRYO-ROP Study）[7]が行われ，ついで光凝固が一般化した。適応時期も，わが国では早くから牽引乳頭/網膜を防止して有用な視力を得ることを目的としていたが，欧米では初めは失明予防を目的としてthreshold ROPが適応とされ，ついで良い視力獲得を目的とするEarly Treatment for ROP（ETROP）Study[8]が行われてprethreshold ROPへと治療時期が移ってきている。

網膜症がさらに進行して網膜剝離を生じ始めると，強膜バックリング[9]か硝子体手術[10]が行われる。この段階では，光凝固や冷凍凝固は無効であ

東 範行：国立成育医療センター

るばかりか，増殖膜の癒着や牽引を増強し，網膜裂孔を形成するなど却って問題が多い。網膜剝離がまだ部分的で黄斑が脅かされる程度であれば，まず輪状締結を行って牽引の軽減を試みるが，増殖膜が広い範囲にわたって存在し，これが強く収縮して網膜襞が形成されるような状態ではあまり効果がない。さらに進行して網膜全剝離に向かえば硝子体手術を行うが，増殖組織内の血管の活動性が高いと，術中に大出血を起こし，眼内操作を妨げるとともに，不十分な切除部位や凝固血液塊に沿って術後再増殖を起こし，予後不良となる。したがって，一般に硝子体手術を行うには，増殖組織内の血管が退縮し外見が白色となるまで待ってから行わなければならない。これには網膜が剝がれてから通常1～2か月を要し，この間に網膜の変性が進んでしまうので，復位が得られても視力は光覚～手動弁にとどまるものが大部分である[11]。

これに対して近年，網膜剝離がまだ進行していない，より早期(厚生省分類4期，国際分類stage 4) に水晶体を温存して硝子体手術 (lens-sparing vitrectomy)[12] が行われるようになった。前もって光凝固を十分に行っておけば，出血も比較的少なく，高率な復位が得られる。既に視力予後の検討が行われ，良好な結果が報告されている[13]。強膜バックリングが強い屈折異常を起こすこと，眼球絞扼予防のために後でバックルを除去しなければならないことを考えれば，これに替わる治療法であるとする主張もある。ただし，小児では眼球内で水晶体が占める比率が高く，硝子体切除を安全に行えるのは後極からやや周辺までの比較的狭い範囲に限られること，血管を多く含む増殖膜がまだ伸展していない相当早期に行わなければならない等，実際にはかなりの制約があると思われる。

Ⅱ．急速に進行するⅡ型未熟児網膜症/aggressive posterior ROP

しかしながら一方で，未熟児網膜症には劇症型とも言えるきわめて重症な病型が存在する。わが国の厚生省分類[1,2]では，活動期の順を追って進行するⅠ型に対し，急激に悪化して網膜剝離にいたるⅡ型を分けて，注意を喚起していたが，1984年に発表された国際分類[14]では，このⅡ型とⅠ型が全く異なる病態であるとの考えに十分な理解が得られなかった。後極血管の拡張と蛇行を伴う場合は重症の兆候であり，stageの後に＋を加えてplus diseaseと称するに止まった。しかしその後，欧米でもわが国の考えが認識されるようになり，2005年に改訂された未熟児網膜症国際分類[3]では，Ⅱ型の概念を全面的に取り入れて，aggressive posterior ROPと規定した。これは非定型的重症型として，(1)通常のstage 1からstage 3への段階的な進行は示さず，急速に悪化してstage 5の網膜剝離にいたる予後不良なもので，(2)多くはzoneⅠ，時にはzoneⅡ後部でも起こり，(3)早期に後極の網膜動静脈が全周で顕著に拡張，蛇行し，(4)方々で血管シャントを形成し，無-有血管領域の境界で出血が起こることが特徴である，と記載されている。このⅡ型あるいはaggressive posterior ROPはきわめて難治であり，光凝固を広汎かつ密に行っても，これに抵抗してしばしば網膜剝離に進行する。

わが国ではⅡ型網膜症が十分に注意喚起されていたこともあり[1,2]，早期に兆候が発見され，診断がつき次第，直ちに広汎かつ密な光凝固を行われてきた。何回か追加凝固を要し，牽引乳頭/網膜や網膜襞等，ある程度の瘢痕を残すにせよ，これで何とか抑えられることもある。しかし，効果なく網膜全剝離へ進行する場合は（図1A～D），これまで手をこまねいて見るに等しく，失明に至ることを覚悟するしかなかった。バックリング手術をⅡ型/aggressive posterior ROPに適応した場合，増殖組織とこれに伴う牽引性網膜剝離がzoneⅠあるいはzoneⅡ後部の眼球後方から立ち上がり，しかも緯線円周方向の範囲がほぼ全周にわたっているので，手技が難しく，眼球壁を圧迫する方向からも牽引を解除する効果はわずかである。硝子体手術の適応にはなるものの，増殖組織内の血管活動性がきわめて高いので，これが退縮して術中出血の危険性がなくなるまで待つと，手術時期までかなり長引いてしまう。しかも網膜剝離の程度が強いので，網膜機能障害が顕著であり，手術手技が難しいばかりか，復位が得られても視力予後が非常に悪い。さらに，広汎に生じた増殖組織は強く収縮して水晶体を前方に移動させるの

で，まもなく前房消失，角膜混濁，緑内障あるいは眼球癆にいたって，手術を行えなくなることもしばしばである。

Ⅲ．Ⅱ型/aggressive posterior ROPに対する早期手術の効果

そこで，増殖組織が立ち上がって網膜剝離が起こり始めた時期に，早めに硝子体切除を行って増殖の足場を無くせば，網膜剝離の重症化を少しでも軽減できるのではないかと考え，2004年後半より国立成育医療センターでは早期硝子体手術を開始した。手術を行う時期の患児の体重は1,500～2,000 g程度で眼球も小さいので，25Gのように繊細で，かつ高速度で網膜を傷つけない安全な硝子体手術システムが開発されたことも，手術に踏み切ることができた一因である。いずれの症例でも，前もって光凝固が無血管領域のみならず，その後方のシャントがあると思われる有血管領域までかなり踏み込んで，十分密に行われていた。それにもかかわらず進行がみられたので，凝固瘢痕の中から増殖組織が緯線円周方向に広く立ち上がって牽引性網膜剝離を起こし始めた段階で，手術を行った。術式等の詳細は原著[5]に譲るが，以後症例をやや増やし，2006年5月現在18例27眼［出生時在胎22～30（平均24）週，体重466～1,676（平均773）g，手術時修正在胎35～41（平均37）週，体重1,560～2,602（平均2,019）g］に手術を行い，予想をはるかに超えた良好な成果が得られている。

最初の4例6眼はlens-sparing vitrectimyを行った。しかし，後極を中心とした限局的な部位でしか硝子体を切除できず，水晶体後面や硝子体基底部はほぼ残ったため，ここに沿って増殖組織が進展し，全例が高度の網膜剝離となった（図1 DE)。これに対し，これまでのように，増殖組織内の血管活動性が鎮静化するまで1～2か月待って再手術を行った。しかし，網膜の復位が得られても，変性は既に高度に進行しており，視反応は光覚にとどまった（図1 F)。

そこで以後は，水晶体を切除して，硝子体基底部まで十分な硝子体切除を行った。ただし，術中の出血を恐れ，有形硝子体の切除にとどめて，増殖組織には極力手をつけないようにした。現在までに行った14例21眼（国際分類stage 4A：12眼；stage 4B：9眼）のうち，19眼でほぼ全復位が得られた。術前の十分な光凝固が奏功したと思われ，術後の再増殖はみられなかった。術前stage 4Bで，既に広汎な網膜剝離に進行していた2眼は，部分復位にとどまった。そして，全復位が得られた19眼中，11眼（58%）で明瞭な黄斑が形成され，6眼（32%）はやや低形成，牽引乳頭/網膜を残した2眼では形成されなかった（図2)。そして，黄斑を認めた17眼は，全例で良好な固視と追視反応が確認され，感覚欠陥型眼振もみられていない。網膜剝離がstage 5へ進行した後に，増殖膜内の血管が枯れるのを待って行っていた従来の硝子体手術では，光覚～手動弁の視覚しか得ら

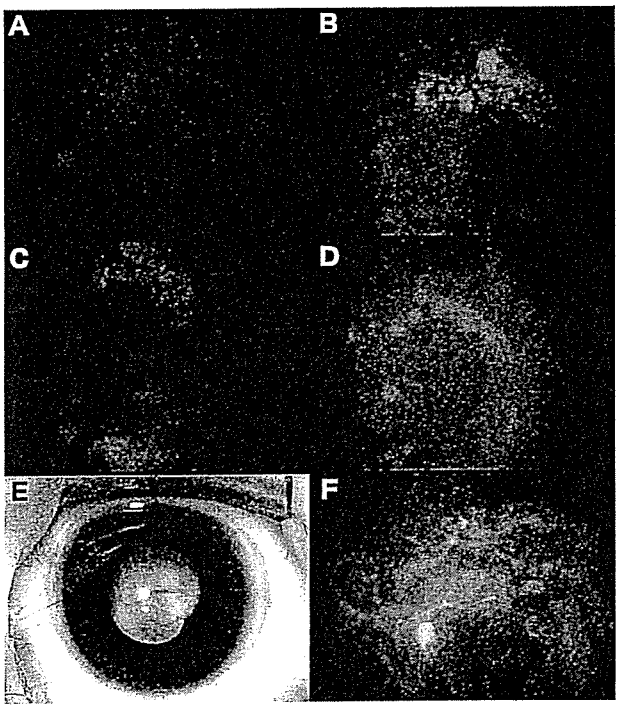

図1 Ⅱ型/aggressive posterior ROPの経過とlens-sparing vitrectomyの結果

22週，479 gで出生。
（A） 生後10週，初回汎光凝固。
（B） 生後13週，凝固瘢痕。
（C） 生後17週，凝固瘢内からの出血，増殖が出現。
（D） 生後18週，増殖が伸展し，牽引性網膜剝離も出現（stage 4A)。この段階でlens-sparing vitrectomyを施行。
（E） 網膜全剝離となり白色瞳孔に進行。
（F） 増殖組織内の血管消退を待って1か月後に硝子体手術を施行。網膜は復位したが，高度の変性を認める。（文献5より，許可を得て，一部改変掲載）

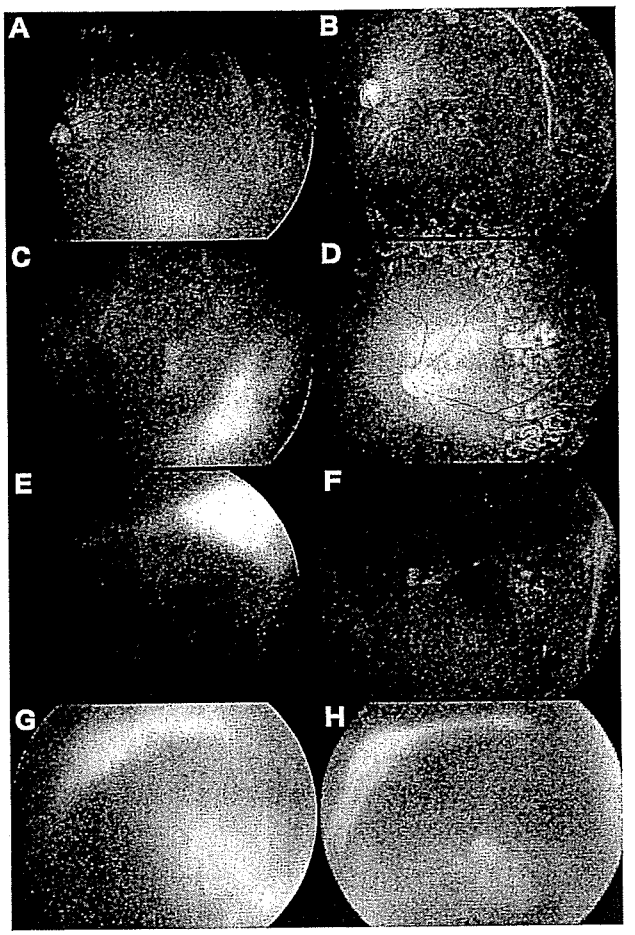

図2　Ⅱ型/aggressive posterior ROPに対するlensectomyを併用した早期硝子体手術の結果

術前の増殖組織伸展度によって予後は大きく異なる。
(AB：左眼　GH：右眼) 24週，760gで出生。
(CD) 26週，897gで出生。(EF) 24週，526gで出生。
(ACEG) 術前。(BDFH) 術後。

(A)　増殖組織は，緯線円周ほぼ全周で，凝固瘢痕部から水晶体後方に向かって伸展。既にこの下で網膜剝離が起こり始めており，stage 4Aごく初期と言える。(広角度眼底カメラ [Nidek RetCam] による撮影，血管の拡張や線維組織・網膜剝離の丈と範囲は実際より軽度に写る)

(B)　線維組織を残すも網膜はほぼ全復位し，網膜血管の拡張蛇行も軽減。黄斑が形成されている。

(C)　網膜剝離は牽引された黄斑に及び始め，stage 4B初期。線維組織は硝子体基底部に向かって倒れこんでいるが，まだ隙間が残っている。

(D)　牽引乳頭/網膜が残り，黄斑は光凝固斑縁にまで引かれているが，一応形成されている。

(E)　Stage 4B。線維組織の一部が硝子体基底部に接着。

(F)　線維組織瘢痕周囲に牽引性網膜剝離を残し，黄斑は形成されていない。

(G)　Stage 4B後期。線維組織が1象限以上にわたって硝子体基底部に接着。

(H)　円周状の網膜襞が残存し，後極網膜も浅く剝離している。(文献5より，許可を得て，一部改変掲載)

れなかったのに比べ，きわめて良好な結果である。重症未熟児網膜症を起こす超低出生体重児あるいは極低出生体重児は中枢神経合併症等のため視力が得られないこともあるが，網膜症の観点からは，この早期手術が奏功すれば，患児は盲学校ではなく普通学校へ行ける可能性が開けたと思われる。

Ⅱ型/aggressive posterior ROPに対して，lens-sparing vitrectomyは無効である。硝子体の切除が足りなければ，これに沿って増殖と網膜剝離が進行するので，硝子体基底部を十分に切除することが重要であり，水晶体を除去しなければならないことが判明した。確かに，水晶体を失うことは視力発育において大きな問題である。しかし，予後に明確な差がある以上，重症網膜症の場合に水晶体除去はやむを得ない。術後早期から眼鏡やコンタクトレンズによる屈折矯正，視能訓練を開始する必要があるが，得られる恩恵は大きい。

Ⅳ．術中，術後合併症

手術の合併症については，増殖組織からかなりの出血を予測していたが，これに反して，術中，術後ともごく僅かに過ぎなかった。増殖組織の切開や切除は，網膜への牽引が強い場所に限ってやむを得ず行ったが，非常に粘性があって切りにくかったものの，中に血管が少ししか含まれていなかったことは予想外であった。これまでは，まず新生血管が硝子体腔内に充満し（活動期），その後これが枯れるとともに膠原線維が産生される（瘢痕期）と考えられていた。しかし，光凝固が十分に行われた後で凝固斑部から立ち上がってくる増殖では，活動期と瘢痕期が混在しており，色がかなり白く，膠原線維に比べて血管成分が少ないと思われる。線維組織が伸展するにつれ，その中の血管が赤く目立つようになるのは，後で新生血管が成熟し太くなるのか，あるいは二次的に血管侵入が起こるためと推測される。したがって本手術では，かなり進行した網膜症でない限り，術中出血はほとんど障害にならない。ただし，光凝固が十分に行われておらず，増殖組織が強く赤みを帯びていたり，硝子体腔に細かく赤い新生血管が充満しているような場合は別で，早期硝子体手術は依然として危険を伴う。

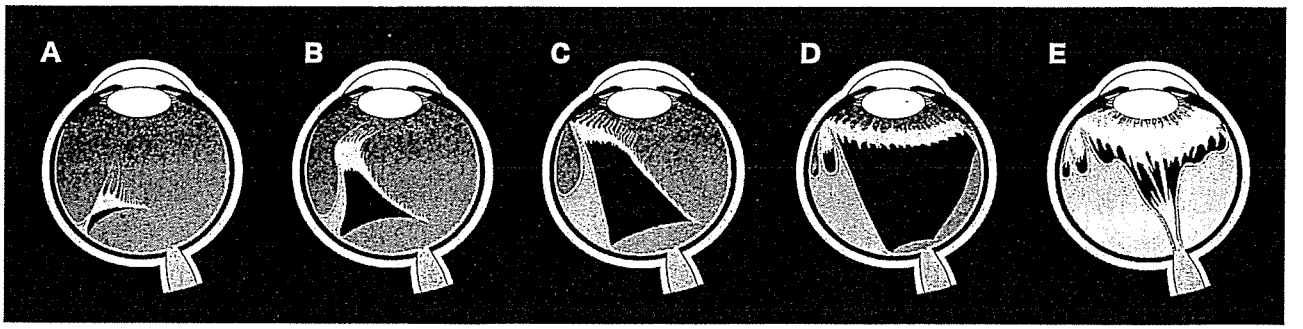

図3 Ⅱ型/aggressive posterior ROP の進行シェーマ
（A） 増殖組織は水晶体後面に向かって伸びる。すでにこの下で牽引性網膜剝離が起こり始めている。
（B） 増殖組織と剝離網膜は硝子体基底部へ向かって倒れ込む。
（C） 増殖組織の先端が硝子体基底部の網膜・毛様体に接着すれば，対側の把持部を得て，牽引性網膜剝離は襞状となり，急速かつ高度に進行する。
（D） 増殖組織と硝子体基底部の網膜・毛様体との接着が強く，広汎になれば，ここを安全に解除することはきわめて難しい。
（E） 増殖は進行し，網膜全剝離（stage 5）となる。増殖膜は血管を多く含み，もはや早期手術の対象ではなく，血管が退縮するのを1～2か月待たなければならない。
図1, 2と対比すると，（A）図1D, 図2A,（B）図2C,（C）図2E,（D）図2G,（E）図1E（ただし血管退縮後）に相当する。光凝固が奏功せず，急速に増殖が始まった場合，早ければ1週間程で（A）から（E）へ進行する。実際は，水晶体後部へ真直ぐ伸びたり，遺残硝子体血管に沿う増殖も加わるので，さらに複雑な形態を示すことも多いが，手術予後はこの図の硝子体基底部の状態が主に反映する。早期手術を行う時点で（A）あるいは（B）の段階であれば復位率が高いが，（D）に至っていれば復位困難である。（C）では，接着範囲がまだ狭ければ，手術は可能であるが，術中の医原性網膜裂孔形成や術後に網膜襞を残す恐れがある。

また，本手術では水晶体を除去するので，術後に血管新生緑内障が発生することが危惧されたが，幸いなことに1例も起こらなかった。これも，術前に，無血管領域のみならず有血管領域にかなり踏み込んで，光凝固が広汎かつ密に行われていたことが奏効したと思われる。

2眼で，術直後一過性に眼圧上昇が起こり角膜浮腫を生じたが，眼圧下降薬点眼によって短期間のうちに治癒した。

Ⅴ．早期手術の適応時期

今回手術を行った網膜症はいずれもstage 4であったが，網膜剝離や増殖組織の形態は多彩であり，これによって手術予後にかなりの差が生ずる。黄斑は，満期産でも出生後3～4か月に完成するので，未熟児の網膜剝離では，形成が障害されやすい。したがって，網膜剝離が高度なstage 4Bの方がstage 4Aよりも，ことに黄斑領域が強く伸展されていた場合は，視力予後が不良であることは明らかである。今回も術前はまだ黄斑の形態が明瞭でなかったが，網膜剝離が軽度であれば（図2AC, 3AB）術後全例に形成され，進んでいれば（図2EG, 3CD）形成は不良であった。

しかし，手術結果の成否は，網膜剝離の程度よりは，線維組織の進展度とその方向に強く左右される。一般に，網膜から立ち上がった増殖組織は，硝子体線維の走行に沿って，まず水晶体後面に向かう。この段階で，増殖組織下の網膜は牽引されて既に垂直方向に剝離し始めている（図2A, 3A）。そのまま水晶体後面に到達し接着することもあるが，増殖の多くは硝子体密度が最も高い硝子体基底部へ向かうので，やや遅れて線維組織と剝離網膜の先端部はこの部位へ倒れ込むように牽引される（図2C, 3B）。もし増殖組織の先端が対側の組織に接着すれば，把持部を得て牽引力が非常に強くなるので，牽引性網膜剝離は水晶体後面あるいは硝子体基底部の網膜・毛様体に向かって，襞状となって急速かつ高度に進行する（図2E, 3C）。したがって，手術ではこの水晶体後面と硝子体基底部の硝子体線維を切除して，ここととの接続を断つことが重要である。水晶体後面との接続は水晶体を切除すれば無くなるが，網膜襞の隙間で硝子体基底部を除去するのは難しい。25Gあるいは23G手術システムは効果的であるが，

それでも，ひとたび増殖組織が硝子体基底部の網膜・毛様体に強くしかも広く接着してしまえば，これを切開することは非常に困難となる（図2G, 3D)。血管の二次侵入が始まっていて出血が多く，線維成分が収縮して硬くなっている上に，網膜との癒着も強い。その奥で網膜がどの様に剝がれているのかを透見できないので，医原性網膜裂孔を形成しやすい。網膜裂孔を作れば，周囲の増殖線維をできるだけ除去し，液－空気置換，眼内光凝固を行わなければならず，手術時間はかなり延長する。手術が長引けば，体重の少ない未熟児では，角膜が混濁して眼内が観察しにくくなるばかりか，全身に悪影響を与える。これまでの印象では，増殖組織－硝子体基底部の接着が緯線円周方向で1象限を超えてしまえば，これを安全に解除することはまず無理である。しかも，網膜復位が得られたとしても，大部分は網膜が強く伸展されているので黄斑が形成されず，視力予後は不良となる。したがって，この早期硝子体手術は，増殖組織が硝子体基底部の網膜・毛様体に接着していない前の段階で行うべきと考える(図2AC, 3AB)。眼底観察においては，網膜剝離の程度だけでなく，硝子体の状態を十分に評価できることが必須である。

Ⅵ．早期手術における時間的制約

この手術には，さまざまな時間的制約がある。まず，網膜症は数日の遅れであっても，増殖組織が硝子体基底部に広く接着し，網膜剝離もstage 4Bまたはstage 5へ急速に進行する恐れがある。未熟児網膜症の硝子体手術を専門にする施設は限られているので，患児の迅速な移送が必要となる。新生児科医が付き添って，比較的近隣なら救急車のみでも可能だが，遠方であれば飛行機・救急車や新幹線・救急車の連携，あるいはヘリコプターによる移送を考えねばならない。ことに，ヘリコプターであれば日本全国からの移送が可能で，国立成育医療センターではこれを採用している。この移送に準備を含めて2〜3日かかる上に，転院後も全身麻酔の術前評価のために最低1日は要する。上述のように，網膜剝離と増殖組織の程度によって予後が大きく左右され，手術を行って良好な視力が期待できる期間は，網膜剝離が起こり始めてからごく僅かに過ぎない。程度の差はあるが，Ⅱ型/aggressive posterior ROPで十分な光凝固を行ったにもかかわらず増殖が始まった場合は，おおむね1週間も猶予はないと考えた方が安全である。

手術自体にも時間制限があり，超低出生体重児あるいは極低出生体重児はストレス障害に陥りやすいので可及的速やかに行う必要がある[15]。国立成育医療センターでは，全身合併症の有無にもよるが，通常は手術時体重が2,000 gであれば2時間，1,500 gであれば1時間半を手術時間の目安としている。抜管後に声帯や気管の浮腫，無呼吸を生じやすいため，短期間の繰り返し麻酔は極力避けたいので，両眼とも網膜症が急速に進行する可能性がある場合には，両眼同時手術を行うこともやむを得ない。体重1,500 gの両眼網膜症では，片眼45分で手術を終える必要がある。したがって，無駄な手術操作を極力排することが第一である。医原性網膜裂孔を作ることは厳に戒めるべきで，止血や光凝固の追加にも時間を取られたくない。増殖組織を積極的に切除しなければ，後に収縮して網膜への牽引を残すことは明らかで，除去したいのはやまやまであるが，出血や医原性裂孔を起こさないために，一歩手前で止まることも大切である。

このことからも，術前に広汎かつ高密度の光凝固が行われていることは，新生血管の活動を抑制し，網膜剝離の進行を遅延させるだけでなく，手術時に追加凝固をしなくても済む点で重要である。ただし，あくまで網膜剝離が起こる前に行っておくべきである。ひとたび増殖膜が硝子体腔に立ち上がり始めれば，ごく初期であっても，その下に既に牽引性網膜剝離が生じている。したがって，かなり離れた不足部位ならまだしも，この付近に光凝固を追加することは，増殖膜の牽引・癒着増強や網膜裂孔形成を惹起するので，禁忌である。まして，この機転を強く起こす冷凍凝固は決して行ってはならない。

Ⅶ．手術眼の選択と家族への説明

Ⅱ型/aggressive posterior ROPは大部分が両眼に起こる。両眼とも早期手術の適応で，全身状態が短期間の繰り返し麻酔を許さなければ，網膜

症の急速な進行を考慮して，両眼同時に手術を行うことが多い。この場合，増殖・網膜剝離が軽度で，手術時間が短くて済み，視力予後が期待できる方の眼を先に行う。全身状態の急変によって手術を早めに切り上げねばならない場合や，出血・医原性網膜裂孔の処置で手術が長引いて麻酔の許容時間を使い果たす場合を危惧するためである。同様に，2回に分けて手術を計画する時も，網膜症が軽度な方を優先している。片眼がまもなく手遅れになるほど悪ければ，両眼にチャンスを与えるため，初回は悪い方を2回目に良い方を手術する選択もある。しかし，全身状態が急変して2回目の手術ができなくなれば，両眼とも視力不良に終わる。いずれも，状態の良い片眼だけでも救うことが目的である。

片眼が光凝固で既に落ち着き，生活に支障ない有用視力が期待できる場合は，他眼が網膜剝離へ進んでも，従来は積極的には治療しなかった。手術で僅かな視力が得られても使うことがなく，良い方が万一失明した場合のspare eyeに過ぎない。しかも，多くは小眼球となり，後に整容目的でコンタクト義眼を装用するからである。しかし，早期手術を網膜剝離の発生初期（図2AC, 3AB）に行えば，かなり有用な視力が期待でき，水晶体を除去しても目立つ程の小眼球にはならない。術後のコンタクトレンズ矯正や視能訓練の労があっても，積極的に手術を勧めるべきと考える。網膜剝離がやや進行しても（図2E，3C），失明することに比べれば，手術を考慮して良いと思う。一方，かなり進んでしまった場合は（図2G，3D），再手術を前提に，状態を良くしておく目的で手術する選択もあるが，慎重さが必要である。

しかし，いずれも，全身麻酔に耐えられるか，手術をどの位の時間・回数で行えるか等，まず全身状態が優先する。インフォームドコンセントは重要で，新生児科医・麻酔科医とともに，保護者に眼と全身の状態を説明し，発展途上の治療法であること，手術にともなう危険性と利点について十分な理解を得た上で，治療の選択を委ねている。

Ⅷ. 今後の展望

この早期手術は開始したばかりで，まだ安易に喧伝すべきでないと思う。無作為化比較試験を行うことは難しく，今後さらに症例を集積して適応や術式を検討し，視力を含めた長期予後を追跡する必要がある。これまでの症例は前もって十分な光凝固が行われていたので，再増殖や血管新生緑内障等の手術直後の合併症は回避できている。しかし，光凝固が少しでも不足していれば，起こる可能性が高いと思う。また，晩期合併症としての緑内障，裂孔原性網膜剝離の発生等に注意しなければならない。

もっと重症例，例えば網膜血管の成長が非常に悪く視神経乳頭近傍にあるだけで，乳頭上や硝子体血管本幹に沿って増殖が起こる症例や，光凝固が十分行われず増殖組織内の血管活動性が非常に高い症例，網膜剝離が高度に進行してしまった症例（図3E）では，たぶん本手術は効を奏さないであろう。I型としては重症だがII型とまでは言えない中間型に対しては，網膜血管が比較的成長していて病変がzone IIにあることが多いので，バックリングを優先するか最初から硝子体手術を行うか，硝子体手術なら水晶体を除去するか温存するかも，今後の検討課題である。一方で，さほど進行せず僅かな瘢痕に止まるであろう軽症例に，誤って手術するのも戒めるべきである。

この早期手術が導入されると，II型/aggressive posterior ROPの治療適応は今後大きく変わると思われるが，これによって，懸念すべき社会的問題が多く生ずることが危惧される。これまでに未熟児網膜症では数多くの訴訟が起こされてきたが，従来はII型/aggressive posterior ROPで網膜剝離に進行すれば，失明に至ってもやむを得ないとされていた。しかし，有用視力が得られる可能性があるとなれば，考え方はまったく変わる。本早期手術は効果が非常に大きいにもかかわらず，さまざまな時間的制約がある上に，奏功するのは経過のごく短期間に過ぎない。少しでも遅れれば予後が非常に悪化するのが，最も懸念される点である。一方で昨今，多くの新生児集中治療室で，II型/aggressive posterior ROPを起こす可能性がある超低出生体重児あるいは極低出生体重児が管理されている。しかも，未熟児網膜症診療に関する教育がなかなか受けにくいので，十分

に対応できる眼科医が少ないことは大きな問題である。いずれにせよ，この時期の眼底検査と治療適応の判断，全身管理，家族への説明には，新生児科，麻酔科とも連携して，細心の注意を払うべきである。

おわりに

重症のⅡ型/aggressive posterior ROPで網膜剝離が進行すれば，従来は失明に至ることを覚悟するしかなかった。しかし，早期硝子体手術で有形硝子体を十分に除去して増殖の足場を取り払えば，重症網膜症であっても進行が抑えられ，良好な予後が得られることが明らかになった。硝子体の牽引は血管新生を亢進すると言われているので，手術による牽引の減弱は血管新生の抑制に寄与するのかもしれない。糖尿病網膜症では，重篤な線維増殖と新生血管が存在する眼で，広範囲の光凝固が既に行われていれば早期硝子体手術が有効と言われているが[16]，これに類似している。重症未熟児網膜症の手術適応は今後大きく変わり，糖尿病網膜症と同じく，光凝固を十分に行って功を奏さなければ，早期に硝子体手術を行う時代になると考える。

付記：「日本眼科学会雑誌」，「日本の眼科」両編集委員会は，協議の結果，本稿の内容をすべての眼科医に周知させることが望ましいため，両誌に同一稿を掲載することとした。

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REGULAR ARTICLE

Yasuko Yamaguchi · Takashi Watanabe ·
Akito Hirakata · Tetsuo Hida

Localization and ontogeny of aquaporin-1 and -4 expression in iris and ciliary epithelial cells in rats

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Abstract The precise localization of aquaporin (AQP)1 and AQP4 was studied in iris and ciliary epithelial cells, in both mature and developing rats, to elucidate the molecular mechanisms underlying aqueous humor balance. Anterior segments of eyes dissected from embryonic day (E)13, E15, E18, and E20, postnatal day (P)0, P7, and P14, and postnatal week 8 rats were subjected to immunofluorescence analysis with AQP isoform-specific antibodies. In adult rat eye, AQP1 was localized to the apical and basolateral plasma membranes of iris epithelial cell layers and of anterior ciliary non-pigmented epithelial (NPE) cells. Conversely, AQP4 was localized to the basolateral plasma membrane of NPE cells in ciliary epithelium and the posterior iris. Developmentally, AQP1 was detected as early as E15 in immature iris and ciliary epithelial cells, and expression persisted throughout development up to adulthood. In contrast, AQP4 was first observed at P7 in the developing pars plicata, and the AQP4-positive area gradually spread to cover the entire pars plicata as development proceeded. These findings indicate that both AQP1 and AQP4 contribute to aqueous humor secretion in the rat eye, thereby maintaining proper intraocular pressure. Moreover, AQP appears to play a major role in aqueous humor secretion in early eye development. This study thus provides a basis for understanding the molecular mechanisms of aqueous humor secretion in pathological and physiological conditions.

Keywords Aquaporin · Iris epithelium · Ciliary epithelium · Aqueous humor · Fluid transport · Immunofluorescence analysis · Rat (Sprague Dawley)

Y. Yamaguchi (✉) · A. Hirakata · T. Hida
Department of Ophthalmology,
Kyorin University School of Medicine,
6-20-2 Shinkawa,
Mitaka, Tokyo, 181-8611, Japan
e-mail: yamaguchi@eye-center.org
Tel.: +81-422-475511

T. Watanabe
Laboratory Medicine,
Kyorin University School of Medicine,
6-20-2 Shinkawa,
Mitaka, Tokyo, 181-8611, Japan

Introduction

Aquaporins (AQPs) are molecular water channels localized in plasma membranes in animals, plants, and microorganisms (Chrispeels and Agre 1994; Calamita et al. 1995). In mammalian cells, 11 isoforms of AQPs (AQP0–AQP10) have been identified to date (Matsuzaki et al. 2002; Verkman 2003). AQPs are present in cells requiring either rapid bulk transport of fluid or transport of fluids against an insufficient osmotic pressure gap. AQPs allow efficient transport of fluid, thereby contributing to the maintenance of proper organ function (Stamer et al. 1994; Patil et al. 1997a; Hamann et al. 1998).

Of the 11 AQP isoforms, AQP0, AQP1, AQP3, AQP4, and AQP5 have been shown to be localized in mammalian eyes (Patil et al. 1997a; Hamann et al. 1998; Verkman 2003). AQP1 and AQP4 are localized in ciliary epithelial cells and appear to be involved in aqueous humor secretion (Patil et al. 1997a, 2001; Hamann et al. 1998; Zhang et al. 2002; Verkman 2003). Within the eye, the watery aqueous humor plays important roles in maintaining proper visual function. Aqueous humor generates a suitable intraocular pressure (IOP) and also conveys nutrients and metabolic waste to and from avascular intraocular tissues such as the lens (Caprioli 1992). Moreover, the aqueous humor appears essential in normal eye development (Reichman and Beebe 1992). Aqueous humor is also important ophthalmologically, as impairments in water dynamics can result in eye disorders such as glaucoma. The molecular

mechanisms involved in aqueous humor dynamics, however, remain largely unclear. The present study has therefore attempted to identify localization of AQP1 and AQP4 in iris and ciliary epithelial cells in rats by using immunocytochemical methods. Developmental expression of AQP1 and AQP4 in rat iris and ciliary epithelial cells has also been examined from embryonic to postnatal stages. This is the first precise analysis of AQP1 and AQP4 localization in iris and ciliary epithelial cells in both mature and developing rats.

Materials and methods

Animals

Timed-pregnant and postnatal Sprague-Dawley rats were obtained from Clea Japan (Tokyo, Japan). Birth usually occurred on embryonic day (E)22, which was considered as postnatal day (P)0. Samples taken on E13, E15, E18, and E20 from fetal rats, on P0, P7 and P14 from postnatal rats, and at postnatal week (PW)8 from adult female rats were

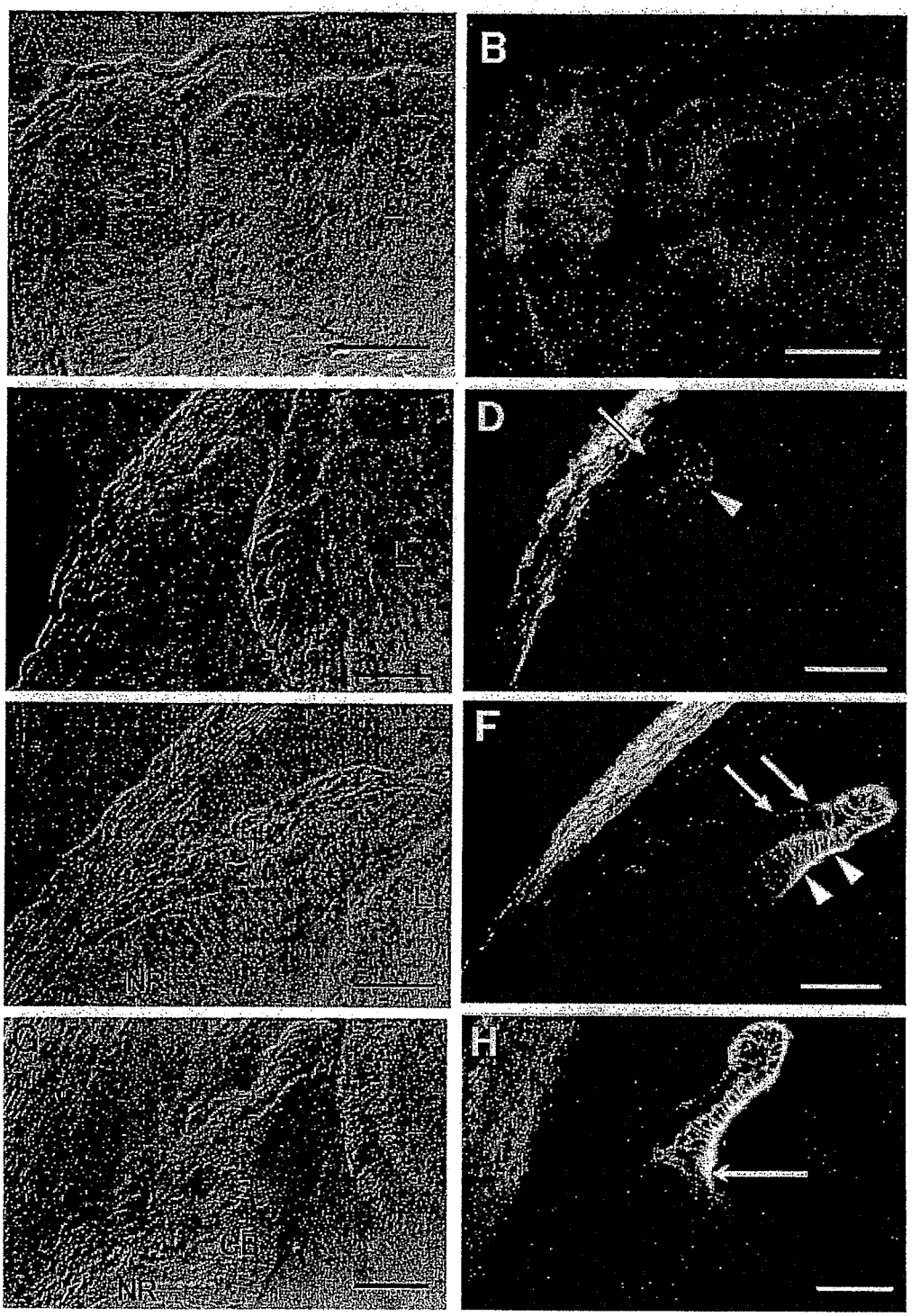

Fig. 1 AQP1 immunolocalization in iris and ciliary epithelial cells in embryonic rat eyes (*C* cornea, *L* lens, *CB* ciliary body, *NR* neural retina, *asterisks* inner plate, *stars* outer plate, *St* iris and ciliary stroma). Vertical sections through developing eyes dissected from E13, E15, E18, and E20 rats were immunostained with anti-AQP1 antibody followed by FITC-labeled swine anti-rabbit immunoglobulin and then photographed using Nomarski (**a, c, e, g**) and fluorescence (**b, d, f, h**) optics. At E13 (**a, b**), no definitive AQP1–IR was observed in either inner or outer plates of the optic cup. AQP1–IR first appeared at E15 (**c, d**). At E15 and E18 (**e, f**), AQP1–IR was confined to the anterior ends of the developing optic cup. Within this region, the optic cup inner plate (**d, f**, *arrowheads*) showed more intense AQP1–IR than the outer plate (**d, f**, *arrows*). The intensity of AQP1–IR increased dramatically as development proceeded from E15 (**d**) to E18 (**f**). At E20 (**g, h**), AQP1–IR was observed in the most anterior region of the ciliary body (**h**, *arrow*) in addition to the entire iris, and AQP1–IR intensity was significantly higher than at E15 or E18. In the cornea, AQP1–IR was first observed at E15 (**d**) and persisted throughout the embryonic stages (**f, h**). *Bars* 25 μm

subjected to immunohistochemical analysis. All animal care and tissue collection procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Preparation of eye specimens

Embryos (E13, E15, E18, and E20) were removed after pregnant Sprague-Dawley rats has been killed by cervical dislocation under deep anesthesia induced by diethyl ether. Eyes were immediately enucleated and immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 2 h at 4°C. Postnatal rats (P0 and P7) were sacrificed by decapitation. Eyes were immediately enucleated, and the anterior segments were dissected. Resultant eye tissues were immersion-fixed in a similar manner to embryonic eyes. For P14 and PW8 rats, animals were perfused transcardially with 0.01 M phosphate-buffered saline (PBS, pH 7.4) followed by ice-cold fixative containing 4% paraformaldehyde in PB under deep anesthesia induced by diethyl ether. Eyes were enucleated and processed in a similar manner to those from P0 and P7 rats. All tissue samples were subsequently cryoprotected by using a graded series of sucrose in PBS at 4°C, embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan), and frozen. Frozen sections (6 μm) were cut in an HM500 cryostat (Zeiss) and transferred onto 3–aminopropyltrethoxy–silane–coated slides. A hydrophobic ring was drawn around sections by using a PAP pen (Daido Sangyo, Tokyo, Japan). Sections were then air-dried for 1 h at room temperature.

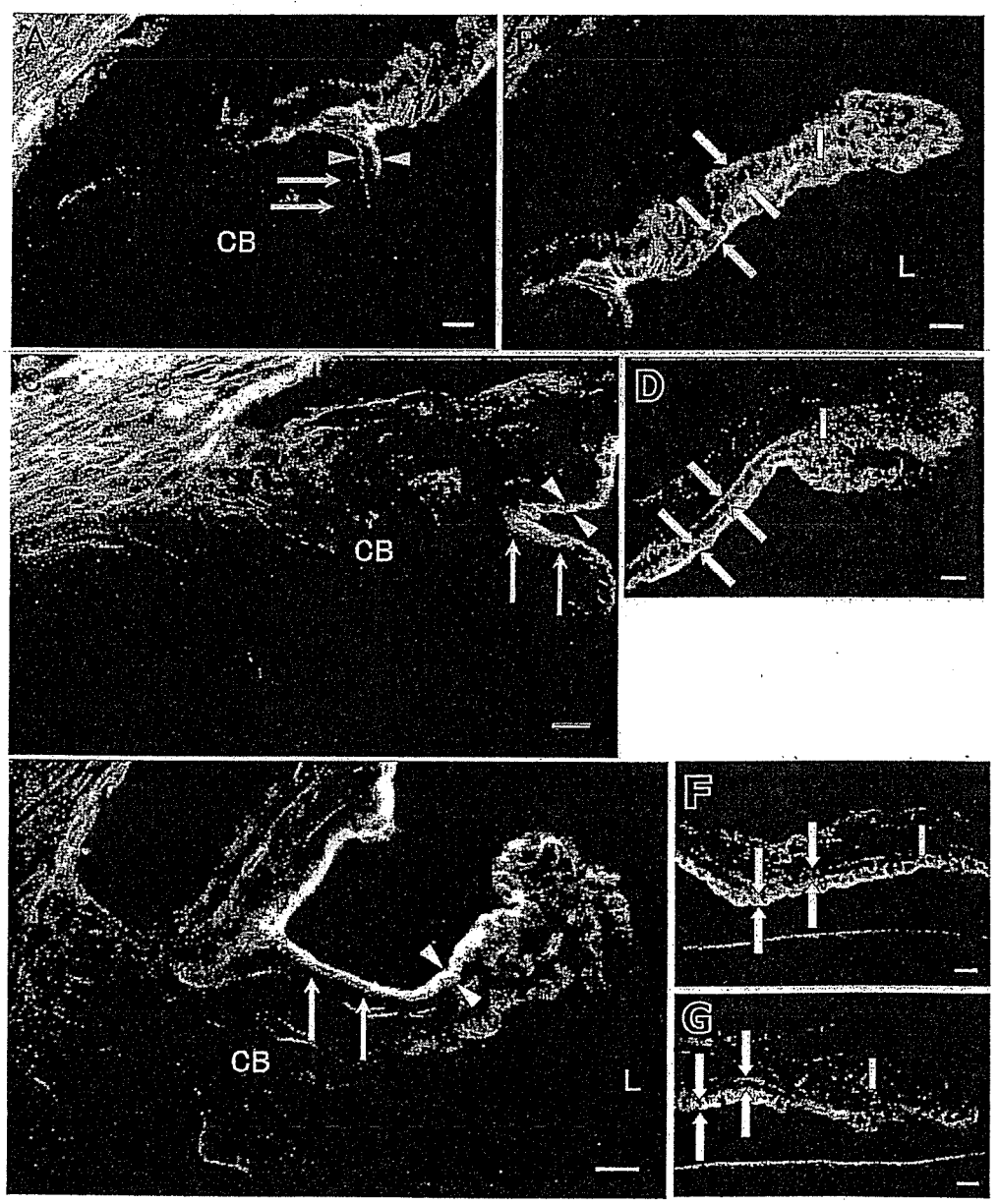

Fig. 2 AQP1 immunolocalization in iris and ciliary epithelial cells of postnatal rat eyes (*C* cornea, *CB* ciliary body, *I* iris, *L* lens). Vertical sections through anterior ocular segments dissected from P0, P7 and P14 rats were immunostained with anti-AQP1 antibody followed by FITC-labeled swine anti-rabbit immunoglobulin and then photographed under fluorescence optics. At P0 (**a**, **b**), both apical and basolateral plasma membranes of the inner plate cells corresponding to NPE cells in the anterior region of the ciliary body, displayed AQP1–IR (**a**, *arrowheads*), whereas outer plate cells corresponding to PE cells were AQP1-negative (**a**, *arrows*). In both layers of iris epithelial cells, both apical and basolateral plasma membranes were stained (**b**, *arrows*). At P7 (**c**, **d**) and P14 (**e**–**g**), the AQP1–IR pattern in the ciliary and iris epithelium was similar to that at P0 (**c**, **e**: *arrowheads* NPE, *arrows* PE; **d**, **g**: *arrows* anterior region of iris; **f**: *arrows* medial region of iris). Cornea was AQP1-positive throughout the postnatal stages (**c**). *Bars* 10 μm

Antibodies

Rabbit anti-rat AQP1 and AQP4 antibodies (Chemicon, Temecula, Calif.) were used. The specificities of these antibodies have been extensively examined by Western blot analysis in rodents (AQP1: Ward et al. 2001; Frigeri et al. 2004, AQP4: Yamamoto et al. 2001; Dalloz et al. 2003).

Immunofluorescence staining

For immunofluorescence staining, sections were rinsed in PBS (3×5 min each), treated with 10% normal swine serum in PBS for 10 min, and then incubated overnight at 4°C with either anti-AQP1 antibody (diluted 1:100) or anti-AQP4 antibody (diluted 1:20). All sections were rinsed in PBS (3×5 min each) and then incubated in swine anti-rabbit immunoglobulin coupled to fluorescein isothiocyanate (FITC; diluted 1:100; DakoCytomation, Glostrup, Denmark) for 1 h at room temperature. The above antibodies were all diluted in PBS containing 4% fetal calf serum, 0.1% sodium azide, and 0.1% Triton X–100. Sections were again rinsed in PBS (3×5 min each), mounted in glycerol containing 0.1% paraphenylenediamine to prevent bleaching, and examined with an Axioplan fluorescence microscope (Zeiss). Photomicrographs were taken by using 400 ASA Tri-X film (Kodak, Rochester, N.Y.).

To test the specificity of immunoreactivity (IR), control sections were processed in the same manner as described above, except that anti-AQP1 and anti-AQP4 antibodies were replaced with primary antibodies pre-adsorbed with either AQP1 or AQP4 oligopeptide (50 μg/ml diluted antibody; Chemicon), respectively, at 4°C for 4 h. These

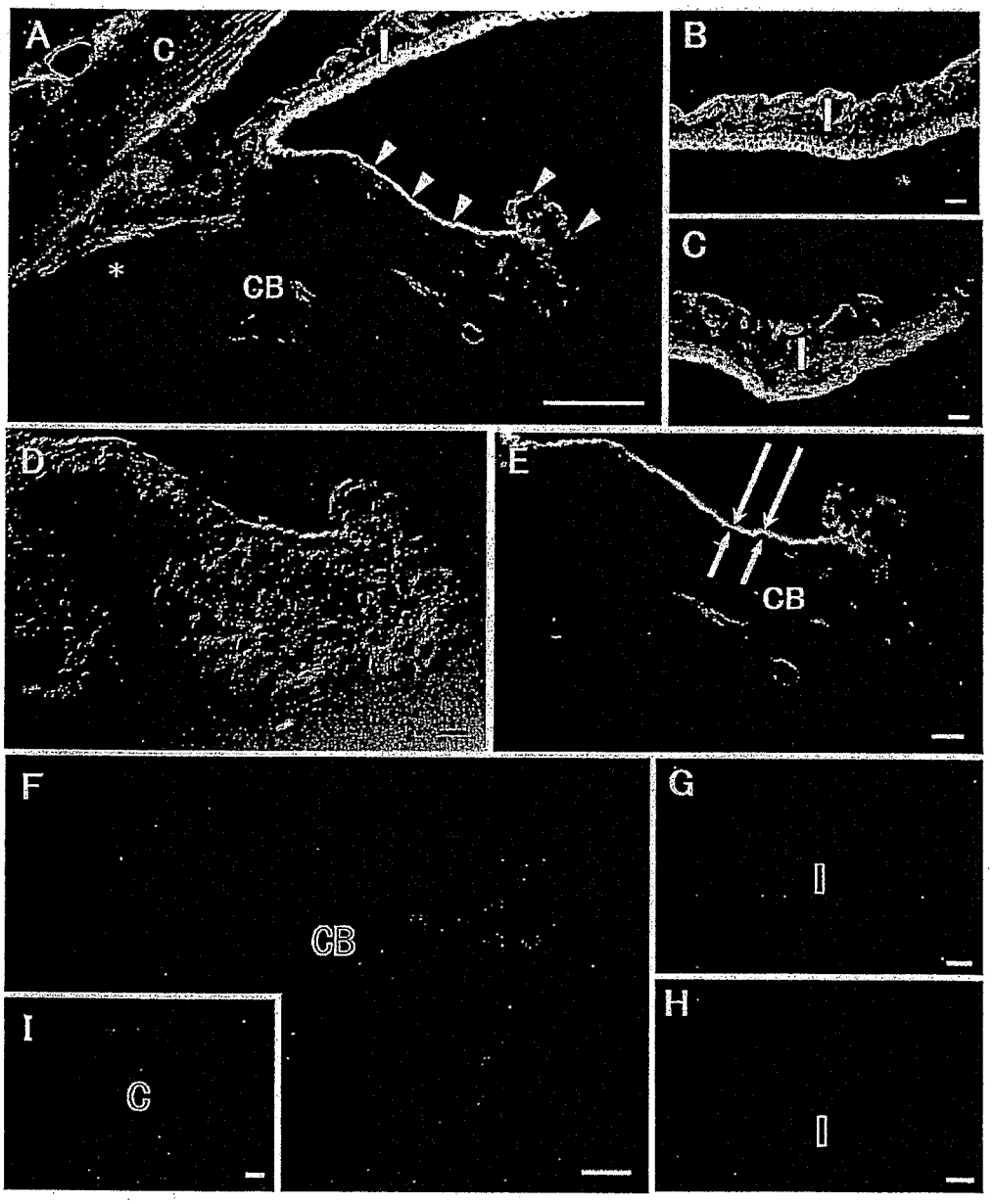

Fig. 3 AQP1 immunolocalization in iris and ciliary epithelial cells of PW8 rat eyes (*C* cornea, *CB* ciliary body, *I* iris, *asterisk* pars plana). Vertical sections through anterior ocular segments dissected from PW8 rats were immunostained with anti-AQP1 antibody followed by FITC-labeled swine anti-rabbit immunoglobulin and then photographed under Nomarski (**d**) and fluorescence (**a–c**, **e**) optics. AQP1–IR for epithelial cells in the iris (**a** posterior region, **b** medial region, **c** anterior region) and ciliary body (**a**, **d**, **e**) basically resembled that seen during developmental stages. In iris epithelial cells, both layers were AQP1-positive. In ciliary epithelial cells, only NPE cells localized anterior to the pars plicata were stained (**a**, *arrowheads*). AQP1–IR intensity was higher on the basolateral side (**e**, *long arrows*) than on the apical side (**e**, *small arrows*). In the remaining pars plicata and pars plana (**a**, *asterisk*), neither NPE cells nor PE cells were stained. Cornea was also AQP1-positive (**a**). **f–i** Pre-adsorption of anti-AQP1 antibody with antigenic peptide. PW8 rat anterior ocular segments were immunostained with anti-AQP1 antibody pre-adsorbed with AQP1-specific antigenic peptide. In ciliary (**f**) and iris (posterior region is not shown; **g** medial region, **h** anterior region) epithelium and in cornea (**i**), the AQP1–IR observed in **a–c** was completely abolished. *Bars* 10 μm

oligopeptides were equivalent to those used as immunogens to raise anti-AQP1 and anti-AQP4 antibodies.

Results

Prenatal AQP1 immunolocalization in iris and ciliary epithelial cells

At E13, no definitive AQP1–IR was observed in either the inner or outer plates of the optic cup (Fig. 1a,b). AQP1–IR first appeared at E15. At this stage, AQP–IR was confined to the anterior margin of the optic cup (Fig. 1c,d), where the inner plate of the optic cup showed more intense AQP1–IR than the outer plate, despite the outer and inner plates representing continuous tissues. In this region, AQP1–IR was observed all around the cell membrane. At E18, the anterior tip of the optic cup continued to grow along the lens anteriorly (Fig. 1e). At the same time, the iris and ciliary stroma began to form. The posterior part of the inner plate thickened markedly to form the neurosensory retina. AQP1–IR in the inner plate was more intense at E18 than at E15 (Fig. 1f). AQP1–IR in the outer plate also became more intense than at E15. However, AQP1–IR at E18 was again much more intense in the inner plate than in the outer plate. In the inner plate, although both apical and basolateral plasma membranes were stained, AQP1–IR intensity was higher on the basolateral side. At E20, when the iris became distinguishable from the ciliary body, AQP1–IR was observed in the most anterior region of the

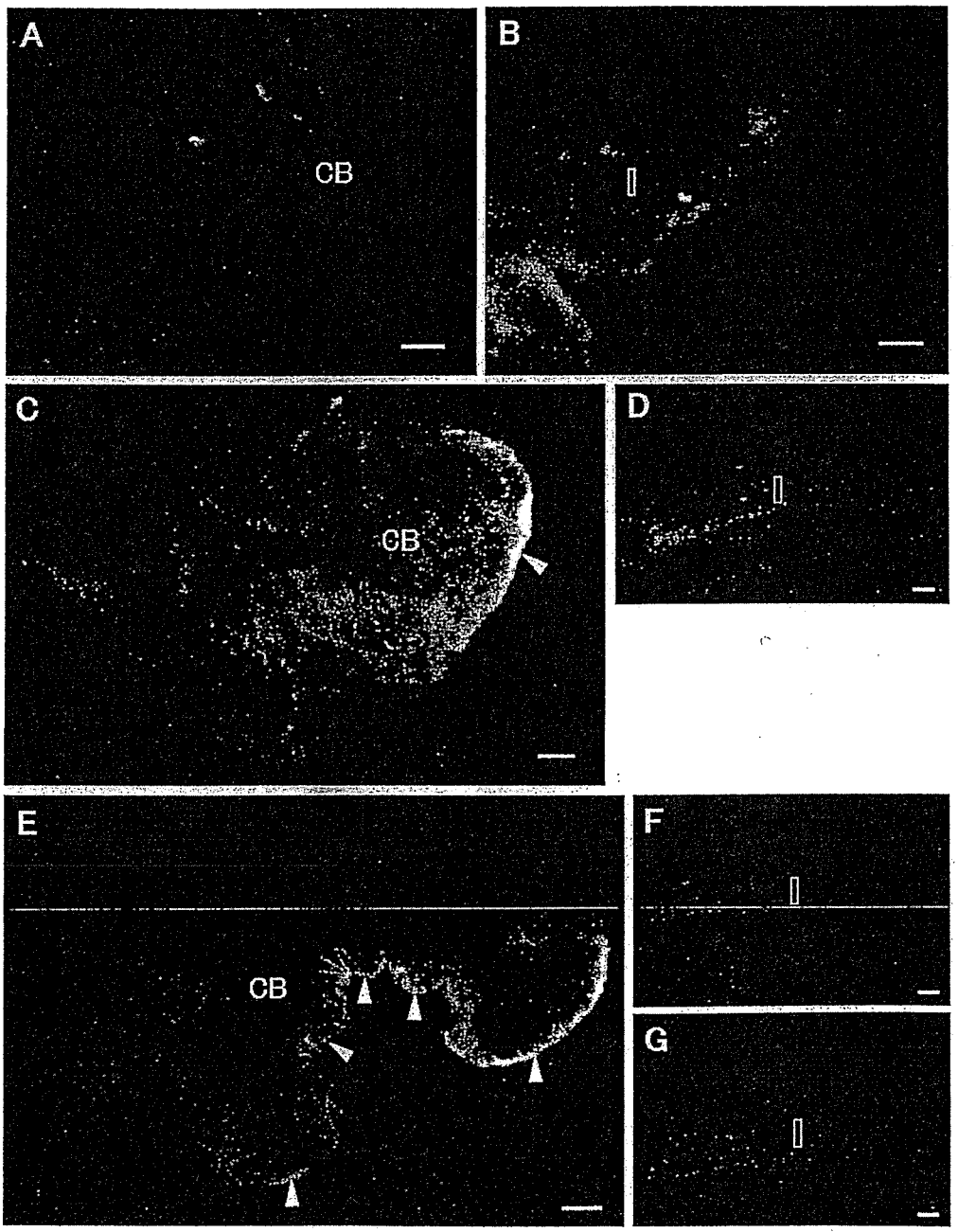

Fig. 4 AQP4 immunolocalization in iris and ciliary epithelial cells of postnatal rat eyes (*CB* ciliary body, *I* iris). Vertical sections through anterior ocular segments dissected from P0 (**a**, **b**), P7 (**c**, **d**), and P14 (**e**–**g**) rats were immunostained with anti-AQP4 antibody followed by FITC-labeled swine anti-rabbit immunoglobulin and then photographed under fluorescence optics. Little AQP4–IR was present in the ciliary body (**a**) or iris (**b**) at P0. Weak AQP4–IR was first observed on NPE cells of the immature pars plicata (**c**, *arrowhead*) at P7. AQP4–IR was more prominent at P14 than at P7 (**e**, *arrowheads*). At both P7 and P14, both iris epithelial layers (**d**, **g** anterior region; **f** medial region) were AQP4-negative. *Bars* 10 μm

ciliary body, in addition to the entire iris (Fig. 1g,h). At this stage, AQP1–IR intensity was significantly higher than at E15 or E18. AQP1–IR in the cornea was first seen at E15 and was observed throughout the embryonic stages (Fig. 1d,f,h).

Postnatal AQP1 immunolocalization in iris and ciliary epithelial cells

The iris and ciliary body remained structurally immature at birth (Fig. 2a). At P0, fold formation in the ciliary body remained incomplete. However, the ciliary body was more readily distinguishable from the iris at this stage than in the embryonic stages. In the anterior region of the ciliary body, both apical and basolateral plasma membranes of the inner plate cells corresponding to non-pigmented epithelial (NPE) cells, displayed AQP1-IR, whereas outer plate cells corresponding to pigmented epithelial (PE) cells showed no AQP1–IR (Fig. 2a). In both layers of the iris epithelial cells that were continuous with ciliary epithelial cells, both apical and basolateral plasma membranes were stained, but AQP1–IR intensity was lower in the anterior part of iris epithelium than in the posterior part (Fig. 2b). As development proceeded, the ciliary body formed more complex folds (Fig. 2c,e), and the iris extended at P7 and P14 (Fig. 2d,f,g). Patterns of AQP1–IR in the ciliary (Fig. 2c,e) and iris (Fig. 2d,f,g) epithelium at P7 and P14 basically resembled that at P0. AQP1–IR in the cornea was also observed throughout the postnatal stages (Fig. 2c).

At PW8, when development of the eyeball had been completed, AQP1–IR for epithelial cells in the iris and ciliary body was basically equivalent to that seen during the developmental stages. In iris epithelial cells, both layers were AQP1-positive (Fig. 3a–c). However, AQP1–IR was more intense in posterior epithelium compared to anterior epithelium. In ciliary epithelial cells, only NPE cells localized anterior to the pars plicata were stained (Fig. 3a). The intensity of AQP1–IR was higher on the basolateral side than on the apical side, again resembling observations made during development (Fig. 3d,e). In the remaining pars plicata and pars plana, neither NPE cells nor PE cells were stained (Fig. 3a). AQP1–IR was also observed in the cornea (Fig. 3a).

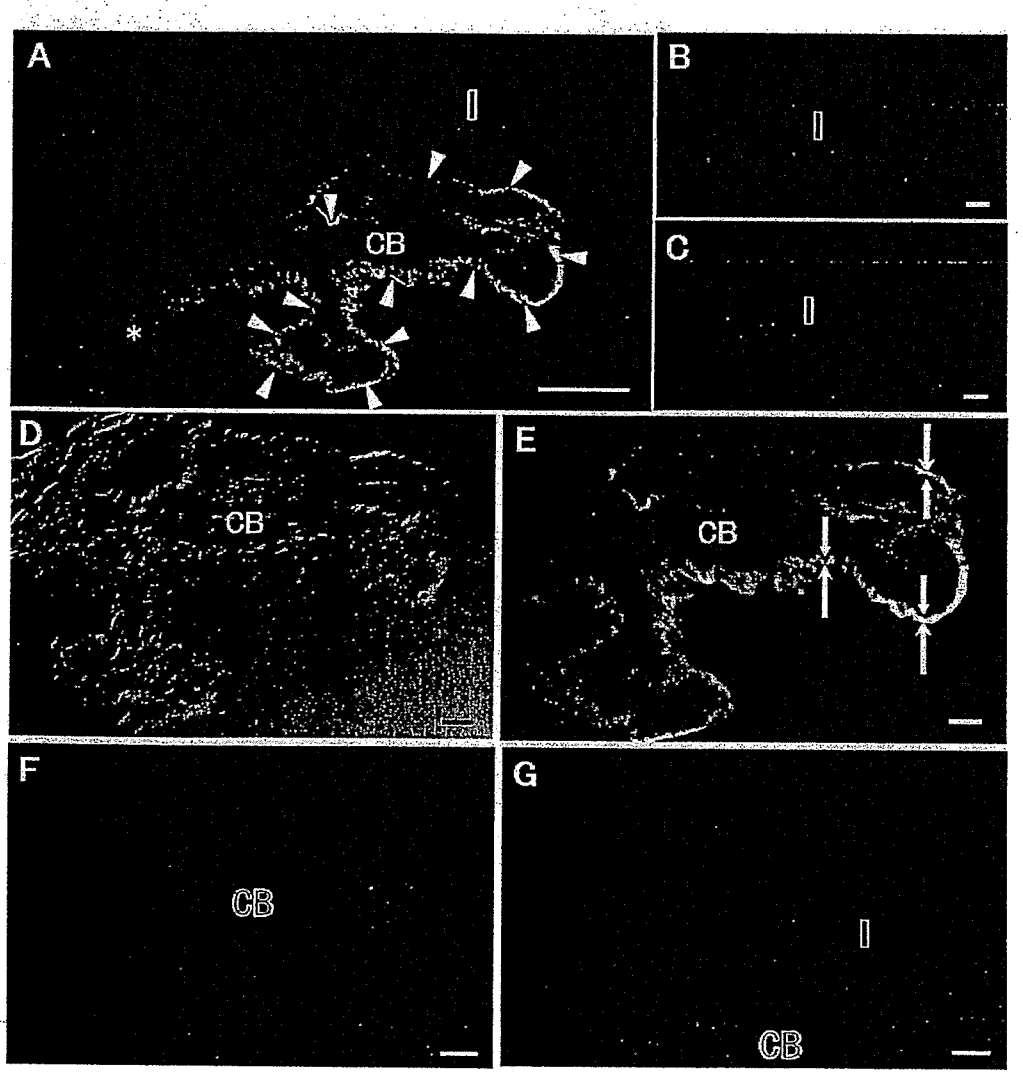

Fig. 5 AQP4 immunolocalization in iris and ciliary epithelial cells of PW8 rat eyes (*CB* ciliary body, *I* iris, *asterisk* pars plana). Vertical sections through anterior ocular segments dissected from PW8 rats were immunostained with anti-AQP4 antibody followed by FITC-labeled swine anti-rabbit immunoglobulin and then photographed under Nomarski (**d**) and fluorescence (**a–c, e**) optics. AQP4–IR was observed in the entire pars plicata region of the ciliary body (**a**, *arrowheads*). In this region, only NPE cells were AQP4-positive, not PE cells. AQP4-IR was observed only on the basolateral plasma membrane (**e**, *large arrows*), and the apical side was AQP4-negative (**e**, *small arrows*). In contrast, the pars plana region of the ciliary body did not show any significant AQP4–IR (**a**, *asterisk*). In the iris (**a**, posterior region; **b**, medial region; **c**, anterior region), only the posterior region continuous with NPE cells displayed weak AQP4–IR (**a**). **f–g** PW8 rat anterior ocular segment stained with anti-AQP4 antibody preadsorbed with antigenic peptide. Note that the AQP4–IR in ciliary and iris epithelium seen in **a** was completely abolished (**f**, ciliary body; **g**, posterior region of iris). *Bars* 10 μm

Pre- and postnatal AQP4 immunolocalization in iris and ciliary epithelial cells

No significant AQP4–IR was noted in the iris or ciliary body through perinatal stages up to P0 (Fig. 4a,b; prenatal period not shown). At P7, faint AQP4–IR was first observed on NPE cells of the immature pars plicata (Fig. 4c). By P14, AQP4–IR was more intense and extensive, and AQP4–IR in NPE cells in the pars plicata was unambiguously observed (Fig. 4e). At both P7 and P14, the two layers of iris epithelial cells were unstained (Fig. 4d,f,g).

At PW8, AQP4–IR was observed throughout the pars plicata. AQP4–IR in PE cells was negligible, and only NPE cells were stained (Fig. 5a). AQP4–IR in NPE cells was observed only in the basolateral plasma membrane and not on the apical side (Fig. 5d,e) or in the pars plana (Fig. 5a). In the iris (Fig. 5a–c), only the iris posterior region continuous with NPE cells displayed weak AQP4–IR (Fig. 5a).

Pre-adsorption of anti-AQP1 or anti-AQP4 antibody with antigenic peptide in PW8 rat eyes

When the primary antibodies against AQP1 and AQP4 were pre-adsorbed with the corresponding immunogenic peptides and then applied to PW8 anterior eye samples, all AQP1–IR and AQP4–IR observed at the ciliary body, iris, and cornea was completely abolished (AQP1, Fig. 3f–i; AQP4, Fig. 5f,g). Similarly, when E18 anterior eye samples were incubated with anti-AQP1 antibody pre-adsorbed with immunogenic AQP1 peptide, AQP1–IR both at the anterior tip of the optic cup and cornea was completely abolished (not shown).

Discussion

The present immunohistochemical study has revealed that iris and ciliary epithelial cells in the adult rat eye express both AQP1 and AQP4 with characteristic distribution patterns. Whereas AQP1–IR is localized to the entire iris and anterior ciliary body, AQP4–IR is localized solely to the ciliary body. On the basis of these AQP1 and AQP4 expression patterns, iris and ciliary epithelial cells can be divided into three regions (Fig. 6): region 1, including the major part of the iris, and expressing only AQP1; region 2, including the posterior iris and anterior pars plicata, expressing both AQP1 and AQP4; and region 3, the medial and posterior pars plicata, expressing AQP4 alone. These expression patterns may offer some insights into the mechanisms controlling intraocular fluid transport. First of all, the present study has confirmed and further extended the findings of previous investigations that AQP is present on iris epithelial cells (Nielsen et al. 1993; Hamann et al. 1998). The results of our study support the idea that iris epithelial cells, in addition to ciliary epithelial cells, are

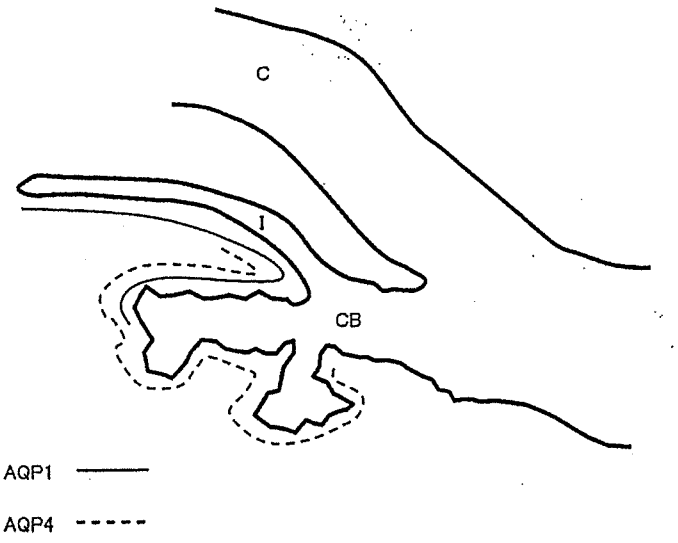

Fig. 6 Representation of expression patterns for AQP1 (*solid line*) and AQP4 (*dotted line*) in iris and ciliary epithelial cells in PW8 rat eyes. Iris and ciliary epithelia are divided into three regions depending on AQP1 and AQP4 expression patterns. Region 1 includes the major part of the iris and expresses only AQP1. Region 2 includes the posterior region of the iris and anterior pars plicata and expresses both AQP1 and AQP4. Region 3 includes the medial and posterior pars plicata and expresses AQP4 alone (*C* cornea, *CB* ciliary body, *I* iris)

involved in the secretion of the aqueous humor (Green and Pederson 1973). Since the precise role of the iris in regulating aqueous humor volume remains unclear, the present findings are expected to provide clues for further analysis of this issue. Secondly, the characteristic AQP1 and AQP4 expression patterns raise the possibility that ciliary epithelial cells in regions 2 and 3 differ with respect to their ability to secrete aqueous humor, although ciliary epithelial cells in these two regions appear morphologically indistinguishable. Studies have yet to determine the way that each of these two regions contributes to aqueous humor secretion within the ciliary body, in which aqueous humor is most actively secreted. Thirdly, no significant AQP1 or AQP4 expression has been detected in the pars plana in this study, consistent with the localization of Na–K–ATPase and Na–K–Cl–cotransporter, both of which are considered to be closely associated with aqueous humor secretion. Both Na–K–ATPase and Na–K–Cl–cotransporter are reportedly more abundant in the pars plicata than in the pars plana (Ghosh et al. 1991; Dunn et al. 2001). The present study thus provides a molecular basis for aqueous humor secretion through an analysis of AQP distribution. Fourthly, the localization of AQP1 and AQP4 also differs significantly at the cellular level. Our results for AQP1 expression on both the apical and basolateral plasma membranes of both layers of iris epithelial cells and ciliary NPE cells confirm the findings of Hamann et al. (1998). Recent studies have shown that cultured NPE cells actively transport liquid in an apical–to–basolateral direction in the absence of PE cells (Patil et al. 2001). AQP1 on both apical and basolateral plasma membranes may play important

roles in transporting water within ciliary NPE cells. In contrast, the present findings on AQP4 expression differ from those of previous studies (Hamann et al. 1998), in which AQP4 reactivity has been demonstrated on both the apical and basolateral plasma membranes of NPE cells. Although the reasons for this discrepancy remain unclear, variable expression of AQP isoforms as described above may contribute to the precise volume regulation of aqueous humor. AQP1–IR has also been found in the cornea from E15 through to adulthood. The observation of AQP1–IR at PW8 in the present study is consistent with that reported previously (Hamann et al. 1998).

The essential role of AQP molecules in regulating aqueous humor balance has been demonstrated by the generation of mice lacking AQP1 and/or AQP4 (Zhang et al. 2002). Significant decreases in IOP have been seen in these mice. Interestingly, aqueous humor secretion is significantly decreased in mice lacking AQP1 alone and also in mice lacking both AQP1 and AQP4, indicating a major role for AQP1 in fluid transport in the murine eye (Zhang et al. 2002). In contrast, humans with complete hereditary deficiency of AQP1 show no significant IOP abnormalities and instead display only a disorder in maximal urinary concentrating ability (King et al. 2001). This may be attributable to the presence of various compensatory mechanisms among AQP isoforms, such as functional redundancy, at least in the eye. Species differences may also warrant consideration.

AQP molecules in iris and ciliary epithelial cells thus appear to play important roles in regulating aqueous humor turnover and thereby in regulating IOP. In future, the manipulation of aqueous humor secretion may be achievable by regulating the kinetics of AQP molecules by using factors regulating AQP1 and AQP4 activity (Patil et al. 1997b; Han et al. 1998; Han and Patil 2000). The present study may represent a starting point for designing novel therapies to treat disorders caused by impaired IOP regulation, such as glaucoma.

The present study also examined the expression of AQP1 and AQP4 in developing rats. AQP1–IR was detected as early as E15, whereas AQP4–IR was first observed at P7. AQP4–IR density increased with development, possibly reflecting increases in the requirement of aqueous humor secretion in the area. Taken together, developing iris and ciliary epithelium expressed only AQP1 during the embryonic and early postnatal stages, but expressed both AQP1 and AQP4 after birth. AQP1 is known to be abundant in the choroid plexus throughout fetal development in the rat, indicating the presence of AQP1-mediated water transport in cerebrospinal fluid secretion during embryonic stages (Bondy et al. 1993). Similarly, AQP1 in the eye appears to play an important role in water transport during the prenatal period. Interestingly, when fold formation of the ciliary body is still immature during embryogenesis, AQP1 appears to be more abundantly expressed in the iris than in the ciliary epithelial primordium. This pattern contrasts with that in the adult eye, where the ciliary body is the dominant site of AQP expression. These results indicate that the iris epithelium is more active in secreting aqueous humor compared with ciliary epithelium during embryonic life. Indeed, one previous study has suggested that AQP1 expression in iris epithelium is related to the regulation of aqueous humor volume (Hasegawa et al. 1994).

IOP in the early prenatal stage has been considered to be maintained primarily by the growing vitreous body (Beebe 1986). If this is really the case, active fluid transport by the ciliary epithelium may play little if any role in this regard (Beebe 1986). The present results, however, suggest that AQP1 found in the inner plate is involved in fluid secretion, even in the prenatal period. Indeed, in chicks, early secretion of aqueous humor from the embryonic stages has been demonstrated (Latker and Beebe 1984; Linser and Plunkett 1989; Reichman and Beebe 1992). Secretion of aqueous humor during the embryonic stages is further supported by experiments on mouse embryos in which the administration of carbonic anhydrase inhibitors suppressed aqueous humor secretion and caused microphthalmia (Scott et al. 1984). The presence of functional tight junctions in rat ciliary epithelium as early as E18 also supports its involvement in secretion (Arguillere et al. 1986). The findings of the present study thus provide a molecular basis for aqueous humor secretion from the iris and ciliary epithelial primordium during the prenatal period.

In conclusion, the study reported here has confirmed that AQP molecules play important roles in the regulation of aqueous humor secretion in rats, in both mature and developing eyes. These results provide a basis for understanding the molecular mechanisms underlying the regulation of normal intraocular fluid balances and offer insights into the pathophysiology of disorders involving the impairment of intraocular fluid balance.

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CLINICAL INVESTIGATION

Vitrectomy for Myopic Posterior Retinoschisis or Foveal Detachment

Akito Hirakata and Tetsuo Hida

Kyorin Eye Center, Kyorin University School of Medicine, Mitaka, Tokyo, Japan

Abstract

Purpose: To evaluate the efficacy of vitrectomy for posterior retinoschisis (RS) or foveal detachment (FD) associated with posterior staphyloma in myopic eyes.

Methods: We reviewed the records of 14 consecutive patients (53–77 years of age; 16 eyes) with progressive visual impairment as a result of myopic RS or FD. Optical coherence tomography demonstrated the presence of a variety of RS and FD characteristics. Five eyes had RS alone, and 11 eyes had RS and FD. Two eyes with RS and severe FD developed retinal detachment in conjunction with a tiny macular hole. Vitrectomy, including posterior vitreous separation in all eyes and internal limiting membrane (ILM) peeling in six eyes, had been performed. The patients were followed postoperatively for 6 to 66 months (mean, 24 months). The anatomical outcome and visual acuity were retrospectively analyzed in this study.

Results: Although the two eyes with RS and severe FD developed retinal detachment with a macular hole after an initial vitrectomy, final retinal reattachment was achieved in all 16 eyes. Visual acuity improved in nine eyes and remained unchanged in seven eyes.

Conclusions: Vitrectomy with posterior vitreous separation is effective for reattaching the macula and preventing a deterioration of vision, although eyes with RS and severe FD may be at risk for the development of a macular hole after the initial vitrectomy. **Jpn J Ophthalmol** 2006;50:53–61 © Japanese Ophthalmological Society 2006

Key Words: high myopia, macular detachment, optical coherence tomography, retinoschisis, vitrectomy

Introduction

Posterior retinal detachment associated with a macular hole is a well-known complication in highly myopic eyes with posterior staphyloma. However, localized shallow posterior retinal detachment inside posterior staphyloma can also exist in the absence of a macular hole. In 1958, Phillips noted that localized posterior retinal detachment over posterior staphyloma might occur in the absence of a retinal hole.[1] Using optical coherence tomography (OCT), Takano and Kishi[2] reported that foveal retinoschisis or foveal retinal detachment occurs frequently in severely myopic eyes with posterior staphyloma, even in the absence of a macular hole. They suggested that retinal detachment may precede the formation of a macular hole in highly myopic eyes. However, the pathological mechanism responsible for posterior retinoschisis and the process of macular hole development are not well understood.

Recently, several authors have reported that vitrectomy, internal limiting membrane (ILM) peeling, and gas tamponade are useful for the treatment of foveal retinoschisis in highly myopic eyes.[3–7] Kobayashi and Kishi[4] also suggested that vitreous surgery might be indicated as a prophylactic treatment in highly myopic eyes at high risk for macular hole development. However, myopic foveal retinoschisis exhibits a variety of profiles,[8,9] and the accumulated data on surgical cases remain insufficient to prove

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Correspondence and reprint requests to: Akito Hirakata, Department of Ophthalmology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
e-mail: hirakata@eye-center.org

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whether vitrectomy is effective for the alleviation of this condition.

The purpose of the present study was to examine the clinical outcomes of vitrectomies performed in the Kyorin University Hospital between September 1998 and February 2004 in 14 consecutive patients (16 eyes) with localized shallow posterior retinal detachment inside a posterior staphyloma who exhibited signs of progressive visual impairment. Different clinical terms have been used to describe this pathological condition in previous reports, including foveal retinoschisis,[2] foveal detachment and retinoschisis,[2,4] macular retinoschisis,[5,9] shallow detachment of the macula,[1] and foveal retinal detachment without a macular hole.[8] In this paper, we use the terms posterior retinoschisis (RS) and foveal detachment (FD) to describe this pathological condition.

Patients and Methods

Fourteen consecutive patients (16 eyes) with myopia and posterior staphyloma who exhibited progressive visual impairment as a result of RS or FD were included in this study. Their records were retrospectively reviewed. Informed consent had been obtained from all study patients. The fundus of each patient had been pre- and postoperatively examined by indirect ophthalmoscopy and slit-lamp biomicroscopy using a Goldmann contact lens and a 90-diopter noncontact fundus examination lens (SuperField Lens). The best-corrected visual acuity (BCVA) was recorded. An OCT system (Zeiss-Humphrey, San Leandro, CA, USA) had been used to observe the posterior retinal changes. In this study the anatomical outcome and BCVA were retrospectively analyzed for all eyes.

Surgery had been initiated once the patient's vision had begun to deteriorate and the macular detachment had persisted or progressed for 3 months or longer. Two eyes (cases 13, 14) with RS and severe FD developed retinal detachment with a tiny macular hole before the vitrectomy. All the operations were performed by the same surgeon (AH) between September 1998 and February 2004. Vitrectomy, including posterior vitreous cortex removal from the retinal surface, was performed in all eyes, and ILM peeling was performed in six eyes. After core vitrectomy, complete removal of the posterior vitreous cortex (typically appearing as a thin membrane) from the posterior retinal surface was initiated by cutting with a microvitreoretinal knife (20-gauge) or a diamond-dusted membrane scraper.[10] A viscodissection technique[11] was used to advance the posterior hyaloid separation gently over the areas of retinoschisis in the posterior staphyloma. Triamcinolone acetonide (TA) was used intraoperatively in five cases to highlight the posterior hyaloid membrane.[12] ILM peeling over the posterior pole was regarded as indicated in eyes in which the presence of posterior hyaloid separation or induction over the macula was uncertain during the vitreous surgery, and was performed after indocyanine green (ICG) staining (5 mg/ml) in six eyes. In 12 eyes, fluid–air exchange was carried out without drainage of the subretinal fluid, followed by gas tamponade with either 20% sulfur hexafluoride (SF_6) or 14% perfluoropropane (C_3F_8). The patients were placed in a facedown position postoperatively. In two eyes [patients 5 (right eye, R) and 10], silicone oil tamponade was used because of poor vision in the opposite eye.

Twelve eyes were phakic before surgery, and four eyes had received phacoemulsification with intraocular lens implantation for the treatment of senile cataracts. Among the 12 phakic eyes, phacoemulsification with intraocular lens implantation was performed simultaneously with the vitrectomy in two eyes, and after the vitrectomy in six eyes.

The patients were followed for 6 to 66 months (mean, 24 months) after surgery.

Results

Case Reports

Patient 1

A 59-year-old man presented with a history of several months of metamorphopsia in his right eye. He also had a

Figure 1A–C. Fundus photograph and optical coherence tomography (OCT) images of the right eye of patient 1. **A** A photograph taken at presentation in an eye with a best-corrected visual acuity (BCVA) of 0.5 and metamorphopsia shows a shallow macular detachment over the posterior staphyloma. **B** An OCT examination confirmed the foveal detachment and posterior retinoschisis (scan length, 5.0 mm). **C** About 4 years after the operation, the BCVA had improved to 0.7. An OCT image shows complete reattachment (scan length, 5.0 mm).

Figure 2A–D. Fundus photograph and OCT images of the right eye of patient 2. **A** During a routine follow-up for macular hole retinal detachment in the left eye, the BCVA of the right eye was found to have decreased from 0.8 to 0.5. The fundus photograph shows a shallow macular detachment. **B** A preoperative OCT image shows a shallow elevation of the macula without splitting of the fovea, creating the appearance of a lamellar hole (scan length, 5.0 mm). **C** Two months after the vitrectomy, an OCT image shows a marked resolution of the retinoschisis (scan length, 3.5 mm). **D** At 4 years postoperatively, the retina had completely reattached (scan length, 10.0 mm).

Figure 3A–C. Fundus photograph and OCT images of the right eye of patient 11. **A** A preoperative fundus photograph shows a shallow retinal elevation over a posterior staphyloma in an eye with a BCVA of 0.4. **B** A preoperative OCT image shows an inner layer separation that appears to be connected to the conus of the optic disc (1), as well as to a large outer layer detachment at the macula (2). A partial posterior hyaloid separation surrounding the posterior retinoschisis is visible (scan length, 10.0 mm). **C** At 6 months after the vitrectomy, an OCT image shows a marked improvement of the retinal detachment (scan length, 10.0 mm).

Figure 4A–C. Fundus photograph and OCT images of the left eye of patient 5. **A** A preoperative fundus photograph shows a shallow retinal elevation over a posterior staphyloma. The BCVA of the eye was 0.06. **B** An OCT image shows a posterior retinoschisis over the posterior staphyloma, with a partial separation of the posterior hyaloid. Outer layer detachment is not visible (scan length, 10.0 mm). **C** Two weeks after the vitrectomy, the retina had completely reattached (scan length, 10.0 mm).