Chapter 9

Future of IOP-Lowering Medication for Glaucoma Therapy

9

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Core Messages

- Glaucoma therapies utilizing compounds that alter the actin cytoskeleton are very promising for the future. They act via a mechanism not targeted by current glaucoma therapies to enhance aqueous humor outflow via the trabecular meshwork (TM).
- Other classes of compounds that are promising for development as topical drop therapies and may act by enhancing aqueous outflow via the trabecular route include steroid antagonists, and adenosine agonists and antagonists.
- Gene therapy targeting the TM will eventually be used to express proteins or peptides that can alter the actin cytoskeleton and interactions of cells with the extracellular matrix.
- Topical drop therapy to enhance outflow via the uveoscleral route will be accomplished by new prostaglandin (PG) analogs targeting the EP receptor subtypes in addition to the FP receptor subtype.
- Other classes of compounds that may be developed as topical drop therapies and affect uveoscleral outflow as well as other aqueous dynamics pathways include serotonin agonists and nitrovasodilators. Gene therapy to enhance uveoscleral outflow will include increased local production of PGs, extracellular matrix degrading enzymes, and ciliary muscle (CM) relaxants.
- The long-term use of inflow suppressants may eventually decline due to the unfavorable effects on outflow pathways which could eventually lead to outflow obstruction.
- New classes of compounds that may be developed as topical drop therapies to suppress aqueous humor formation include opioids and cannabinoids.
- Prolonging contact with the cornea for enhancing drug delivery may be accomplished by entrapment and encapsulation of the drug in liposomes, niosomes, nanoparticles, microparticles and contact lenses, and incorporation of bioadhesives into the vehicle solutions, as well as combinations of these approaches.
- Continuous intraocular pressure (IOP) monitoring may soon be available via contact lenses fitted with strain gauges and transmitters.

Note: Due to the citation limitation, in most cases when multiple citations are available, only the most recent representative citation or review article is listed since other relevant citations may be found within it.

9.1 Introduction

One of the primary goals of glaucoma therapy is to control intraocular pressure (IOP). IOP reduction by just a few mmHg can have a significant effect on disease progression. The first line of therapy in lowering IOP is usually pharmacological in the form of topical eye drops. Over the course of time, most patients will use more than one medication, singly and in varying combinations, experimenting with differing classes of compounds with varying mechanisms of action.

The goal of the current chapter is to look at what lies ahead in the next 20 years for IOP-lowering drug therapy, rather than to dwell on the numerous possible formulations and combinations that can be made using currently available medications. Enhancing outflow and suppressing inflow will likely remain the general mechanisms of action targeted by future therapies as they have been in the past; however, since long-term use of drugs that reduce IOP by decreasing aqueous humor formation may have a negative effect on the eye [24], enhancing outflow may become the preferred therapeutic approach.

9.2 Outflow Enhancement

9.2.1 Trabecular Outflow

9.2.1.1 Basic Structure

The angle of the anterior chamber is bounded anteriorly by the corneal endothelium, and posteriorly by the root of the iris and ciliary body. At the apex of the angle lies the TM, suspended between Descemet's membrane and the anterior portion of the CM (Fig. 9.1). The TM commences just posterior to the point where Descemet's membrane terminates. This transition zone is identified gonioscopically as Schwalbe's line, but is less easily seen histologically. The TM continues posteriorly until it joins the scleral spur and CM. The inner portion of the meshwork (that closest to the anterior chamber) is called the uveal meshwork, and the outer portion closest to Schlemm's canal constitutes the corneoscleral meshwork, which is itself separated from the endothelial lining of Schlemm's canal by the juxtacanalicular tissue, or endothelial meshwork [30]. There is, however, no sharp dividing line between the portions. Some of the meridional CM fibers insert into the TM.

The TM harbors 60–80% of the resistance to aqueous outflow with the remainder residing in the CM, sclera, collector channels, and intrascleral aqueous veins. Current evidence suggests the juxtacanalicular or subendothelial region adjacent to Schlemm's canal is the primary location of resistance to aqueous humor drainage within the TM. Quantitative morphological studies revealed a significant increase in extracellular material in the subendothelial region of the meshwork adjacent to Schlemm's canal in glaucomatous eyes compared with age-matched normal controls. In eyes with primary open-angle glaucoma (POAG), material derived from (or adhering to) the thickened sheath of the elastic-like fibers predominates and presumably contributes to the increase in outflow resistance. Factors influencing the formation of sheath-derived plaques may also contribute to optic nerve fiber loss before or in conjunction with IOP elevation. Cell number diminishes at these sites in POAG and pigmentary glaucoma, but it is difficult to correlate cell loss with increased resistance to outflow [30].

Cytoskeletal and junctional proteins may be especially important in the maintenance and modification of outflow resistance. Agents that interfere with dynamics of the actin cytoskeleton (Fig. 9.2) alter the cell shape, contractility, and adhesion to neighboring cells, and to the extracellular matrix in culture, and decrease trabecular outflow resistance in the living monkey eye by expanding the areas available for fluid drainage (reviewed in [19]).

9.2.1.2 Myosin Light-Chain Kinase Inhibitors

Recent studies have revealed a number of novel agents that reduce outflow resistance in the living monkey or rabbit eye and/or the enucleated porcine and human eye, probably by cytoskeleton-related mechanisms [2, 19]. With some agents, the lowered resistance is accompanied by, and perhaps caused by, changes in cellular contractility in the TM (e.g., cellular relaxation)

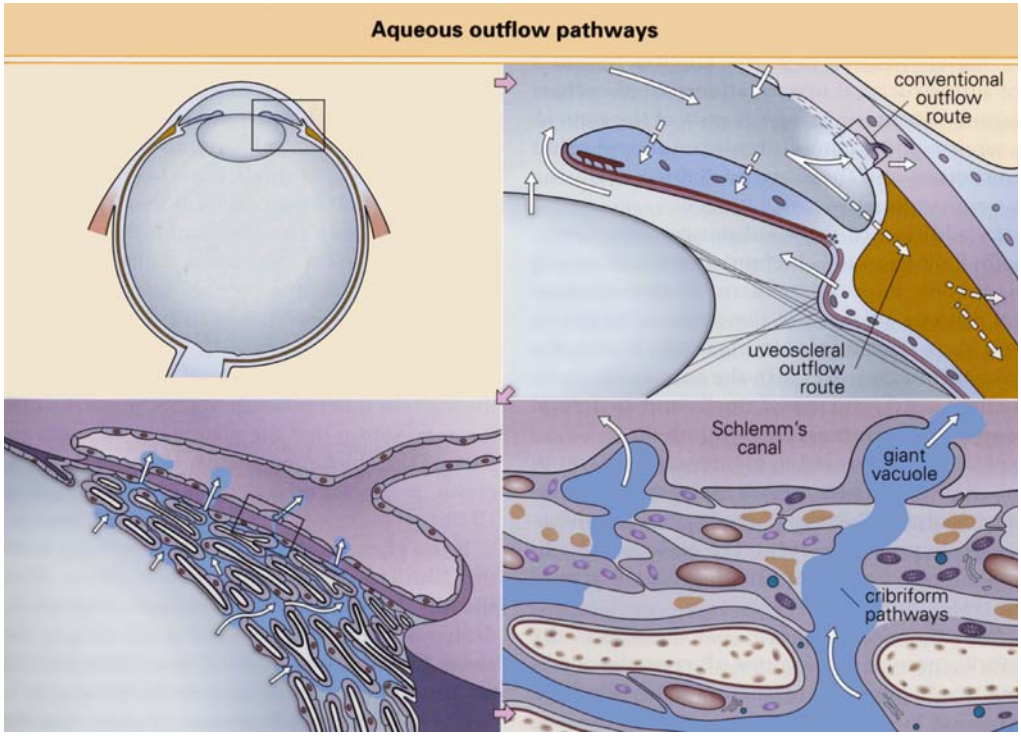

Fig. 9.1 Aqueous outflow pathways. The aqueous humor leaves the anterior chamber via the trabecular meshwork and Schlemm's canal (the so-called conventional outflow route) or via the ciliary muscle and sclera into the orbit, the so-called uveoscleral outflow route. (From [29])

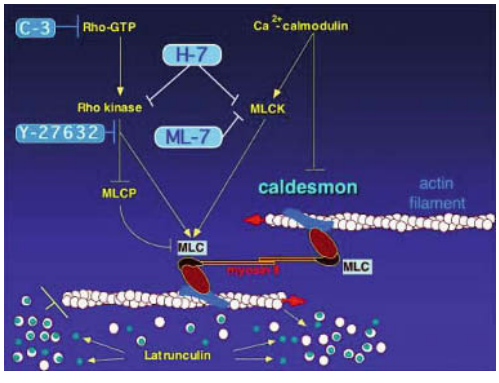

Fig. 9.2 Pathways targeting actomyosin contractility to enhance aqueous humor outflow through the TM. *MLCK* myosin light chain kinase, *MLCP* myosin light chain phosphatase, *MLC* myosin light chain. (From [11])

without apparent cell–cell separations. H-7, a serine–threonine kinase inhibitor, inhibits actomyosin-driven contractility and induces general cellular relaxation by inhibiting myosin light chain kinase or Rho kinase. Although H-7 does not directly affect actin polymerization, the inhibition of contractility leads to deterioration of the actin microfilament bundles and perturbation of its membrane anchorage at matrix adhesion sites in human TM and other cultured cells [19]. In living monkeys, H-7 administered intracamerally or topically increases outflow facility and decreases IOP. Multiple topical doses of 2–5% are effective in decreasing IOP without adversely affecting corneal thickness. Morphological studies in the living monkey eye show that H-7 expands the intercellular spaces in the juxtacanalicular meshwork, accompanied by removal of extracellular material. The inner-wall cells of Schlemm's canal become highly extended, yet cell–cell junctions are maintained (Fig. 9.3) [42]. H-7 also increases outflow facility in human [2] and pig eyes in vitro.

Summary for the Clinician

- Due to the lack of specificity of H-7 to inhibit actomyosin contractility and the high concentrations needed, it is unlikely that this compound, per se, will be further developed in the future; instead, it has served as a tool for investigating prospective mechanisms and identifying characteristics to be targeted for further development.

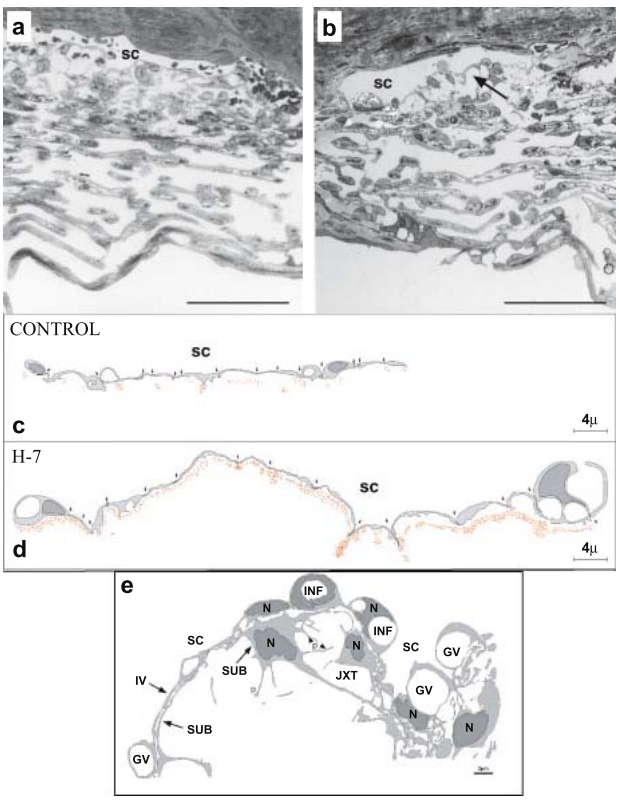

Fig. 9.3 Morphology after perfusion of monkey eyes in vivo with H-7 or LAT-B. Light micrographs of vehicle (**a**) and H-7 (**b**) treated eyes shows expanded intercellular spaces (*arrow*), extended IW cells, and maintained cell–cell junctions after H-7 (*bar* = 50 μm). Drawings depict 15-cell stretches (cell–cell junctions marked by *arrows*) along the Schlemm's canal (*SC*) of control (*C*) and H-7-treated (**d**) eyes. The location of individual gold particles is represented by *red dots*. (From [42] with permission) **e** A long "montage" of transmission EM images, depicting the inner wall IW–JXT regions of the TM following LAT-B. Massive "ballooning" of the JXT region is shown along with retention of close contact between IW and SUB, the irregular diameter of P of IW cells, and the prominent GV. It is difficult to state whether LAT-B increased GV prominence due to the apparent variability in the prominence of GV in the vehicle-treated eye, as well as their non-homogeneous distribution along the canal's wall. *GV* giant vacuoles, *INF* membrane infoldings, *IW* inner wall, *JXT* juxtacanalicular region, *OW* outer wall, *P* cellular processes, *SC* Schlemm's canal, *SUB* sub-canalicular cells, *TM* trabecular meshwork

9.2.1.3 Rho Kinase Inhibitors

Compounds which are more selective in targeting the Rho kinase pathway also show promise for future therapeutic development. Pharmacological studies show that H-7-induced cellular relaxation in the TM and subsequent enhancement of outflow facility may be partially related to its Rho kinase inhibition. A more specific ROCK inhibitor, Y-27632, induces reversible changes in cell shape and decreases in actin stress fibers, focal adhesions, and protein phosphotyrosine staining in human TM cells and Schlemm's canal cells, altering flow pathways through the juxtacanalicular tissue and increasing outflow facility two- to threefold in monkey eyes in vivo [47]. A derivative of Y-27632, Y-39983, also decreases IOP, and increases outflow facility and optic nerve head blood flow, as reported in monkey and rabbit studies recently conducted in Japan [18].

9.2.1.4 Latrunculins

A more potent and very promising group of compounds for future development have been isolated from marine sponge macrolides, such as latrunculins A and B. These compounds alter cell shape and disrupt microfilament organization by sequestering G-actin, leading to disassembly of actin filaments. Latrunculins A and B increase outflow facility and decrease IOP in living monkeys (Fig. 9.4; Table 9.1) [19] and in pig eyes in vitro. A preliminary morphological study in the living monkey eye shows that latrunculin B induces massive "ballooning" of the juxtacanalicular region, leading to a substantial expansion of the space between the inner wall of Schlemm's canal and the trabecular collagen beams (Fig. 9.3). No detrimental effects on tight junctions and cell–cell and cell–extracellular matrix adhesions are observed in the TM [48], although latrunculins interfere with cell–cell adhesions in cultured

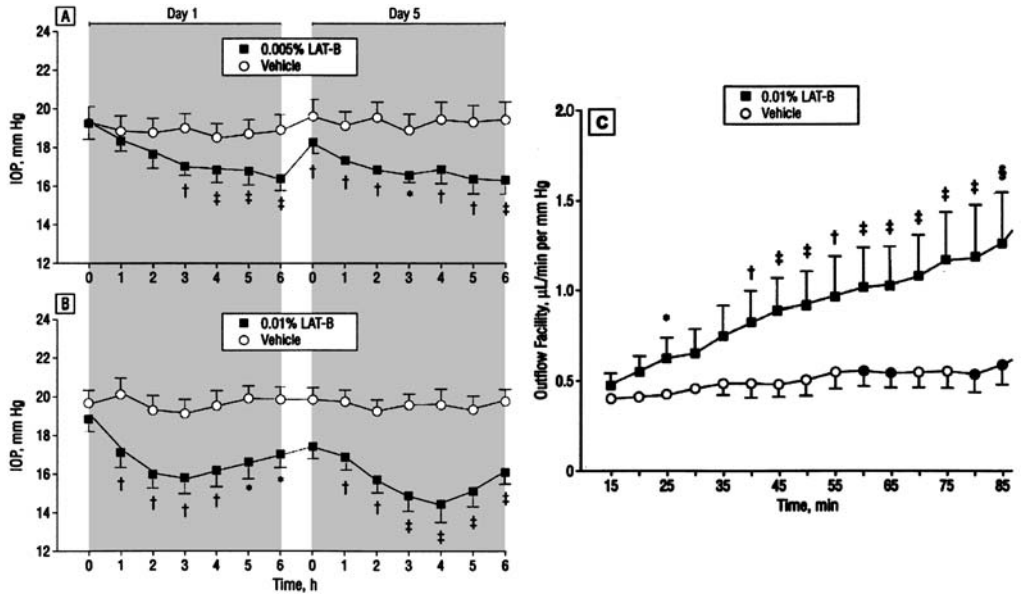

Fig. 9.4 Effects of Latrunculin (LAT) B on IOP and outflow facility in monkeys. **A,B** 0.005/0.01% Lat-B and vehicle (4×5 or 2×10 µl) were administered to opposite eyes topically twice daily for 4.5 days. Intraocular pressure (*IOP*) was measured before and after the first (on day 1) and ninth (on day 5) treatment. **C** Outflow facility was measured by two-level constant pressure perfusion for 90 min on day 9 (2 h after the fifteenth treatment with 0.01% LAT-B once or twice daily). Data are expressed as mean ± SEM: $n = 8$ (IOP); $n = 7$ (outflow facility). The IOP difference between eyes corrected for baseline was tested for differences vs 0.0 by the two-tailed paired *t*-test: * $p < 0.01$; † $p < 0.005$; ‡ $p < 0.001$. Outflow facility difference between eyes was tested vs 0.0 by the two-tailed paired *t*-test: * $p < 0.05$; † $p < 0.03$; ‡ $p < 0.05$; § $p < 0.01$. (From [34])

Table 9.1 Effect of Latrunculin B (*LAT-B*) on outflow facility in monkeys. (From [34])

	Outflow facility (μl/min mmHg^{-1})		LAT-B/vehicle
	LAT-B	Vehicle	
90 min	0.93 ± 0.19	0.51 ± 0.08	1.75 ± 0.13**
First 30 min	0.58 ± 0.10	0.43 ± 0.05	1.35 ± 0.14*
Second 30 min	0.89 ± 0.19	0.51 ± 0.08	1.69 ± 0.14***
Third 30 min	1.19 ± 0.28	0.57 ± 0.11	2.00 ± 0.14***

Following 15 doses of 0.01% LAT-B/vehicle, outflow facility was measured by two-level constant pressure perfusion for 90 min. No baseline outflow facility was determined, but all monkeys were selected from those that had similar baseline facilities in both eyes per previous studies. Data are mean ± SEM for seven animals. Ratios are unitless. Difference between eyes was tested for ratios ≠ 1.0 by the two-tailed paired *t*-test: * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$

TM cells. Multiple topical treatments with low doses of latrunculin-B are effective in increasing outflow facility, relaxing the iris sphincter and CM without adversely affecting the cornea in nonhuman primate eyes.

Latrunculin A or B may also be useful in treating steroid-induced glaucoma. Concurrent administration of latrunculin A prevents the dexamethasone-induced reorganization of the actin cytoskeleton in human TM cells and reverses existing dexamethasone-induced reorganization [26]. Latrunculin and actin depolymerizing agents may also affect outflow facility by activation of matrix metalloproteinases as a result of actin cytoskeletal reorganization and changes in cell morphology [43].

9.2.1.5 Steroid Antagonists

The aqueous humor of control and POAG patients has levels of cortisol in excess of what is in the general circulation, due to the activity of 11β-hydroxysteroid dehydrogenase 1 in the ciliary epithelium where it may be involved in regulating aqueous secretion. The cortisol levels could also reduce aqueous outflow facility to a level that is detrimental in susceptible individuals. In the normal population, 34–42% of patients treated with topical or systemic corticosteroids are termed "steroid responders," and develop moderately to markedly elevated IOP after several weeks. This contrasts to patients with POAG, 90% of whom are considered strong steroid responders. The oral administration of the glucocorticoid biosynthesis inhibitor metyrapone to glaucoma patients or the 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone to ocular hyperpertensive patients [39] elicit small, transient reductions in IOP.

Topically applied 3α, 5β-tetrahydrocortisol (3α, 5β-THF), an intermediate metabolite of cortisol, decreases IOP and increases outflow facility in glaucomatous human eyes, and 3α, 5β-THF antagonizes dexamethasone-induced cytoskeletal reorganization in normal human-cultured TM cells. Interestingly, cultured TM cells from patients with POAG metabolize cortisol predominantly to 5β-dihydrocortisol (5β-DHF), which potentiates the facility-decreasing and IOP-increasing effects of dexamethasone. These cells produce relatively little 3α, 5β-THF from cortisol.

Possible mechanisms for steroid-induced elevation of IOP have been proposed and include: accumulation or deposition of extracellular matrix material; decreased protease and stromelysin activities; reorganization of the TM cytoskeleton; increased nuclear size and DNA content; decreased phagocytic capacity; and changes in the synthesis of specific proteins. The progressive induction of one major steroid product in human TM cells matches the time course of clinical steroid effects on IOP and outflow facility. This molecule, known as myocilin (MYOC), appears to be a secreted glycoprotein with aggregation- and

extracellular matrix-binding groups interacting with extracellular components such as fibronectin [8].

Alluded to previously, LAT-A can reduce or prevent the formation of actin networks in TM cells exposed to dexamethasone. Other compounds are currently being developed to be effective in reducing IOP in the steroid glaucoma model; these may have broader application for glaucoma IOP-lowering therapy in general.

9.2.1.6 Adenosine Agonists/ Antagonists

Adenosine is a common signaling molecule often associated with cellular responses to stressful situations. Ischemia, in many tissues including the eye, can lead to rapid increases in adenosine concentration. Intravenous infusion of exogenous adenosine into healthy human subjects causes an ocular hypotensive effect and significantly increases choroidal and optic nerve head blood flow [35]. The vasodilatory effects of adenosine in vascular beds is mediated primarily by adenosine A1 and adenosine A2 receptors. The ocular hypotensive effect is presumably due to activation of the adenosine A1 receptors which is correlated with an increase in outflow facility in nonhuman primates. Stimulation of the adenosine A1 subtype in TM cells in vitro results in secretion of matrix metalloproteinase (MMP)-2 which could contribute to extracellular matrix remodeling and enhancement of outflow facility. Other functional adenosine receptor subtypes (A2A and A3) are also present on TM cells. All three subtypes, upon stimulation, produce similar responses of Ca^{2+} release and cell volume changes, making it unlikely that differential effects of adenosine subtype selective agonists on aqueous humor outflow are mediated through TM cells alone.

In ocular hypertensive patients, adenosine levels are elevated compared with normotensives and correlate with IOP [7]. The elevated levels of adenosine may be due primarily to the reduction in ocular blood flow identified in glaucomatous individuals and may represent an adaptive response to enhance blood flow and decrease IOP by increasing outflow facility. Ciliary epithelium contains stores of ATP which, upon release, may be degraded to adenosine by ecto-adenosine triphosphatase. Delivery of adenosine downstream to TM and Schlemm's canal inner-wall cells could represent a mechanism for regulating outflow [6]. Conversely, stimulation of the adenosine A2 and A3 receptors is known to increase IOP; thus, elevated adenosine levels could possibly contribute to the elevation in IOP. The functional adenosine receptor subtypes in glaucomatous and normal eyes needs to be evaluated to help clarify this issue.

Summary for the Clinician

- Future development of glaucoma therapies targeting adenosine receptors would likely utilize adenosine A1 agonists and/or adenosine A3 antagonists.

9.2.1.7 Gene Therapy

Another approach to increase aqueous humor outflow (trabecular outflow) is to use gene therapy to inhibit or enhance the molecular pathways involved in regulating trabecular cell contractility or to block cellular interactions with the extracellular environment that enhance actomyosin contractility and the formation of actin stress fibers. The recent discovery of TM-specific promoters may allow more specific targeting of this tissue [13]. Delivery of genes to the anterior segment of the living primate eye using viral vectors has not yet been successful due to the accompanying inflammatory response which precludes assessment of the effect of the expressed transgene on aqueous humor dynamics. Glaucoma is a chronic disease; thus, long-term expression of the transgene will be required for any gene therapy approach for this disease.

Once the vectorologists can modify their constructs to eliminate these confounding issues (not a trivial matter), some of the genes that will be of therapeutic interest for anterior-segment gene therapy may include the following:

Rho kinase and C3. Rho kinase, targeted for pharmacological therapy, as described above

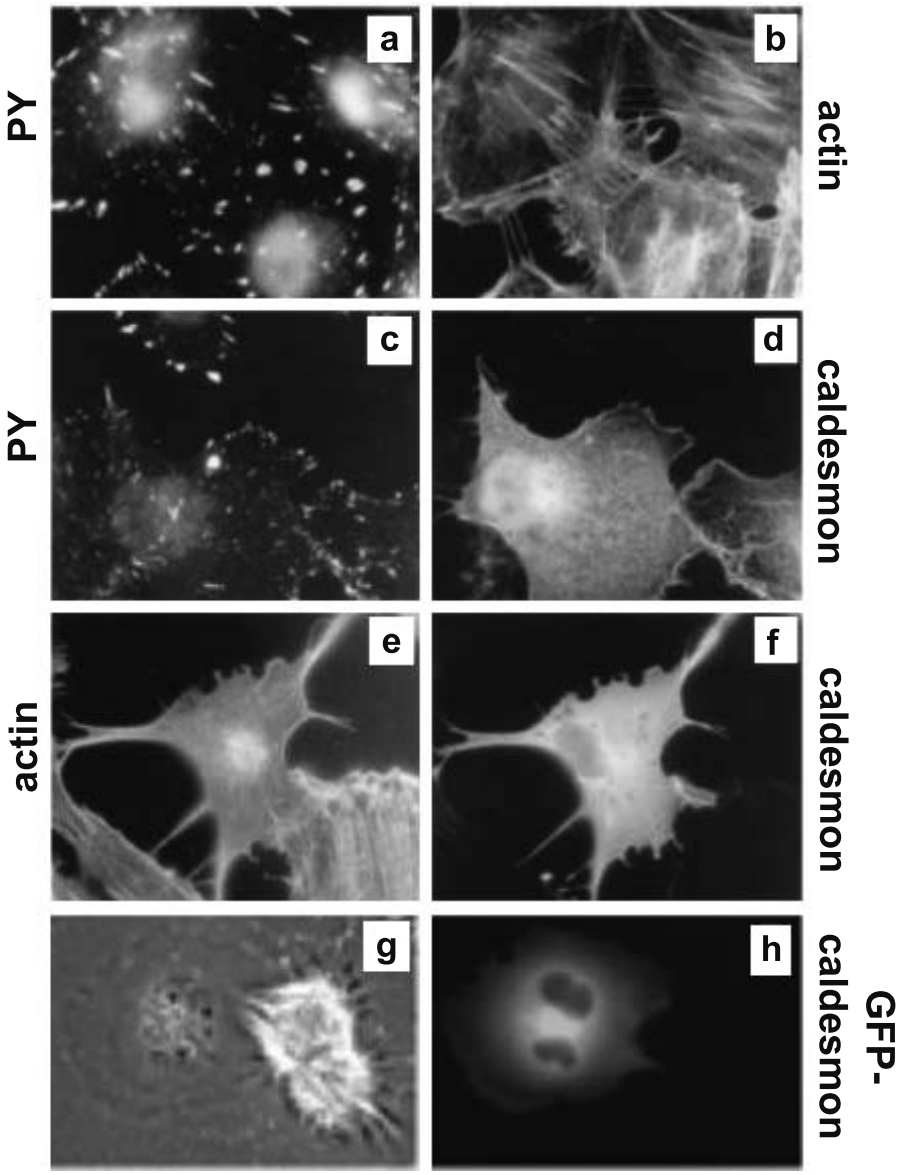

Fig. 9.5 Caldesmon gene therapy in vitro. **A–F** Effect of caldesmon overexpression on focal adhesions and the actin cytoskeleton of SV80 cells. **A,B** Cells in control culture. **C–F** Cells transfected with full-length caldesmon. Staining for focal adhesions was performed with anti-phosphotyrosine (*PY*) antibody (**A,C**); for actin with FITC-phalloidin (**B,E**); and with antibody to visualize transfected caldesmon (**D,F**). Note that focal adhesions (**A,C**) in cells expressing full-length caldesmon (**C**) are much smaller than those in nontransfected cells (**A**). Stress fibers are abundant in nontransfected cells (**B**) but disappear in cells expressing full-length caldesmon (**E**). Some caldesmon-transfected cells show an increase in the formation of long processes (**E,F**) **G,H**. Caldesmon prevents development of traction forces in cells attached to an elastic silicone-rubber substrate. Cells were plated onto a fibronectin-coated silicon-rubber film 7 h after transfection with green fluorescent protein (GFP)–caldesmon. **G** Phase-contrast images of substrate-attached cells and wrinkles they produce. **H** Green fluorescent protein fluorescence images of the same fields show that cells expressing full-length GFP–caldesmon cannot deform the substrate, whereas cells expressing truncated caldesmon readily form wrinkles. (From [15])

for H-7, and rho kinase inhibitors, such as Y-27632 and Y-39983, may also be inhibited by a gene therapy approach. The GTPase Rho, which activates Rho kinase, can be inhibited by proteins, including the dominant negative Rho A (RhoN19) and C3 transferase (a botulinum exoenzyme known to inactivate Rho and disrupt actin filaments and cellular adhesions). Overexpression of C3 transferase can increase outflow facility in monkey-organ-cultured eyes in vitro [27].

Construction of a dominant negative Rho vector will allow targeting of a specific Rho protein and may have different effects than the C3 protein. Dominant negative Rho transduced into human TM cells and organ-cultured human eyes results in reductions of actin stress fibers, focal adhesions and intercellular junctions, and small increases in outflow facility [38].

Caldesmon. Another intracellular protein involved in regulating actomyosin contractility is caldesmon. This protein has been studied mainly in smooth muscle cells and acts by blocking the interaction of actin with myosin, inhibiting actin-activated myosin ATPase activity. Caldesmon affects several actomyosin-based processes, including the movement of actin filaments over myosin heads in vitro.

In nonmuscle cells in culture, caldesmon overexpression leads to suppression of cellular contractility, manifested by a reduced capacity to develop traction forces applied to the underlying extracellular matrix (Fig. 9.5) [15]. This relaxation of the cells resembles in many ways the effect of H-7 on the contractility of cells. Overexpression of caldesmon in human TM cells in vitro following adenoviral vector transduction results in a loss of actin stress fibers and focal adhesions. Outflow facility is enhanced in human and monkey anterior segment organ cultures following transduction with adenoviral vectors carrying nonmuscle caldesmon [10]. It is important that caldesmon overexpression prevent focal adhesion and stress fiber formation, even if the cells express constitutively active Rho [15], showing that caldesmon is operating downstream from the Rho signaling pathway (Fig. 9.2).

α-Catenin. Another protein to target using gene therapy to potentially enhance outflow facility is α-catenin. α-Catenin is a protein found exclusively in cell–cell adhesion complexes (adherens junctions) where it plays a key role in linking cadherins (transmembrane proteins that directly mediate adhesions between neighboring cells) to the actin cytoskeleton [41]. Blocking normal α-catenin function disrupts cell adhesion. Vectors containing a dominant negative version of α-catenin are currently under construction and will be tested for their effects on the cytoskeleton and cell junctions of human TM cells and for their outflow facility effects in organ-cultured anterior segments.

Hep II. Among the signaling pathways that maintain the actomyosin cytoskeleton in vivo are integrin-mediated mechanochemical signaling events via the extracellular matrix. Included in the matrix proteins identified in the TM are laminin, fibronectin, types I, III, IV, V, VI, and VIII collagen, chondroitin, dermatan and heparan sulfate proteoglycans, hyaluronic acid, and a small amount of keratan sulfate. Fibronectin is distributed throughout the TM along the trabecular beams and is especially prevalent in the juxtacanalicular tissue next to Schlemm's canal. It is also found in the basement membrane of the inner wall of Schlemm's canal. The other major components of the basement membrane on the inner wall of Schlemm's canal are laminin and type-IV collagen. During aging and glaucoma, the expression of some matrix proteins is altered. For example, fibronectin levels are upregulated. In contrast, laminin levels are reduced in glaucomatous eyes. Myocilin, a protein that is upregulated during glaucoma, may also be a component of the extracellular matrix. Myocilin is secreted into the media of human TM cells treated with glucocorticoids and is found in the extracellular matrix of TM cells in culture as described previously [8].

Two domains of fibronectin can affect the organization and the contractility of the actin cytoskeleton. They are the central cell binding domain and a heparin-binding domain called the Hep-II domain. Both these domains contain a binding site for members of the integrin receptor family. In addition, Hep-II domain contains a binding site for another family of cell surface receptors called the syndecans, which are cell surface heparan sulfate proteoglycans. All the members of the syndecan family bind fibronectin.

Using recombinant integrin-binding domains from fibronectin to block integrin-fibronectin interactions, it has been shown that a binding domain called Hep II significantly lowers IOP in human eye organ cultures. Treatment of human TM cells with the Hep-II domain disrupts cell–cell junctions and causes a disruption of the cadherin/β-catenin complex of cell–cell junctions, and subsequently, a disassembly of actin filaments [12]. This suggests that gene therapy that interferes with cell–extracellular matrix mediated signaling events could be an approach for modulating aqueous humor outflow in vivo.

Summary for the Clinician

- Gene therapy approaches to enhance outflow through the TM will likely target pathways which alter the TM cytoskeleton and TM interactions with the extracellular matrix.

9.2.2 Uveoscleral Outflow

9.2.2.1 Basic Structure

The anterior chamber and the spaces within the TM are continuous with those between the CM bundles (Fig. 9.1). Water and larger molecules from the anterior chamber can pass into and through the CM via its anterior face, and from there, into the suprachoroidal space to be carried away, some perhaps by the choroidal vessels, but most actually *through* the sclera into the orbit. Indirect measurements in young, health-conscious humans, although incorporating some assumptions, indicate that uveoscleral outflow may routinely account for nearly 50% of total aqueous drainage. This decreases somewhat with age (reviewed in [11]). Aqueous draining via the uveoscleral route takes 2 h or more before it reaches the general circulation.

This system likely evolved to protect the eye in several ways during inflammation. The TM may become compromised by inflammation or obstructed by inflammatory debris, and the choroid may be overloaded with debris and extravasated proteins that must be removed from the eye. In this situation, prostaglandins would be released and, as autacoids or hormones that are synthesized, released, and locally acting, would induce the production of matrix metalloproteinase enzymes that would break down some of the extracellular matrix in the uveoscleral pathways to allow greater flow via this route. Since the eye has no lymphatics, uveoscleral outflow may serve as an analog to an intraocular lymphatic drainage system. Redirection of aqueous outflow from the trabecular to the uveoscleral pathway would both rid the eye of excess proteins and maintain physiological IOP [19].

9.2.2.2 Prostaglandin Analogs

Prostaglandin (PG) analogs are the most potent and efficacious topical ocular hypotensive agents currently known for the treatment of human glaucoma. The most effective PGs for lowering IOP in humans are derivatives of $PGF_{2\alpha}$-isopropyl ester (ie), modified structurally to enhance ocular penetration and specifically activate the FP-prostanoid receptor. Side effects of early analogs included ocular irritation, conjunctival hyperemia, and headache; these have been largely eliminated with latanaprost, a 17-phenyl-substituted isopropylester prodrug derivative of $PGF_{2\alpha}$-ie, which maintains an ~30% IOP reduction with once daily topical application of a 30-μl drop of 0.005% solution in ocular hypertensive patients with starting IOP of ~26 mmHg. Other analogs with similar IOP-lowering efficacy, but slightly higher prevalence of side effects, include 0.03% bimatoprost and 0.004% travoprost.

A new $PGF_{2\alpha}$ derivative, AFP-168, has been developed in Japan. Its affinity for the FP receptor and IOP-lowering response in monkeys exceed those of latanoprost, and it has less stimulating effect on melanogenesis in melanoma cells [46]. This compound is currently in clinical trials in Japan.

Other PG subtypes are being targeted for future anti-glaucoma drug development as well. The EP2-receptor agonist, butaprost, increases uveoscleral outflow approximately twofold in normotensive cynomolgus monkeys without an effect on outflow facility [32]. These findings

are in agreement with the enlargement of the uveoscleral pathway observed after long-term treatment (1 year) of normotensive cynomolgus monkeys with bimatoprost, latanoprost, sulprostone (EP3/EP1 agonist), or AH13205 (EP2 agonist). Similar morphological changes are observed in all groups as well as in the contralateral untreated eyes. Uveoscleral outflow pathways are enlarged and appear organized. More myelinated nerve fiber bundles are found. Changes in the TM are also noted [40].

The selective EP4 receptor agonist ONO-123A may represent a novel anti-glaucoma drug that directly enhances pressure-dependent outflow, perhaps indicating an effect on the TM. In monkeys a single topical dose increases outflow facility by 43% [23]; however, further studies, including multiple treatments, are needed before claims can be made that this receptor subtype acts through mechanisms different than all other PG subtypes to date.

Summary for the Clinician

- Drugs targeting different PG subtypes will be forthcoming, although the mechanism of action will still likely be via an enhancement of uveoscleral outflow.

9.2.2.3 Serotonin Agonists/ Antagonists

Serotonin (5-HT) receptors were identified in ocular tissues of the anterior segment of the eye in several species, including human. These findings suggest that 5-HT might play a role in regulating aqueous humor dynamics and IOP.

There are conflicting reports on the effects of 5-HT receptor subtype ligands on IOP in various species and as a consequence of activity at other classes of receptors [31].

Of particular interest, one study demonstrates that 5-HT_2 agonists, but not 5-HT_2 antagonists or 5-HT_{1A} agonists, are involved in locally mediated control of IOP in conscious cynomolgus monkeys [31]. The mechanism by which a selective 5-HT_2 agonist, R-DOI, lowers IOP in normotensive monkeys is primarily by increasing uveoscleral outflow [33].

Summary for the Clinician

- The 5-HT2 receptor stimulation represents another pathway for IOP-lowering drug therapy development that is currently being pursued; however, the possibility of an overlapping effect via PG-related mechanisms must first be ruled out.

9.2.2.4 Nitric Oxide

Nitric oxide (NO) synthases are detected in ocular structures involved in fluid (aqueous humor) drainage from the anterior portion of the eye (CM and TM) as well as in layers of the retina and its circulation supply. Nitric oxide has the potential to be involved in both protective and damaging functions related to glaucoma. Inhibition or enhancement of its production in selected locations in the eye could be used to therapeutic advantage.

In human glaucoma eyes there are dramatic reductions in staining indicative of NO synthase activity in CM and outflow pathways compared with control eyes unrelated to general ocular decrease, the use of multiple glaucoma therapies, or the severity of the disease [4].

Nitric oxide-mimicking nitrovasodilators can act at various sites in the anterior segment of the eye to potentially decrease IOP by increasing outflow facility, decreasing episcleral venous pressure, decreasing aqueous humor flow, and relaxing the CM to potentially increase uveoscleral outflow. In human eyes the TM and CM are enriched sites of NO synthesis. Topical and intracameral administration of nitrovasodilators to monkey eyes in vivo decreases IOP and possibly increases outflow facility, respectively; however, the outflow facility increase is devoid of a clear-cut dose-response relationship, making the mechanism for the IOP lowering unclear. Nitrovasodilators relax TM and CM strips precontracted with carbachol in vitro. The IOP

and aqueous humor formation are decreased in isolated pig eyes perfused with nitrovasodilators [44], suggesting mechanisms independent of ocular vasculature.

Summary for the Clinician

- Development of nitrovasodilators for topical drop glaucoma therapy has not yet been initiated. This class of compounds has the potential to alter both aqueous humor inflow and outflow.

9.2.2.5 Gene Therapy

Gene therapy has the potential to alter the extracellular environment to enhance aqueous outflow via the uveoscleral route. Overexpression of PG synthesizing genes is being targeted for this type of therapeutic approach. Genes for all prostanoid receptors are expressed in human postmortem TM. Prolonged treatment of human TM cells with latanoprost or $PGF_{2\alpha}$ ethanolamide increases expression of genes for IGF-1 and fibroleukin. IGF-1 can increase the level of matrix metalloproteinase (MMP) enzymes in TM cells that can degrade components of the extracellular matrix. The protease activity of fibroleukin may also be active against extracellular matrix elements [50].

The MMP upregulation via gene therapy is another approach to enhance outflow through the TM and CM. It is one mechanism by which PGs are believed to enhance uveoscleral outflow through the CM. The TM also expresses a spectrum of MMPs. MMPs directly control outflow resistance in organ culture. MMP-3 (stromelysin) in an adenoviral vector construct transduces and shows expression in human TM cells in vitro and rat TM, iris, and uveoscleral pathways in vivo [22].

In the eye there are multiple sites of action for nitrovasodilators or NO donors as described above.

Selective stimulation of the endothelial form of NO synthase (NOS-3) could increase blood flow to the retina. It may also be possible that overexpression of NOS-3 in the anterior segment could potentially increase outflow facility, uveoscleral outflow, and decrease aqueous humor formation.

Stimulation of the neuronal and inducible forms of NOS (NOS-1 and NOS-2, respectively) most likely should be avoided since NO produced via these enzymes is often associated with the formation of highly destructive peroxynitrite.

Summary for the Clinician

- The gene therapy approach to enhance aqueous outflow via the uveoscleral route may advance rapidly as soon as vectorologists can develop vectors that produce long-term expression in the anterior segment without inducing inflammation. Genes that will be targeted for overexpression will likely include those whose products relax the CM and break down its extracellular matrix.

9.3 Inflow Suppression

Long-term use of drugs that decrease IOP by decreasing aqueous humor formation could have a negative effect on the eye. Some patients who have well-controlled IOP with timolol show evidence of reduced pressure control with continued administration. In cynomolgus monkeys treated with topical timolol for over 7 months, underperfusion of the TM results in meshwork densification, activation of meshwork endothelial cells, and increased extracellular material within the cribriform region. Unilateral aqueous flow suppression in monkeys with timolol (β1,2 antagonist) + dorzolamide (carbonic anhydrase inhibitor) and redirection of aqueous outflow in the same eye with topical $PGF_{2\alpha}$-ie (enhanced uveoscleral outflow) significantly decreases outflow facility [24]. Humans receiving oral acetazolamide over a several-week period demonstrate restoration of IOP but reduction in tonographic outflow facility.

Even though aqueous flow suppression is not the optimum approach for IOP reduction, it will continue to be a mechanism that can be targeted

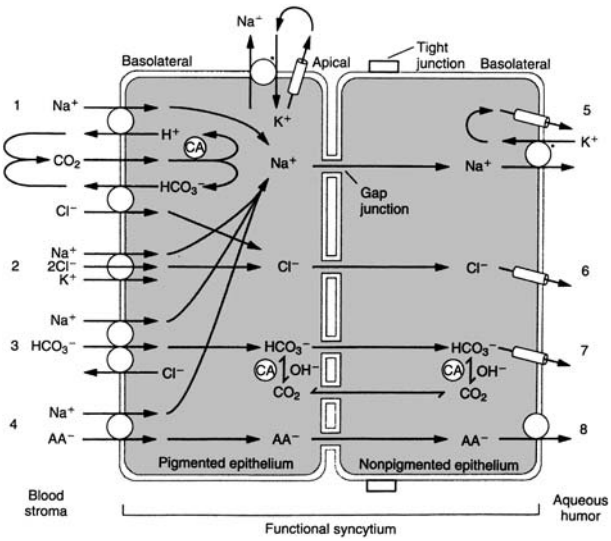

Fig. 9.6 Possible secretory pathways in the ciliary processes. *AA* ascorbic acid, *CA* carbonic anhydrase. (From [49])

for glaucoma therapy development at least for the immediate future.

9.3.1 Basic Structure

The ciliary processes consist of a central core of highly vascularized connective tissue stroma and a specialized double layer of epithelium that covers the stromal core. The inner epithelial layer (nonpigmented epithelium, NPE) is in direct contact with the aqueous humor, and the outer pigmented epithelium (PE) lies between the NPE and the stroma (Fig. 9.6).

Three physiological processes contribute to the formation and chemical composition of the aqueous humor: diffusion; ultrafiltration; and active secretion. Under normal conditions active secretion accounts for 80–90% of total aqueous humor formation. The active process of aqueous humor secretion is mediated by selective transport of certain ions and substances across the basolateral membrane of the NPE against a concentration gradient. Two enzymes abundantly present in the NPE that are involved in this process are sodium-potassium adenosine triphosphatase (ATP) and carbonic anhydrase. Sodium-potassium ATPase provides the energy for the metabolic pump, which transports sodium into the posterior chamber. As a result of active transport, aqueous humor in humans exhibits increased levels of ascorbate, some amino acids, and certain ions such as Cl^-. There is also passive transport of HCO_3^-.

Carbonic anhydrase is abundant in the basal and lateral membranes and cytoplasm of the pigmented epithelium and NPE of the ciliary processes. Inhibition of the production of HCO_3^- also leads to an inhibition of the active transport of Na^+ across the NPE, thereby reducing active aqueous humor formation (reviewed in [9]).

9.3.2 Opioids

Opioid receptors can modulate various functions in the eye. The kappa opioid receptor agonists, bremazocine and dynorphin A, lower IOP bilaterally following unilateral topical administration by suppressing aqueous humor formation in rabbits. The IOP and aqueous flow suppression are antagonized by the relatively selective kappa opioid receptor antagonist, nor-binaltorphimine (nor-BNI). However, species differences may exist, since the IOP-lowering response in monkeys following topical administration of bremazocine, could be completely blocked by maintaining the mean arterial pressure by simultaneous intravenous infusion of angiotensin II. Also, there is no effect of bremazocine on outflow

facility in monkeys, in contrast to rabbit studies [37].

Summary for the Clinician

- The efficacy of other opioid subtypes in modulating aqueous humor dynamics in primates has yet to be determined. Derivatives must be developed which minimize central and systemic effects.

9.3.3 Cannabinoids

A number of well-done studies show that in normal people, smoking a marijuana cigarette reduces IOP by ~24%, an effect comparable to other glaucoma medications. However, the duration of action of smoked or ingested marijuana, Δ9-THC or other cannabinoids, is unacceptably short – approximately 3.0–3.5 h. Decreased blood pressure, decreased optic nerve blood flow, and short duration of the IOP-lowering effect are significant actual and potential problems irrespective of the psychotropic effects. Another issue is whether cannabinoids can work topically. Δ9-THC, the supposedly active compound, applied topically, whether in single or multiple doses, whether once or four times daily, does not lower IOP.

The demonstration of a wide distribution of cannabinoid CB1 receptors in the human anterior eye segment and retina suggest that cannabinoids may influence several physiologcial functions in the human eye. The CB1 mRNA levels were significant in the human retina, ciliary body, and iris. Cannabinoid subtype selective compounds are currently being identified and studied.

Small reductions in IOP after topical administration of WIN 55,212-2, an aminoalkylindole with CB1 activity, to normal monkeys are attributed to reductions in aqueous humor flow [5]. Larger reductions in IOP are produced in glaucomatous monkeys after multiple topical treatments. In human glaucoma resistant to conventional therapies, topical WIN decreases IOP within the first 30 min [36]. WIN had no effect on in vitro monkey or dog CM resting tension or the contractile response to carbachol. Conversely, induction of bovine CM contraction in vitro by an endogenous and a synthetic cannabinoid results from CB1 receptor activation [28].

The cannabinoid HU-210 suppresses cell proliferation and cell viability in differentiating pheochromocytoma cells, in association with altered distribution of microtubules and microfilaments. The potential of an effect on the actin cytoskeleton suggests that one target may be outflow through the TM. Intracameral injection of HU-210 into monkey eyes in vivo produced a dose-dependent decrease in IOP but inflammation and corneal toxicity have delayed mechanistic studies.

Some endogenous cannabinoids, known to decrease IOP following topical application, may be hydrolyzed to arachidonic acid and thus act via PG pathways.

9.4 Drug Delivery

The main aim of pharmacotherapeutics is to attain effective drug concentrations at the intended site of action for a sufficient period of time to elicit a response. Major problems contributing to poor bioavailability with current ocular therapeutics include precorneal loss factors such as tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and relative impermeability of the corneal epithelium. Various approaches, such as viscosity enhancement, use of mucoadhesives, particulate drug delivery, vesicular drug delivery, prodrugs, and controlled release systems, such as Ocuserts, are being explored.

9.4.1 Vesicular Drug Delivery

Vesicular systems not only help in providing prolonged and controlled action at the corneal surface, but also help in providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can cross cell membranes. In ophthalmics, ve-

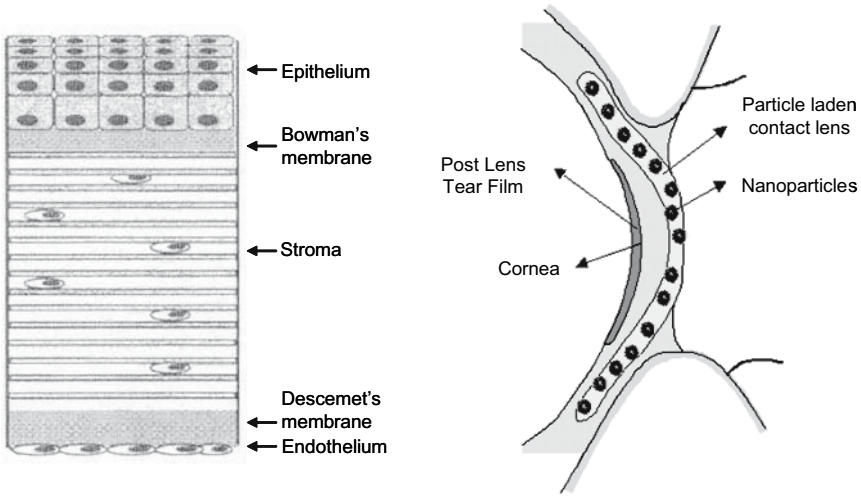

Fig. 9.7 The cornea (*left*) and particle-laden contact lens (*right*). (From [14, 45])

sicular drug delivery (reviewed in [21]) systems include liposomes and niosomes.

Liposomes are microscopic vesicles with a diameter ranging from 80 nm to 10 μm and are composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments. These vesicles can entrap both hydrophilic and hydrophobic drugs. Positively charged liposomes seem to be preferentially captured by the negatively charged corneal surface but may cause initial irritation. Liposomes used in combination with bioadhesive polymers, collagen matrices, gel-forming solution, and gangliosides prolong the residence time of the preparation in the precorneal region; however, these combinations have short half-lives, limited drug capacity, and problems with sterilization that must be overcome before they can be used clinically.

Niosomes are non-ionic surfactant vesicles and are also bilayered structures that can entrap both hydrophilic and lipophilic drugs. They have the advantages of liposomes but are lower in cost, and have greater stability and ease of storage. Surfactants also act as penetration enhancers.

Nanoparticles are polymeric colloidal particles, ranging from 10 nm to 1 μm, in which the drug is dissolved, entrapped, encapsulated, or adsorbed. They are further classified into nanospheres (small capsules with a central cavity surrounded by a polymeric membranes) or nanocapsules (solid matricial spheres). Nanocapsules show a better effect, possibly because the drug is in a non-ionized form in the core and can diffuse at a greater rate into the cornea. Nanocapsules also have better bioadhesive properties.

Encapsulation of drug in microspheres can prolong drug concentration in the aqueous humor by twofold.

9.4.2 Contact Lenses

Another form of encapsulated drug delivery is via soft contact lenses laden with drug formulations in nanoparticles dispersed in the lens material. Contact lenses made with the particle-laden hydrogels release therapeutic levels of drug for a few days. The drug delivery rate can be controlled by varying the loading of nanoparticles in the gel. Drug will diffuse from the particles, travel through the lens matrix, and enter the post-lens tear film (Fig. 9.7). This type of lens has advantages over soaked contact lenses in that more drug can be incorporated into the lens and release is continuous for longer periods of time. Also, it takes a few hours to load lenses with drug from aqueous solutions with a large fraction of the drug that is left in the solution going to waste.

Further development of particle-laden lenses for drug delivery is likely to be forthcoming [14].

9.4.3 Penetration Enhancers

Current topical drop therapy has begun to utilize penetration enhancers (reviewed in [20]) or absorption promoters to transiently increase the permeability characteristics of the cornea. Classes of penetration enhancers include calcium chelators (e.g., EDTA), surfactants (e.g.,. Brij), bile acids and salts (deoxycholate), preservatives (e.g., benzalkonium chloride), glycosides (e.g., Saponin), fatty acids, azone, cytochalasins, and ionophores. Caution must be exercised in the use of these agents since they themselves can penetrate the eye and may therefore produce unknown toxicological effects.

9.4.4 Bioadhesives

The capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surfaces of the eye by non-covalent bonds forms the basis of ocular mucoadhesion. Clearance time of bioadhesive polymeric systems is much slower since it now depends on the rate of mucus turnover rather than the tear turnover rate. The most commonly used bioadhesives are macromolecular hydrocolloids with numerous hydrophilic functional groups capable of forming hydrogen bonds. These do not cross biological membranes. Some examples of polymers used in ophthalmics for their mucoadhesive properties include: hyaluronic acid; hydroxypropyl methylcellulose; chitosan; DEAE-dextran; and polyacrylic acid derivatives (e.g., carbopols, polycarbophils, and carboxymethylcellulose; reviewed in [20]).

Mucoadhesive polymers, such as chitosan, have been used to coat nanoparticles to increase the time associated with the ocular mucosa and consequently prolong the penetration of drug into the ocular structures. Additional studies are needed to investigate the interaction and internalization of these particles and their toxicity following repeated administration. Other properties that make chitosan a good candidate for ocular drug delivery include its biodegradability, ocular tolerance, good rheological properties, and adaptability for designing different delivery systems (reviewed in [1]).

Receptor-mediated bioadhesion may also be accomplished with the use of lectins. Lectins are proteins that recognize and bind to sugar complexes attached with high specificity to proteins and lipids. Drug delivery to the eye may be prolonged by conjugation to lectins that adhere to the corneal and conjunctival epithelia, which is covered by mucin. Lectin-binding sites exist on the corneal and conjunctival epithelia of human and other species. The use of lectins in drug targeting is an area that will likely grow in years to come (reviewed in [3]).

9.4.5 Ocular Inserts

Ocular inserts placed in the cul-de-sac of the eye have the advantage over liquid formulations of prolonged retention and controlled release, allowing effective drug concentration in the eye over an extended time period with more accurate dosing and decreased risk of systemic side effects. However, ocular inserts have not been widely used in ocular therapy due to the foreign-body sensation that occurs. Ocular inserts prepared from mucoadhesive thiolated polymers are well tolerated by patients and may represent a promising new solid device for ocular drug delivery [17].

Summary for the Clinician

- Prolonging contact with the cornea for enhancing drug delivery may be accomplished by entrapment and encapsulation of the drug in liposomes, niosomes, nanoparticles, microparticles, contact lenses, and incorporation of bioadhesives into the vehicle solutions. Combinations of bioadhesive and nanoparticles are also promising approaches. Use of penetration enhancers may have undesirable toxicological effects.

9.5 IOP Monitoring

In contrast to the single point-in-time aspect of our current IOP measurement techniques, continuous monitoring of IOP would greatly improve managing glaucoma, testing of drugs that could lower IOP, and basic research into mechanisms of glaucoma. Increased IOP and wide diurnal IOP variations are considered major risk factors for glaucoma progression.

9.5.1 Contact Lenses

In humans, an IOP change of 1 mmHg causes a change of central corneal radius of curvature of approximately 3 μm. A soft contact lens has been developed with an embedded microfabricated strain gauge that allows measurement of changes in corneal curvature that correlate with IOP [25]. Incorporation of a telemetry chip and an antenna into this device will allow for wireless power and data transfer. Extended testing in humans will then be feasible.

9.5.2 Implantable Sensor

A completely encapsulated IOP sensor equipped with telemetric signal and energy transfer integrated into the haptics of an intraocular lens is being developed [16]. This approach will remain limited to patients who need intraocular surgery. Other telemetry-based sensors have been used in animal studies, but these have required some component to be implanted under the skin as well as into the eye.

Summary for the Clinician

- Continuous monitoring of IOP via a contact lens or implantable sensor may allow for better regulation of IOP during glaucoma therapy; however, a contact lens would need to be removed for topical drop therapy administration.

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