"Pharmacologic or medical trabeculectomy" is a term first used in the 1970s. This describes a process by which the trabecular meshwork (TM) can be biochemically manipulated in order to reduce the outflow resistance and, thus, the intraocular pressure (IOP). The TM is now regarded as a complex organization of multiple components, which play a major role in aqueous humor (AH) dynamics. Indeed, it is largely a function of AH dynamics to maintain IOP in a steady state.

In cases of primary open angle glaucoma (POAG) and normal-tension glaucoma (NTG), the IOP can be controlled by either reducing the ingress of AH or by increasing its outflow through the ciliary body. Most of the medications in use now reduce aqueous production or the aqueous outflow through the unconventional uveoscleral pathway. According to one school of thought, decreasing the aqueous production and thus reducing aqueous outflow through the TM will progressively be detrimental to the trabecular function. This could be further accelerated by concurrent prostaglandin analog use, which directs aqueous away from the meshwork to the ciliary body.

In order to overcome this, and to develop a new line of approach to the management of glaucoma, interest is developing on the trabecular outflow pathways, and the modulation of which, can be used to reduce IOP. In this review we take a look at the agents that have been investigated to improve the facility of AH outflow.

Glycosaminoglycanisases
Glycosaminoglycans (GAGs) are negatively charged particles found on the surface of TM cells, in the extracellular matrix (ECM) and the surface of cells lining the Schlemm’s canal (SC). GAGs are responsible for cell matrix interactions, growth factor binding, and the sequestration and maintenance of tissue structural integrity. GAGs may induce hydration (edema) of the TM, leading to the obstruction of the trabecular structure. Agents such as testicular hyaluronidase (TH), chondroitinase ABC, alpha-chymotrypsin, and cytochalasins result in IOP reduction by degrading GAGs in the ECM, disruption of actin assembly, or by preventing the polymerization of microtubules.

Hyaluronic acid (HA) was first discovered by Meyer Palmer in 1934 in bovine eyes. Soon thereafter, the enzyme hyaluronidase was discovered in pneumococci in 1936 and this was found to be identical to the spreading factor of Hoffmann and Duran-Reveals, which was obtained from testicular extracts. In comparison to TH, streptomyces hyaluronidase (SH) is a more specific HA-degrading enzyme, active over a wide pH range, it is temperature stable, and is not inhibited by sulfated GAG polymers.

Testicular Hyaluronidase
TH was first demonstrated by Barany to reduce the outflow resistance in enucleated eyes. This agent appears to bring about structural changes...
in the juxtacanalicular tissue (JCT). It also facilitates movement of aqueous solutions through the connective tissues by acting along a pressure gradient. The initial action of TH is to reduce the interaction of mucopolysaccharide solution with the confining structures and later, when considerable degradation of the tissues has occurred, the interaction of water with the mucopolysaccharides is affected. This affects AH dynamics, leading to increased outflow. Studies have found that the TM has hyaluronidase-sensitive and hyaluronidase-resistant areas, which respond differently from hyaluronidase.

A rapid exchange of anterior chamber contents with hyaluronidase was found to produce a rapid fivefold increase in facility with stabilization of measured facility after perfusion for 3 hours or more. The changes in the facility of outflow (the so-called “washout-phenomenon”) are assumed to be due to the gradual dissolution of the hyaluronidase-sensitive component of the JCT. Some other studies found only a moderate decrease in the HA in perfused, control eyes. They concluded that the “wash-out” effect of prolonged infusion is the result of other cellular or extracellular changes in the aqueous outflow pathway, rather than due to removal of GAGs per se.

In another study, perfusion of TH was associated with a 50% decrease in aqueous outflow. It has been suggested that in nonprimate species there is a GAG barrier to aqueous outflow, which is sensitive to TH infusion. In human eyes, infusions of TH have given variable and inconclusive results. Infusion of 100 units of TH resulted in a moderate overall effect. However, a significant increase was observed after 30 minutes. The maximum effect was a 29% decrease in outflow resistance. However, anterior chamber injection of bovine TH in rabbit eyes in amounts ranging from 1.5–150 IU was found to produce persistent corneal edema, severe anterior chamber fibrin, and endothelial necrosis in majority of eyes.

**Streptomyces Hyaluronidase**

Infusion of SH was found to result in a significant increase in the facility of outflow after 15 minutes. The total decrease in outflow resistance was 4–6% for 1 unit and 50% for 10 units of SH. This study showed SH is more effective than TH in degrading HA.

**Alpha-chymotrypsin**

Alpha-chymotrypsin is a protease that was used to lyse lens zonular fibers during intracapsular cataract extraction. Anterior chamber perfusion of 50 µg/ml alpha-chymotrypsin in monkey eyes caused a marked rise in outflow facility. Structurally, the intertrabecular spaces in the angle were found to be wider than normal, openings in the uveal and corneoscleral meshwork appeared enlarged, and there was ballooning of the juxtacanalicular TM and innerwall of the SC into the canal, and also splitting of the innerwall of the SC into the canal, and also splitting of the innerwall of the canal. Bill reported that a 30-minute perfusion of 50 µ/ml alpha-chymotrypsin lead to a marked rise in outflow conductance, which was well maintained over 2 hours. No side effects were noted during the experiment or for a few weeks subsequently. However, Melena noted an increase in the AH protein levels attributed to the breakdown of the blood aqueous barrier leading to ocular hypertension in rabbits.

**Calcium Chelators**

Calcium ions are required for cell adhesion and permeability in many systems. Thus, removal of calcium ions (Ca++) from the AH can alter the biochemical properties of the TM. This would affect AH dynamics and “might be a procedure of interest in the treatment of glaucoma.”

Calcium-free fluid containing sodium ethylene diamine tetracetic acid (Na2EDTA) or (ethylene glycol-bis-β-amino-ethyl ether)-N,N,N,N'-tetracetic acid (EGTA) have been used as a chelating agents to bind Ca++ entering the anterior chamber through the AH or by diffusion from the tissues.

Experiments have shown that infusion of Na2EDTA led to a marked rise in gross outflow facility at 4 mM as well as at 6 mM. The outflow facility was found to increase 20 minutes after the start of the perfusion and reached a maximum at 40–50 minutes. The adverse events noted were pupillary dilatation and corneal cloudiness, which remained for 1–2 weeks before subsequently subsiding. Similarly, 80–90 minutes of EGTA infusion also caused a marked increase in gross outflow facility, which was however partly reversible. These effects have been attributed to the binding of Na2EDTA to Ca++ and Mg++, while EGTA is a rather specific Ca++ chelator.

The decrease in outflow resistance has been attributed to: loss of cell processes from trabecular cells, damaged trabecular cores, distortion of the cribriform meshwork with loss of ECM, ballooning of the IW of the SC into the SC, and ruptures in the IW.

Hamanaka reported the AC perfusion of 0.5 mM of Na2EDTA in monkey eyes to be effective in increasing the facility of aqueous outflow within 60 minutes. However, after interruption of perfusion there was rapid reduction in outflow facility, which is presumed to be due to occlusion of the ruptures in the SC by thrombocytes.

The commercial application of calcium chelators in glaucoma management is yet to be seen. Na2EDTA suffers from the disadvantage of not being able to penetrate the corneal epithelium. Thus, it has to be infused directly into the AC or possibly by the use of iontophoresis.

**Cytochalasins**

Cytochalasins are fungal metabolites that were found to interfere with the polymerization process by which globular cytoplasmic actin aggregates into microfilaments of actin. Anterior chamber infusion of cytochalasin-B and -D in monkey eyes was found to cause distention of the cribriform meshwork, separation of its cells, and ruptures in the IW endothelium, leading to washout of the extracellular material. This also lead to a sixfold increase in outflow.
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in conventional outflow facility. These agents also cause disruption of actin filaments. Studies have found that a continuous exposure of cytochalasin B in a concentration of more than 15 μg/ml for 30 minutes causes sufficient disruption of the endothelial lining leading to extensive washout of the extracellular material in the trabecular meshwork.14

Johnson reported around 40 % increase in the facility of aqueous outflow after infusion of 0.6 and 1.1 mg/ml of cytochalasin D. The onset of effect started within 40 minutes, peaked at 2 and 6 hours, and lasted at least 6–14 hours.15 Cytochalasins also act by capping the filaments and preventing their elongation, thereby affecting a wide variety of actin-dependent cellular events including cell morphogenesis, motility, and endocytosis. Svedbergh, in his study, also reported a consistently large increase in outflow facility with 5 μg of intracameral cytochalasin B in cynomolgus monkey eyes.16

Cytochalasin B increases the hydraulic conductivity of cultured monolayers of human TM cells, accompanied by a retraction of the cells and a widening of the intercellular spaces. It is also reported to cause a disruption of endothelial lining and a washout of extracellular material in the TM tissue. These actions contribute to the increased AH outflow.17,18

Cytochalasins bind to the growing end of the microfilaments, capping them and prevent the polymerization of actin monomers into F-actin containing microfilaments.19 Actin filaments are normally in a state of polymerization and depolymerization. Cytochalasin interferes with the polymerization, leading to shifting of the equilibrium towards eventual depolymerization of the microfilaments. Cytochalasin D in humans has been reported to produce changes that were similar to those seen in primates with cytochalasin B.

The mechanism of action of colchicine is through an increase in the rate of outflow across the TM and, consequently, reduce the IOP.22 It blocks the phosphorylation activity of diverse serine-threonine kinases including protein kinase C, myosin light chain kinase (MLCK), and Rho kinase. It also reduces the actinomyosin-driven contractility and leads to deterioration of the actin microfilament system.23,24

Adhesions between cells and between cells and the ECM in the TM contribute to the geometry and flow resistance within the TM. These adhesions include complex transmembrane interactions between the external cell surface and the actin-based cytoskeleton. This is mediated by specific adhesion receptors (integrins and cadherins) and a variety of submembrane anchor proteins (e.g. vinculin and catenins), which link the microfilaments to the membrane in these sites.25

H7 treatment has been found to weaken cell–cell or cell–ECM junctions in cultured cells. H7-treated cells appear to round or thicken towards the centre in a dose- and time-dependent manner. H7 can destabilize the overall architecture of the TM and SC by inducing changes in cell shape and cellular attachments. This opens the flow pathways and leads to formation of new flow routes, thereby reducing flow resistance and IOP.

A study by Liu, treatments with 100 µM of H7 led to a mild effect of cell rounding and apparent thickening towards the centre of cells, within 1 hour of exposure. After exposure to 300 µM of H7 for 1 hour the cells became more refractile towards the centre. After 24 hours of exposure to 100 µM or 300 µM of H7, nearly all treated cells showed these changes. Also, actin-containing bundles were found to deteriorate rapidly after 30 minutes of exposure to H7, especially at higher doses. With longer exposures (2 hours), the stress fiber network was obliterated leaving only a diffuse mesh of actin filaments, mostly at the cell periphery.22

Side effects noted in the above study include a transiently increased protein concentration in the anterior chamber on treatment with 400 mM of H7. There was also an increased entry of intravenous fluorescein into the AH and cornea. There was an increase in the corneal thickness, with the corneal endothelial cell borders found to be indistinct on specular microscopy.

Tian noted a significant IOP reduction during 1 to 6 hours after topical instillation of H7 at a concentration of 400 mmol/l with a maximal decrease of 3.25 mmHg at hour 2 compared with that in the contralateral control eyes. The authors did not note any anterior chamber or lens abnormalities on topical or intracameral administration of H7.25

**Colchicine**

Colchicine is a plant alkaloid that binds specifically with tubulin on topical or intravitreal injection. Thus, it prevents polymerization of microtubules in various locations in the eye.22,26 A topical application of 50 or 100 µg of colchicine was found to produce significant lowering of IOP after 24 hours. The mechanism of action of colchicine is through an increase in the rate of aqueous outflow. However, despite continuous administration of colchicine, the IOP returns to baseline. Colchicine administration is also associated with significant ocular toxicity to the cornea and other anterior segment structures. Subsequently, IOP becomes elevated due to obstruction or disorganization of the outflow pathways. Lumicolchicine is an inactive isomer with insignificant affinity for tubulin. This isomer does not affect aqueous outflow, thus the mode of action of colchicine is through its interaction with tubulin and cytoskeletal elements in the trabecular meshwork.22

A study by Williams on albino rabbits showed that topically administered colchicine can also decrease the AH production in a dose-dependent manner. The mean rate of AH formation in control eyes was 3.68±0.09 μl/min (n=16). While, it decreased to 1.8 μl/min in eyes treated with 40 µg colchicine. In eyes treated with 10 μg/eye, the gross outflow facility was found to be 0.49±0.03 μl/minute/mmHg, which was significantly greater than that of the contralateral control eyes (0.39±0.03 μl/minute/mmHg).21

**H7**

H7 (1-(5-isoquinolinyl-sulfonyl)-2-methylpipperazine) is a broad-spectrum serine-threonine kinase inhibitor. It has been found to reversibly increase outflow facility across the TM and, consequently, reduce the IOP.22 It blocks the phosphorylation activity of diverse serine-threonine kinases including protein kinase C, myosin light chain kinase (MLCK), and Rho kinase. It also reduces the actinomyosin-driven contractility and leads to deterioration of the actin microfilament system.23,24

**Latrunculins**

Latrunculins are macrolides isolated from the marine sponge *Negombata magnifica* (formerly *Latrunculia magnifica*). Macrolides are compounds
whose activity stems from the presence of a macroclide ring, a large macrolactone ring to which one or more deoxy sugars might be attached. The two common latrunculins are latrunculin-A (LAT-A) and latrunculin-B (LAT-B). They differ structurally in the macrolide part, which could play a part in permeation into cells. LAT-A has a diene moiety, while LAT-B incorporates a monoene moiety. LAT-B, unlike LAT-A, is found to be slowly inactivated by an unknown serum component in cell culture medium. Thus, 48 hours after exposure to LAT-B, the cells completely recover, exhibiting a well-developed system of stress fibers. LAT-B also has a milder effect on corneal endothelial permeability compared with LAT-A. Unlike cytochalasin B and ethacrynic acid (ECA), LAT-B does not induce cell—cell separations in the live monkey eye, although it disrupts human TM cell—cell junctions in culture.26–28

LAT-A is a specific actin-disrupting agent that disassembles and disrupts polymeric actin filaments (F-actin) by sequestering monomeric actin (G-actin). It also causes cell separation and cell loss. Topical or intracameral LAT-A reversibly and in a dose- and time-dependent manner increases the outflow facility and lowers the IOP. LAT-A directly binds with G-actin and disrupts the actin microfilament network and subsequently interferes with cell contractility and adhesion. (H7 affects contractility, with secondary deterioration of actin microfilamentary system). LAT-A may also work by affecting other outflow-relevant cells in the chamber angle (e.g. collector channels, episcleral veins), as well as through other uncertain physiologic consequences.29

LAT-B is about 10 times more potent than LAT-A in increasing the facility of outflow, but has fewer effects on corneal endothelial permeability. Thus, LAT-B is assumed to be “gentler” to the ocular tissues. On perfusion of porcine eyes with LAT-B and LAT-A no signs of cellular toxicity were observed. In living monkey eyes, LAT-A was found to reduce IOP after 6 hours of administration. Contrarily, LAT-B brought the IOP down within 1 hour. A study by Ether showed that perfusion with LAT-B in a concentration of 1µM had a dramatic improvement in outflow facility in enucleated human eyes. This was equivalent in magnitude to that seen in enucleated porcine eyes, but less than that observed in living monkey eyes.26 LAT-B produces cytoskeletal disorganization in TM cells and IW cells of SC producing a substantial expansion of the space between the IW of SC and the trabecular collagen beams.27 Under baseline conditions, the IW and JCT cells are in a contracted state. A reduction in their contractility expands the tissues, altering the routes and extent of fluid flow.

Okka and colleagues studied the effects of single versus multiple doses of 0.005/0.01 % topical LAT-B on the outflow facility, IOP, and/or central corneal thickness in normotensive monkey eyes. Fifteen treatments of 0.01 % LAT-B were found to significantly increase the outflow facility in monkey eyes. The effect of the drug was found to last for more than 16 hours and no corneal toxicity was noted.28 Similarly, Peterson noted a dose-dependent two to threefold increase in outflow facility after a topical 21 µg instillation of LAT-A.29

LAT-A and LAT-B produce multiple small, brightly refractile, granule-like spots, or pseudogutta on the corneal endothelium.21 LAT-A and –B have been found to increase protein concentration in the anterior chamber, induce cell shape changes and increased permeability in the corneal endothelium, and lead to overall corneal thickening. These changes are usually mild and reversible. However, Peterson and colleagues studied LAT-A topical/perfusion in monkeys and did not notice any corneal changes on biomicroscopy.29

**Rho-associated Coiled Coil-forming Protein Kinase Inhibitors**

The Rho-associated coiled-forming protein kinase (ROCK) is a kinase that regulates the movement and shape of cells by its action on the cytoskeleton. It is a downstream effector of Rho in the Rho-dependent signal transduction pathway. ROCKs also lead to an abnormal accumulation of ECM (ECM hypothesis) and changes in contractile activity and cell adhesion interactions of the cells of aqueous outflow pathway (contractility hypothesis) leading to an increased resistance to drainage of AH through the conventional pathway. Specific inhibitors of ROCK modulate changes in the actin cytoskeleton and affect cellular motility at the level of trabecular meshwork, SC, and ciliary muscle, augmenting aqueous outflow.30

ROCK inhibitors are a potential new class of ocular hypotensive drugs. While more than a dozen ROCK inhibitors are known, only a few have been studied for their potential role as antiglaucoma medications. These agents include Y-27632, AR-12286, SNJ-1656/Y 39983/RKI 983, K 115, and INS 117548. However, as of 2014, only some of them were undergoing human trials, the development of others having been discontinued. These agents include: AMA 0076, AR-13324, K-115, and PG-324.33

AMA 0076 is a novel, locally acting Rho kinase inhibitor. It is structurally related to Y-27632. Previous studies have reported Y-27632 to produce Rho/ROCK dependent F-actin reorganization, impairment of focal adhesion formation and decreased MLC phosphorylation in TM and SC endothelial cells. This results in an increase in the facility of aqueous outflow.30,33 AMA 0076, developed by Amakem Therapeutics, Diepenbeek, Belgium, involves a soft drug approach, involving predictable metabolic inactivation of an active compound to a nontoxic metabolite, thus improving tolerability. Compared with latanoprost and bimatoprost, this compound was found to be more potent in preventing IOP spikes in an acute hypertensive rabbit model. No significant side effects, especially hyperemia were noted.34,35

AR13324 inhibits both Rho-kinase and norepinephrine transporter to increase trabecular outflow and decrease aqueous production as well. Topical AR13324 0.02 % and latanoprost in once-daily dosing were found to be equally effective in controlling IOP. The only side effect noted was mild to moderate conjunctival hyperemia.36

The AR-13324-CS202 Study Group performed a double-masked study comparing AR-13324-0.01 % and –0.02 % with latanoprost. AR-13324 0.02 % was found to be less effective than latanoprost by approximately 1 mmHg in patients with unmedicated IOPs of 22 to 35 mmHg. The major adverse event was ocular hyperemia, which was more common for both concentrations of AR-13324 than for latanoprost.37

Aerie Pharmaceuticals (Bedminster, New Jersey, US) has developed an agent named PG324 that combines AR13324 and latanoprost. The phase II trials aim to detect the effectiveness of this drug. This is regarded as the first antiglaucoma medication with three distinct mechanisms.

K-115 is a highly selective and potent isoquinoline sulfonamide compound. It is undergoing phase II evaluation. Preclinical testing of the compound in...
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monkey eyes showed a 2.3—4 mmHg decrease in the IOP compared with a 2.5 mmHg decrease with 0.005 % latanoprost.40

Iodoacetamide

Metabolic inhibitors have been used in the past to modify AH outflow. Experiments by Barany on iodoacetamide injection into the anterior chamber of calf eyes showed an increase in the facility of outflow by affecting the hyaluronidase-insensitive component of outflow resistance.41 Studies also show that iodoacetate can have specific effects on passive cellular membrane permeability, which are independent of their metabolic effects. It is assumed that membrane sulfhydryl groups in the trabecular endothelium may be directly involved in aqueous outflow dynamics and are modified by iodoacetamide.

Studies by Epstein showed that iodoacetamide causes an increase in the facility of aqueous outflow in a dose-response manner in calf and monkey eyes. This could be attributed to the inhibitory effect on glycolysis and energy production in the trabecular meshwork. However, TM excised from perfused as well as fresh, nonperfused eyes demonstrated a similar rate of glycolysis. A 15 and 30 µmol infusion of iodoacetamide significantly increased the facility of aqueous outflow. A single dose of 30 µmol of iodoacetamide was effective but not as much as those eyes that had been treated previously with multiple lower doses of iodoacetamide.42

N-methylmaleimide

Sulfhydryl (-SH) groups are one of the most prevalent and reactive ligands in biological tissues and are present in most cellular proteins. -SH also affect the aqueous outflow, presumably by an inhibitory action on energy production in the trabecular meshwork. But, more likely, there is a direct effect on aqueous outflow by the cellular -SH groups and related membrane activities. The state of cell membrane protein sulfhydryls may also affect the cellular or intercellular permeability to fluid flow in the aqueous outflow channels. Epstein and colleagues used N-ethylmaleimide (NEM), a specific -SH agent to study its effect on aqueous outflow. They found that a dosage of 4.7 mM or greater of NEM produced a significant increase in the facility of outflow in the calf and monkey eyes.43

Ethenacrynic Acid

Ethenacrynic acid (ECA) is an -SH-reactive agent that has been used as a systemic diuretic. It causes a dramatic reversible change in the cell shape in different cell lines including human trabecular, calf, and bovine pulmonary artery endothelial cells associated with changes in actin, α-actinin, vimentin, and vinculin. These shape changes are preceded by disruption and subsequent loss of β-tubulin staining. ECA is found to bind with tubulin and may affect tubulin metabolism directly.44 AC perfusion with this agent lead to breaks in endothelial lining of the SC and loss of cell–cell attachments between the IW and the subendothelial trabecular cells and between trabecular cells themselves.45

Ethier and colleagues, in their studies on living monkey eyes, found a 63 % increase in the outflow facility after intracameral perfusion with ECA. They also noted increased platelet adhesion to the IW of SC, decreased I pore density, and a tendency of increasing density of large B pores.46 Conversely, Epstein reported only a 20 % increase in the net facility shortly after a bolus injection of 10 µl of 2.5 mM ECA into the anterior chamber.47 However, Croft reported a 71 % increase in the outflow facility 1 hour after drug infusion.43 Melamed and colleagues injected intracameral ECA in patients with advanced OAG. They reported a drop in IOP ranging from 9–31 mm Hg after 5–15 µl ECA injection. The effect was noted 3–24 hours after treatment and lasted for 3 days. The IOP gradually returned to pre-treatment values 1 week later.47

No clinically apparent side effects have been reported in the anterior segments of cynomolgus/rhesus monkeys after intracameral ECA injections. Since these were young, healthy monkeys, it remains to be seen if the eyes of elderly glaucoma patients would behave similarly.48 However, Melamed in his study of intracameral ECA in OAG patients did not observe any adverse effects on the cornea or anterior chambers. This led him to suggest that ECA could be used as a new class of drugs to treat OAG.49

Some new drugs are being developed by modifying phenoxacyetic acid and acrylol moieties. These compounds were evaluated on the basis of effects produced on TM cell morphology, actin cytoskeleton, focal adhesions, and transcellular fluid flow. Based on these evaluations a compound SA9000 was found to produce a maximal reduction in IOP with minimal side effects compared with ECA, on either intracameral injection or topical instillation.50

Conclusion

Pharmacologic management of glaucoma has been successful in controlling IOP by reducing aqueous inflow through suppression of aqueous production or by increasing the aqueous outflow primarily via the unconventional uveal pathway. However, recently a new frontier in pharmacologic modulation of the facility of aqueous outflow through the TM has opened up. This concept of pharmacologic trabeculectomy raises the possibility of a more physiologic control of the aqueous outflow and achieving better control of the IOP. The present review sheds some light on these exciting developments that may be another weapon in our armamentarium to manage glaucoma.
Pharmacologic Trabeculectomy