

# Special Focus on Glaucoma

## Chapter 6

# Up-To Date of the Pharmacological Therapies for Glaucoma

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## Abstract

Glaucoma, a multifactorial degenerative disorder, affects more than 60 million people worldwide. The treatment of this disease relies on the use of drugs able to reduce/control intraocular pressure (IOP), one of the main risk factors for glaucoma. Current pharmacological therapies are based on the use of drugs belonging to well known categories such as adrenergic agonists and antagonists, cholinergic agonists, prostaglandin analogs, carbonic anhydrase inhibitors (CAIs) and Rho kinase (ROCK) inhibitors, alone or in fixed combinations. These classes of drugs are generally effective in reducing IOP; however, not all the patients respond to the therapy and important side effects impair the compliance, accounting for the necessity of novel therapeutic approaches. Therefore, other targets have been focused, such as the melatonin receptors, the fatty acid amide hydrolase (FAAH), the adenosine receptors and nitric oxide (NO), alone or in combination with classic pharmacological agents to validate new therapies for the management of glaucoma.

## 1. Introduction

Glaucoma, a multifactorial, degenerative disorder of the optic nerve, is a leading cause of irreversible visual impairment and blindness, afflicting more than 60 million people worldwide, with a discouraging estimate of 80 million in 2020 [1]. Glaucoma is a group of diseases resulting in progressive ocular neuropathy, characterized by loss of retinal ganglion cells (RGC) and morphological alterations of optic nerve head, often associated with high intraocular pressure (IOP). The increase in IOP is connected to a malfunction of the ciliary processes and of the trabecular meshwork (TM) in the anterior chamber of the eye [2-5]. These tissues physiologically support adequate pressure in the eye by regulating aqueous humor (AH) production and its drainage [6, 7]. Aqueous humor is a transparent liquid, rich in bicarbonate, which fills the region between the cornea and the lens and it is continuously secreted by the ciliary body around the lens and constantly flows from the ciliary body to the anterior chamber. From the anterior chamber the AH is filtered through the TM into the Schlemm's canal and after into a multitude of aqueous veins which merge with the blood carrying veins. Elevated IOP is a consequence of an imbalance between production and outflow of AH, whose effect leads to a steep increase of the IOP to value major than 20 mmHg [8, 9].

This increased IOP if untreated, can lead to a reduction of local perfusion, chronic ischemia leading to the reduction of RGC by autophagy and apoptosis [10]. When the number of RGC is no longer suitable for neuronal transmission, the visual field becomes narrower.

There are different form of glaucoma, the two main types are open-angle (OAG) and closed-angle (CAG) glaucoma [11]. In OAG, the anterior chamber angle is open but the AH drainage through trabecular meshwork is almost shut; in CAG the peripheral iris blocks the anterior chamber angle by apposition of synechiae, preventing AH drainage [10,12]. In normotensive glaucoma, the optic nerve head becomes damaged even though IOP is within the normal range. There is no consensus on the pathogenesis of this kind of glaucoma but the most reasonable explanation is the reduction of ocular perfusion with less oxygen and nutrients supplied to the optic nerve. This limited blood flow could be caused by atherosclerosis or other conditions that impair circulation. Long course studies showed that IOP reduction, pharmacologically or surgically obtained, is beneficial on visual field conservation also in these patients [13]. Glaucoma can arise and progress in a silent way, making the diagnosis feasible uniquely at later, advanced stages, when important and irreversible losses of visual field have occurred. The diagnosis of this complex disease is made by measuring IOP and checking optic nerve fibers and visual field defects. The ineffectiveness of several drugs has sometime been attributed to the lack of reliable markers supporting the clinical diagnosis in the early stages of the disease. Many genetic mutations have been associated with high IOP [14] and epigenetic individual factors can modify the presentation and the progression of the disease [15]. Many efforts are currently made in these fields for improving the early diagnosis of the disease and

monitoring the progression.

The treatment for glaucoma includes three different approaches for lowering the elevated IOP associated with the disease: a) pharmacologic therapy with different drugs b) laser therapy and c) surgical management [5,16], considering the wealth of new data in glaucoma research.

The current chapter presents an overview of the pharmacological treatments of glaucoma. Nowadays there are six classes of agents clinically used for the treatment of glaucoma: adrenergic agonists and antagonists [17]; systemically or topically acting carbonic anhydrase inhibitors (CAIs) [5]; cholinergic agonists [18]; prostaglandin analogs [19] and the recently introduced in clinical practice Rho kinase (ROCK) inhibitors. It is important to note that other targets have been focused, such as the melatonin receptors [20], the fatty acid amide hydrolase (FAAH) [20], the adenosine receptors [21] and nitric oxide (NO), alone or in combination with other pharmacological agents to validate new therapies for the management of glaucoma.

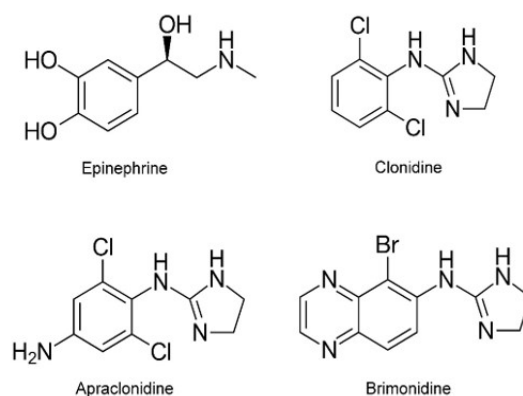
## 2. The Adrenergic Agonists and Antagonists

### *a) $\alpha_2$ agonists*

$\alpha$ -Adrenoceptors are abundantly present in the eye, mostly in the smooth muscle cells of the iris, in the blood vessels of the conjunctiva and of the ciliary bodies and the aqueous outflow tract [22]. These drugs lower the IOP through a stimulation of  $\alpha$  receptors, which induces a constriction of blood vessels thus decreasing the AH production. Epinephrine (adrenaline) has been the first representative of this group of drugs. A 0.5 to 2% concentration of the L-isomer or the racemic mixture is used, but tolerance is developed in prolonged treatments, moreover side effects and low bioavailability limits the use of adrenaline in clinic. Dipivalyl epinephrine is a pro-drug of epinephrine, more lipophilic, which penetrates the cornea where an esterase forms epinephrine and reduces of about 1% IOP in the anterior segment [23]. Selective  $\alpha_2$ -adrenergic agonists are generally used. Clonidine, a selective  $\alpha_2$  agonist, with some  $\alpha_1$  effect, decreases IOP by reducing AH production; clonidine induces systemic hypotension and lowered the pressure in the ophthalmic artery, inducing visual field defects, for these reasons its clinical use has been abandoned [24]. A clonidine derivative, apraclonidine, which has lower lipophilia than clonidine and thus lower permeability of blood brain barrier was introduced in therapy. This drug reduces IOP mainly decreasing AH production and increasing trabecular blood flow and it is used after post-laser and post-surgical IOP elevation [25]. Chronic treatment is endowed with several side effects such as allergic reaction, blepharo- and follicular-conjunctivitis, hyperemia, itching and tearing [26].

Brimonidine, the last of this class of drug, is highly  $\alpha_2$  selective molecule and it is administered in lower concentrations. Brimonidine reduces IOP decreasing AH production and increasing uveoscleral outflow. It is mainly used to prevent hypertension after laser

trabeculoplasty and also for chronic treatment of OAG patients. The patient compliance for this drug is not very good for its short pharmacological action: eye drops must be applied 3-4 times daily. The side effects are similar to apraclonidine [27]. In **(Figure 1)** are reported the structures of  $\alpha_2$  agonists.



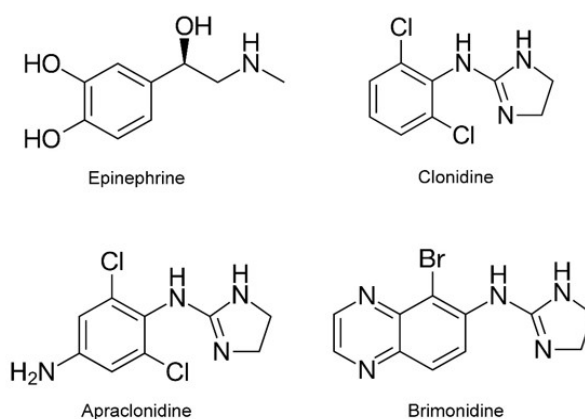
**Figure 1:** Structure of  $\alpha_2$  agonists

### b) $\beta$ -blockers

After the approval of the first  $\beta$ -blocker for the treatment of glaucoma by USA-FDA, timolol became the first choice of treatment for the disease [28]. Topical  $\beta$ -blockers reduce IOP by blockade of sympathetic nerve endings in the ciliary epithelium causing a fall in AH production. Two types of topical  $\beta$ -blockers are available for use in glaucoma: non-selective, which blocks both  $\beta_1$ - and  $\beta_2$ -adrenoceptors; and cardioselective, which blocks only  $\beta_1$  receptors. Among the  $\beta$ -blockers commercially available, timolol, levobunolol, metipranolol and carteolol are non-selective, and betaxolol is cardioselective. Timolol, the most active compound of the class, requires at least twice a day administration to maintain the pharmacological effect, although levobunolol is equally effective and can be used once daily with little difference in effect. Many studies have shown that timolol, available in 0.1, 0.25 and 0.5% solution reduces from 27 to 35% IOP during long term treatment [29]. A gel formulation permits a once daily administration. Of note, patients with dark iris need a higher concentration of the drug for the binding of timolol to the iris pigment.

Timolol has few local side effects, such as hyperemia, but it may induce systemic adverse effect by blocking  $\beta_1$ -adrenoceptors of the heart, thus inducing bradycardia, arrhythmia, congestive heart failure and Adam-Stokes syndrome. Moreover, blocking the  $\beta_2$  receptor at lung level, timolol can induce bronchospasm in asthmatic patients [30]. Carteolol is a non-selective  $\beta$ -blocker with some isoprenaline activity (ISA) and local anesthetic activity and it has been found to act as a serotonin 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor antagonist. It is used twice daily and any theoretical advantage in diminished side effects conferred by its partial  $\beta$  agonist activity compared with timolol has not been fully substantiated [31]. Metipranolol is a non-selective  $\beta$  blocker without ISA; it is administered in 0.1, 0.3 and 0.6 % solutions, twice daily. Plasma high density lipoprotein cholesterol levels are increased with chronic treatment [32].

Betaxolol has an effect comparable to timolol in lowering IOP, but is less effective in some patients. Local stinging can be a problem in some patients with betaxolol, although it seems relatively free of adverse respiratory effects, but this may be dose-related and extreme caution should still be exercised in patients with any history of respiratory illness [33]. Because of the lower risk of precipitating side effects, betaxolol is probably the  $\beta$ -blocker of first choice for use in glaucoma; timolol or levobunolol are reserved for patients who do not respond satisfactorily to betaxolol and are quite free of respiratory disease [34]. The structure of clinically used  $\beta$ -blockers are reported in (Figure 2).



**Figure 2:** Structures of  $\beta$ -blockers

In ophthalmology, many types of new drug delivery systems have been investigated for the clinical use of  $\alpha_2$ -agonists and  $\beta$ -blockers [35]. *In situ* gel systems show a good adherence permitting an enhanced permeation and increasing the availability of the drug in the eye. Timolol maleate loaded *in situ* with a gelling system using carbopol and chitosan to increase the viscosity was proposed in 2010 [36]; later brimonidine tartrate with carbopol was evaluated in comparison with conventional eye drops [37].

Several recent studies evaluated the combination of different drugs with polymers to improve drug delivery. Timolol maleate with temperature sensitive or with pH sensitive polymers were evaluated and an improve drug permeation was achieved. Niosome, bilayered vesicles similar to liposomes, are preferred in comparison to other delivery system for lower toxicity. This system increases significantly drug permeation in comparison with conventional eye drop [38]. Recently a preparation of timolol maleate combined with hydrogel containing gelatin and silk elastin, has been proposed; this preparation increased the retention of the drug in the cornea, reducing IOP for a longer period of time [39]. Betaxolol with chitosan, timolol with galactosylated chitosan, both natural polysaccharides, have been formulated, showing a sustained drug release leading to a longer IOP decrease. De Souza and coworkers [40] incorporated brimonidine tartrate into mucoadhesive chitosan ocular inserts providing a sustained drug release up to 30 days. A unique new patent, JP2014139175.2014, an  $\alpha$ -adrenergic agonist, has been claimed in the last 5 years for treating pain and glaucoma [41]; while all the other patents describe new formulations, combinations and drug delivery systems.



### 3. Carbonic Anhydrase Inhibitors

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes. In mammals, 16 alpha-CA isozymes or CA-related proteins have been described [42], with different catalytic activity, subcellular localization, and tissue distribution. There are five cytosolic forms (CA I, CA II, CA III, CA VII, and CA XIII), five membrane-bound isozymes (CA IV, CA IX, CA XII, CA XIV, and CA XV), two mitochondrial forms (CA VA and CA VB), and a secreted CA enzyme (CA VI) [43,44]. CAs catalyze a physiological reaction that converts  $\text{CO}_2$  into bicarbonate ion and protons. Many of these isoenzymes are important therapeutic targets for the treatment of a range of diseases including glaucoma. The main constituent of AH is bicarbonate and CA in ciliary processes is responsible bicarbonate secretion and after the discovery of this information [45], acetazolamide, a sulfonamide CAI was shown to decrease IOP in animals and humans. This was the beginning of glaucoma treatment with CAIs. Discovered in the 1950s, the heterocyclic sulfonamides acetazolamide, methazolamide, and ethoxzolamide, as well as the aromatic compound dichlorophenamide, represent the first generation of clinically used CAIs.

The use of systemic inhibitors, such as acetazolamide (Diamox) is useful for decreasing elevated IOP characteristic of many glaucoma forms, however their use leads to several side effects, including metallic taste, depression, fatigue, weight loss, decreased libido, gastrointestinal irritation and metabolic acidosis [46]. Acetazolamide and dichlorophenamide are still used for the treatment of refractory glaucoma, which does not respond to adrenergic antagonists or prostaglandin analogues. The idea to administer topically sulfonamide CAIs was addressed in the 1950s but only many years later in 1995, the first pharmacological agent, dorzolamide, was launched by Merck (Trusopt) for clinical use, as 2% eye [47]. Brinzolamide was discovered at Alcon Laboratories and approved in 1999 for the clinical use (Azopt) [48]. Dorzolamide and brinzolamide are nanomolar CA II/CA XII inhibitors, possess good water solubility, and are enough liposoluble to penetrate through the cornea. A multicenter, double-masked, prospective study showed that brinzolamide 1.0% caused less ocular discomfort than dorzolamide 2.0%. The incidence of ocular discomfort, burning and stinging, on instillation of brinzolamide, twice daily, was significantly less compared with the treatment with dorzolamide. The most common side effects are stinging, burning of the eye, pruritus, and bitter taste. These two formulations are still in use; dorzolamide is often selected as reference drug for the development of new CAIs.

Novel type of sulfonamide CAIs with good water solubility and IOP-lowering effects have been developed with a chemical approach defined "*tail approach*," which consist to combine water-solubilizing functionalities with aromatic and/or hetero aromatic sulfonamides incorporating amino, imino, hydroxyl, or sulfonyl moieties. Such moieties include pyridine-carboximido, carboxypyridine-carboxamido, quinoline-sulfonamido, picolinoyl, isonicotinoyl,

perfluoroalkyl/aryl/sulfonyl, and amino acyl groups. These new drugs are 2 or 3 times more effective than dorzolamide, possess good water solubility, good penetrability through the cornea, inhibition in the low nanomolar range against hCA II and hCA IV, and very good IOP-lowering properties in both normotensive and glaucomatous rabbits [49].

Successively, a series “click”-tailed benzenesulfonamides was reported in 2016, which acted as low to subnanomolar inhibitors of hCA II and XII. Some such compounds demonstrated remarkable efficacy in lowering IOP in a rabbit model of glaucoma induced with hypertonic saline [4]. New sulfonamides, sulfamates, and sulfamides with CA inhibitory properties have been synthesized in the last 10 years, with a supposed IOP effect [49]. Pfizer reported tricyclic derivatives with CA II and CA IV inhibitory effect, but no IOP-lowering data were published. Solvay Pharmaceuticals reported sulfamates and sulfamides together with bicyclic or polycyclic/spiro scaffolds. Both types of compounds were low micromolar CAIs and were claimed to be effective for several diseases including glaucoma [50].

The CAIs in clinical use (acetazolamide, dorzolamide, and brinzolamide) probably act as local vasodilators, improving the local blood circulation and consequently clearing metabolic waste products, an effect mediated by CAI inhibition.

New chemotypes that inhibit CA in the same manner than sulfonamides emerged in the last years; among them, the dithiocarbamates (DTCs) are very interesting. These compounds were discovered after the report that trithiocarbonate-CS3-2 was an inhibitor of CA at very low concentrations [51].

DTCs are compounds that incorporate the zinc binding group with new moieties and have been the subject of a large synthetic campaign by Supuran group [52]. These molecules were evaluated for their inhibitory activity against different CA enzymes, among which those involved in glaucoma. The highly water-soluble morpholine DTC was very effective in decreasing IOP in an animal model of glaucoma, when administered topically, directly in the eye of carbomer-induced hypertensive rabbit [53] and might be included in the future antiglaucoma drug armamentarium.

#### **4. Cholinergic Agonists**

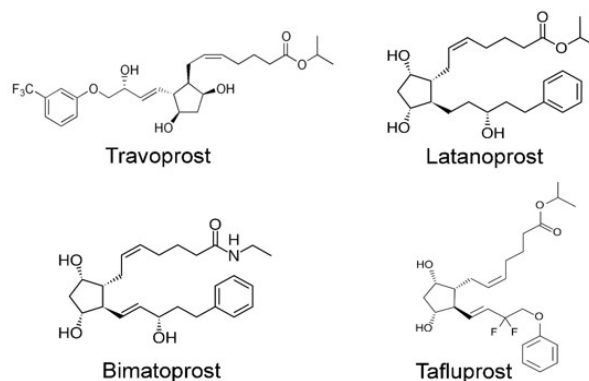
Cholinergic drugs were introduced over 100 years ago and they were the first class of agents used for the treatment of glaucoma and, still now they are useful for the short-term management of CAG associated with pupillary block. Pilocarpine is a muscarinic alkaloid obtained from the leaves of tropical American shrubs, from the genus *Pilocarpus*. It and other miotic agents help to prepare an eye for iridotomy, but these drugs are not a substitute for it in CAG pupillary block. Cholinergic agonists cause contraction of the longitudinal ciliary muscle, tighten the iris, decrease the volume of iris tissue in the angle and pull the peripheral iris away

from the TM, increasing outflow of AH. This results in a 15-25% reduction in IOP. If the IOP is quite elevated, i.e about 40-45 mmHg, the pupillary sphincter may be ischemic and may not respond to cholinergic stimulation [54]. While over a century of experience have confirmed the utility of pilocarpine in the treatment of acute glaucoma attacks, recent evidence suggests that the mechanism of action of this drug is not completely understood and caution is warranted with the long-term use of these agents in narrow-angle eyes with iridotomy [55]. A randomized study with a combination of pilocarpine 1% and clonidine 0.125% showed an IOP reduction comparable to that achieved with timolol 0.25% twice daily [56]; this combination could be used in patients where  $\beta$ -blockers are contraindicated. Systemic side effects of pilocarpine are rare; however, ocular side effects are common and are brow ache, induced myopia, miosis, leading to decreased vision, shallowing of the anterior chamber, retinal detachment, corneal endothelial toxicity, breakdown of the blood-brain barrier, hypersensitivity or toxic reaction, cicatricial pemphigoid of the conjunctiva and atypical band keratopathy. Indirect-acting para-symphomimetics inhibit the enzymatic destruction of acetylcholine by inactivating cholinesterase, leaving acetylcholine free to act on the effector cells of the iris sphincter and ciliary muscle, causing pupillary constriction and spasm of accommodation. Physostigmine is one of the oldest drugs and was successfully used for the treatment of glaucoma since 1864. Anti-cholinesterase agents are generally more potent than pilocarpine, but they have more intense side effects. In addition, indirect agents can cause iris cysts in children and cataract in adults. Finally, prolonged respiratory paralysis may occur during general anesthesia in patients who are in treatment with cholinesterase inhibitors because of their inability to metabolize paralytic agents such as succinylcholine [57]. Echothiophate iodide 0.06% is an indirect agent that inhibits acetylcholinesterase and its maximal effect occurs within 24 hours, lasts up to 2 weeks, and induces intense miosis. Carbachol is a mixed direct agonist and acetylcholine releasing agent.

## 5. Prostaglandin Analogs

Nine known different prostaglandin (PG) receptors are expressed in different cell types; they are G-protein-coupled receptors and modulate a large number of biological responses in the human body due to the fact that these receptors are expressed in various tissues including the eye [58]. All four PGE<sub>2</sub> receptors are expressed in human cornea, conjunctiva, trabecular meshwork, iris, ciliary body and retina [59], but the first molecules acting on these receptors had several side-effects; however the IOP-lowering action of PGs attracted the attention of the scientific world and led to investigate different PG receptors and to develop the first clinically used FGF<sub>2 $\alpha$</sub>  derivatives, such as latanoprost, bimatoprost, travoprost and tafluprost for the treatment of glaucoma (**Figure 3**).





**Figure 3:** Structures of Prostaglandin Analogs

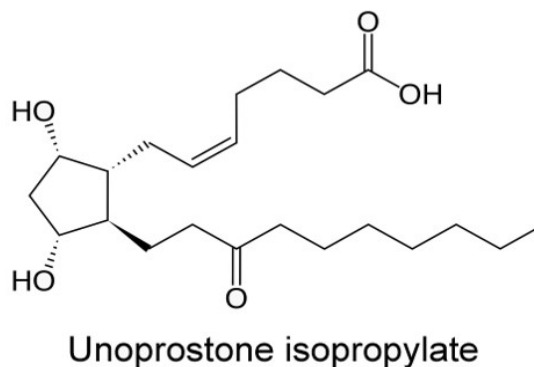
These molecules activate both TM and ciliary muscle cells, increasing the outflow of AH [60]. The mechanism by which PGs increase uveoscleral outflow is not yet completely understood. Several studies suggest that the antiglaucoma effect is probably due to two different mechanisms: the first is the unconventional pathway, increasing AH outflow by binding to prostaglandin E and F receptors in the ciliary muscle, resulting in ciliary muscle relaxation and increasing AH outflow [61]. The second is the conventional pathway, where PG analogs modulate outflow pathway throughout the increase of TM cells contractility and the decrease of cell contractility of the inner wall of Schlemm's canal [62]; of note PG analogs up-regulate metalloproteinases, enzymes involved in extracellular matrix remodeling, making the area more permeable to AH.

Since its introduction in the clinical market in 1996 in the U.S.A., latanoprost has become the most popular drug for the treatment of glaucoma in the world. Latanoprost has been compared with timolol in several multicentric trials and a single drop of 0.005% solution once daily was found more effective in lowering IOP than twice daily timolol [63]. A long-term study of five years with latanoprost has shown no loss in efficacy and for this reason USA-FDA approved this drug as first-line use for glaucoma treatment. Latanoprost showed good ocular tolerability. Conjunctival hyperemia occurs within the first days of treatment which diminishes with time. A history of intraocular inflammation may predispose some glaucoma patients in therapy with latanoprost to cystoid macular edema and/or uveitis.

Unoprostone isopropylate is a structural analog of an inactive biosynthetic cyclic derivative of arachidonic acid, 13,14-dihydro-15-keto-prostaglandin  $F_{2\alpha}$ . It differs structurally from other PGs for the presence in its structure of a 22-carbon chain backbone instead of the typical 20-carbon structure found in other compounds (**Figure 4**). Unoprostone decreases IOP by increasing the outflow facility without affecting AH production. Used in monotherapy, this drug has an effect similar to betaxolol but less than timolol or latanoprost [64], however a six-month study evidenced that unoprostone provide additive IOP lowering effect when associated with  $\beta$ -blockers in patients with OAG [65]. In healthy volunteers, unoprostone increases microcirculation in the optic nerve head. Bimatoprost is a prostamide analog with ocular hypotensive activity whose effect are due to an increase outflow facility. A multicentric

trial of six months evidenced a superiority of bimatoprost in lowering IOP in comparison to timolol or to latanoprost, while its IOP lowering effect was similar to timolol-dorzolamide combination [66,67]. Mild conjunctival hyperemia is the most frequent side effect. Travoprost is a synthetic prostaglandin  $F_{2\alpha}$  analogue and diversely from the other compounds that are partial agonist, it is a full agonist of the  $PG F_{2\alpha}$  receptor.

In clinical studies, travoprost once daily produced an IOP reduction similar to bimatoprost or latanoprost, with little diurnal fluctuation and results in a large percentage of patients the most active compound [68]. Tafluprost is the last prostaglandin analogue introduced in the clinical therapy. It is used topically to control the progression of OAG and in the management of ocular hypertension, alone or in combination with other medication. As all the other PGs, it reduces IOP by increasing the outflow of AH from the eyes. Trafluprost, as a lipophilic ester, easily penetrates the cornea and is then activated to the carboxylic acid, tafluprost acid. Onset of action is 2 to 4 hours after application, the maximal effect is reached after 12 hours, and ocular pressure remains lowered for at least 24 hours [69].



**Figure 4:** Structure of unoprostone isopropylate

## 6. Rho-Kinase Inhibitors

Rho-kinase (ROCK) inhibitors have been evaluated for their IOP lowering effects in animal model of glaucoma starting from the evidences that molecules active on cytoskeleton such as latrunculin can affect the contractile properties of TM, influencing AH outflow [70]. ROCK is one of the best characterized effector of RhoA, a small GTPase belonging to the subfamily of Ras protein superfamily. The activation of the binding of Rho to its GTP protein is controlled by GTPase activating proteins and by guanine nucleotide exchange factors which are controlled by TGF- $\beta$  and endothelin-1. Once GTP is bound, Rho activates a number of effectors including ROCKs, serine/threonine protein kinases existing in two isoforms: ROCK-I and ROCK-II both expressed throughout the body. The crucial role of Rho/ROCK pathway in cell proliferation, cell migration and cellular contraction makes ROCK inhibitors promising therapeutic agents for the treatment of several diseases including glaucoma [71]. Relaxing TM tissue, ROCK inhibitors directly decrease resistance in the conventional AH outflow, thus resulting in a significant IOP-lowering effect [72].

Ripasudil, was introduced in the therapy of glaucoma in 2014. In 2014, ripasudil, a ROCK inhibitor, gained approval in Japan to be used for treatment of ocular hypertension and glaucoma. As recently as December 2017, Rhopressa, a Rho kinase inhibiting drug, consisting of netarsudil, gained Food and Drug Administration (FDA) approval; the first of this kind of drug to be approved in the United States of America [73]. Ripasudil was shown to lower IOP within two hours after instillation of the drop solution, and was proven to do so consistently over a period of a full year [74]; however, this drug caused conjunctival hyperemia in the majority of subjects in each clinical trial reviewed. This is a dose-dependent side effect and it is seen in the majority of patients treated with ripasudil [74].

Netarsudil, is a ROCK inhibitor with norepinephrine transport inhibitory activity. This compound decreases IOP within two hours of instillation and maintains this decrease for a 24-hour period after dosing [75]. Netarsudil uses two mechanisms to lower IOP: by acting as both a ROCK inhibitor and a norepinephrine transport inhibitor. The latter helps to prolong reduction in IOP by constriction of vascular structures in the eye. This reduces blood flow to the ciliary processes, inhibiting production of AH. Two phase-3 clinical trials compared the safety and efficacy of netarsudil to timolol in patients with elevated IOP and as ripasudil the most commonly seen side effect is conjunctival hyperemia [76].

## 7. Combined Therapies For The Therapy Of Glaucoma

Combining ocular hypotensive drugs is indicated when the target pressure for a patient cannot be reached with a monotherapy. The first fixed combination product was a PG with a  $\beta$ -blocker (latanoprost 0.005% plus timolol 0.5%) followed by other PGs like travoprost, bimatoprost or tafluprost with timolol. All these fixed combinations effectively controlled IOP for 24 hours and had a similar effect on diurnal and nocturnal IOP variation. The efficacy and the safety of a triple fixed combination, timolol-brimonidine-bimatoprost was evaluated in a clinical trial [77,78], showing a superiority with an acceptable safety and tolerability. Combination therapy of  $\beta$ -blockers is also additive with miotics, topical CAIs and  $\alpha$ -agonists. Timolol 0.5% plus dorzolamide 2% induced an increased reduction of IOP of 13 to 29% in comparison to timolol alone [79]. In 2015, two multicenter, randomized studies evaluated for the first time ripasudil hydrochloride, a ROCK inhibitor in combination with  $\beta$ -blockers or prostaglandin analogs [79]. These clinical trials found additive IOP-lowering effects of ripasudil from placebo at peak levels either in combination with timolol or with latanoprost.

## 8. From Combination Therapy To Multi-Target Strategies For The Treatment of Glaucoma.

In recent years, an approach consisting of multi-targeted compounds is emerging: the design of hybrid molecules incorporating moieties able to interact at different biological levels for lowering IOP. Derivatives of PGs, agonists of  $\text{PGF}_{2\alpha}$  receptors, with several linkers

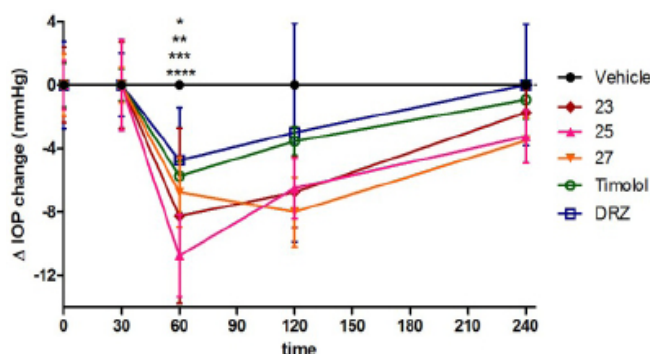
bearing nitric oxide (NO)-releasing moieties have been patented [20]. Latanoprostene-bunod (LBN) is composed of latanoprost acid (LA) linked to a NO-donating moiety and it is the first NO-releasing prostaglandin analog to be submitted for marketing authorization in the United States. The role of latanoprost in increasing uveoscleral outflow of AH is well established [80]. Pharmacokinetic studies in rabbits and corneal homogenates indicate that LBN is rapidly metabolized to LA and butanediol mononitrate; NO is subsequently released as shown by increased cyclic guanosine monophosphate (cGMP) levels in the AH and iris-ciliary body in rabbits and in human TM cells. LBN reduced myosin light chain phosphorylation, induced cytoskeletal rearrangement, and decreased resistance to current flow to a greater extent than latanoprost in TM cells, indicating that NO released from LBN elicited TM cells relaxation [80]. These data indicate that LBN has a dual mechanism of action, increasing AH outflow through both the uveoscleral (using LA) and TM/Schlemm's canal (using NO) pathways. Long-term efficacy and safety of this new drug were demonstrated in the open-label safety-extension phases of the phase 3 pivotal studies and a phase 3 52-week open-label study. LBN 0.024% has demonstrated both short-term and long-term IOP-lowering efficacy in patients with OAG or ocular hypertension, without apparent clinically limiting safety or tolerability concerns [81].

Recently derivatives of the two series of benzenesulfonamides incorporating 2-hydroxypropylamine moieties with striking efficacy against the target hCA II and XII, and significant modulation of  $\beta_1$  - and  $\beta_2$  - receptors have been proposed [83]. The dual agents 23, 25 , and 27 were more effective than standard DRZ and timolol with IOP decreases of 8.25, 10.75, and 6.75 mmHg at 60 min post- administration, respectively (**Figure 5**) [82].

The neurohormone melatonin (MLT) has been shown to decrease IOP. It is primarily produced by the pineal gland with highest concentrations reached at night. A number of biological activities have been ascribed to MLT, ranging from central nervous system (CNS)-related activities, to regulation of the immune system, glucose handling, and cardiovascular homeostasis. In mammals, the majority of MLT actions are driven by the activation of the two G-protein coupled receptors named MT1 and MT2 expressed in the CNS and in the periphery. Moreover, MLT exerts direct antioxidant effects at high concentrations and it is produced locally in the eye and its receptors have been identified in several areas, such as in the retina, ciliary body, cornea, lens, and sclera [83]. Topical and systemic administration of MLT has been shown to transiently reduce IOP in normotensive and hypertensive/glaucomatous animals [84-85].

Hybrid compounds, activating melatonin receptors and inhibiting fatty acid amide hydrolase (FAAH) have been recently synthesized [20] and the topical administration of these compounds reduced elevated IOP in rabbits, with a longer action and improved efficacy compared to the reference compounds melatonin and URB5, a FAAH inhibitor. The activity of endocannabinoid system is controlled through the balance between production and degradation

of endocannabinoids and the enhancement of the endocannabinoid tone with FAAH inhibitors improve IOP; this pathway is at moment widely investigated by medicinal chemistry point of view. Adenosine-based analogs have been recently patented acting as selective  $A_1$  agonists [86] and  $A_3$  antagonists [87]. The compounds can be used alone or in combination therapy and have shown IOP reduction in New Zealand White rabbit.



**Figure 5:** Drop of intraocular pressure ( $\Delta$ IOP, mmHg) versus time (min) in hypertonic saline-induced ocular hypertension in rabbits, treated with 1% solution of compounds 23, 25, and 27. Timolol 0.25% and DRZ 1% were used as reference drugs[85].

Data are analyzed with 2-way Anova followed by Dunnett's multiple comparison test. \*  $p < 0.05$  DRZ vs vehicle at 60 min; \*\*  $p < 0.005$  timolol vs vehicle at 60 min;

\*\*\*  $p < 0.001$  27 vs vehicle at 60 min; \*\*\*\*  $p < 0.0001$  23 and 25 vs vehicle at 60 min.

## 9. Conclusion

Glaucoma is one of the main leading causes of irreversible blindness in the world. The treatment of this disease relies on the administration of drugs able to reduce IOP, the main risk factor of glaucoma. The drugs now in use belong to well-known categories of compounds effective in reducing IOP. However, important side effects and no responding patients account for the necessity of novel therapy approaches. New innovative pharmacological targets have been explored in the last decade, discovering new approaches and new molecules for the future treatment of glaucoma.

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