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REVIEW

The Multifarious Effects of Various Glaucoma Pharmacotherapy on Corneal Endothelium: A Narrative Review

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ABSTRACT

Corneal endothelium is a single cell layer that is mainly responsible for maintaining corneal clarity. Endothelial damage secondary to toxicity, stress, or genetic predisposition are common and in conjunction with the low regenerative ability of the cells, making their preservation critical for maintaining visual acuity. Patients with glaucoma, who are estimated to be close to 80 million worldwide, have a plethora of reasons for developing endothelial damage, being exposed to a spectrum that extends from various medical and surgical interventions to the disease itself. The wide spectrum of glaucoma pharmacotherapy that has been recently extended by addition of newer classes of medications has been the focus of extensive research on its effects on corneal endothelium. Both basic and clinical research have attempted to shine a light on the complex mechanisms associated with the effects of glaucoma medication on corneal endothelium and to answer the important question as to whether these findings are clinically significant. The aim of this review is to summarize and present current literature of the various findings, both from in vivo and in vitro studies that have focused on the complex relationship between different classes of glaucoma medication and their effect on corneal endothelium.

Keywords: Corneal endothelium; Endothelial toxicity; Glaucoma pharmacotherapy; Cornea; Glaucoma

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Key Summary Points
Timolol could have an additional protective role against endothelial oxidative stress.
Betaxolol and carteolol have been found to cause cytotoxicity in human corneal endothelial cells in vitro, stimulating different cell death pathways at different concentrations.
Carbonic anhydrase inhibitors should be carefully considered and administered in corneas with reduced functional reserve.
Further studies are needed in order to fully understand the mechanisms triggered by α_2-agonists and prostaglandins and whether clinical significance is reached.
Rho-kinase inhibitors may stimulate cell cycle progression, enhance cell migration, and augment barrier and pump function characteristics that may benefit patients with Fuchs' endothelial corneal dystrophy.

INTRODUCTION

Glaucoma pharmacotherapy remains in the frontline of treatment for glaucoma and includes categories of the most commonly prescribed drugs among ophthalmologists [1, 2]. It incorporates classes of medications that act at three main directions: (1) decrease of aqueous humor production (e.g., β-adrenergic blockers, carbonic anhydrase inhibitors, α_2-adrenergic agonists), (2) increase of drainage through trabecular meshwork (e.g., rho-kinase inhibitors), and (3) increase of aqueous humor outflow through the uveoscleral tract (e.g., prostaglandin analogues, α_2-adrenergic agonists) [3, 4]. Among the different categories, multiple publications aim to investigate whether these drugs are safe for use and whether chronic administration may lead to toxicity in different parts of the eye [4]. Corneal endothelium has been the target of many studies since it has specific characteristics that make it susceptible to multiple types of toxicity [5]. Furthermore, patients with glaucoma have a plethora of reasons for developing endothelial damage, being exposed to a spectrum that extends from various medical and surgical interventions to the disease itself [4]. These observations are verified by solid data that show increased endothelial cell loss in patients that have undergone glaucoma surgery and at the same time decreased corneal graft survival in patients with glaucoma who have undergone penetrating keratoplasty [5]. In this setting, it is of high importance to clarify whether pharmacotherapy for glaucoma may have negative side effects in healthy or compromised corneas and for research to further explore the various pathophysiologic mechanisms that may arise as potential hazards. Meanwhile, newer glaucoma drugs such as rho-kinase inhibitors have been described to have possibly beneficiary effects on corneal endothelium, with mechanisms that are still not sufficiently understood [6]. Despite the well-established benefits of glaucoma pharmacotherapy in reducing intraocular pressure (IOP), the potential impact of these drugs on the corneal endothelium remains a concern [7]. Several studies have investigated the relationship between different classes of glaucoma medications and corneal endothelial damage, but the results have been conflicting [5, 7–14]. In this context, the present review aims to provide an overview of the current literature on the complex relationship between glaucoma pharmacotherapy and corneal endothelial damage. Specifically, we will highlight the challenges and issues associated with this relationship and discuss its clinical relevance in terms of patient outcomes. By synthesizing the available evidence from both in vivo and in vitro studies, this review will provide valuable insights into the potential risks and benefits of glaucoma medications and help clinicians to make informed decisions when selecting appropriate treatment options for their patients.

This article is based on previously conducted studies and does not contain any new studies

with human participants or animals performed by any of the authors.

METHODOLOGY OF LITERATURE SEARCH

To complete this article, a search was performed using Pubmed and Scopus databases with the following terminology: glaucoma and corneal endothelium, glaucoma pharmacotherapy and cornea, β-blockers and corneal endothelium or endothelial cells, carbonic anhydrase inhibitors (CAIs) and cornea endothelium or endothelial cells, rho-kinase inhibitors and cornea endothelium or endothelial cells, prostaglandins and corneal endothelium or endothelial cells, and brimonidine and corneal endothelium or endothelial cells. Only studies in English or with English translations were included. No studies were excluded on the basis of the date of publication nor the type of the journal.

β-Adrenergic Blockers

β-Adrenergic blockers are a widely used antiglaucoma drug class. They reduce intraocular pressure by decreasing aqueous humor formation [4, 15]. Their more common ocular adverse effects are ocular discomfort, keratitis, and conjunctivitis [3, 4, 16].

It is known that β-adrenergic receptors exist in corneal endothelium [17–19]. However, the exact effects of β-adrenergic blockers on corneal endothelial cells (CEC) still remain unknown and are controversial. Wang et al. showed that timolol, betaxolol, and levobunolol induced cyclic adenosine monophosphate (cAMP) concentration increase in cultured porcine CEC in vitro [19]. Cyclic AMP has been reported to regulate a wide range of physiologic functions in cultured animal corneas, such as stimulation of mitosis, differentiation, maintenance of cell shape, and regulation of DNA synthesis [18, 19]. Furthermore, it has been reported to decrease permeability of the corneal endothelium [19, 20]. This study also demonstrated that propanolol increases intracellular calcium in cultured porcine CECs. The same effect was reported later by Wu et al. as being induced by timolol, betaxolol, carteolol, and levobunolol with 1/100 and 1/1000 concentrations in cultured bovine CECs in vitro [21]. Intracellular calcium plays a key role in significant cellular responses, such as cell migration and pump function, thus also affecting the endothelial wound healing procedure and the swelling rate of the cornea [19, 21]. However, follow-up research by Steinle et al. attributed this effect exclusively to β3-adrenergic receptors [22]. This specific isotype of adrenergic receptors is the least studied due to a lack of appropriate animal models; although recently research interest for the β3 isotype has increased and so far, researchers are focusing on its function on rodent retinal vessels [23].

Moreover, although clinical and experimental studies have shown that timolol has a possible antioxidant role in glaucoma [24], its neuroprotective effect is arguable [23]. Izzotti et al. provided data to support the favorable effect of timolol, demonstrating its protective activity against oxidative stress induced by exposure to iron/ascorbic acid (Fe/Asc) in cultured human CECs in vitro [25]. Specifically, pretreatment with timolol counteracted cytotoxic effects and morphological changes, decreased membrane lipid peroxidation, and inhibited 95.6% of the oxidative DNA damage induced by Fe/Asc in endothelial cells. In addition, Izzotti et al. showed that timolol per se did not have any toxic effect on corneal endothelium. The authors suggested that the antioxidant activity of timolol may be related to its metabolism, involving the oxidation and hydrolytic cleavage of its morpholine ring, which can be a target for reactive oxygen species [25].

On the contrary, data have been provided that question the beneficial effect of β-adrenergic blockers on corneal endothelial cells since multiple studies reported adverse effects of a number of β-adrenergic blockers including timolol, betaxolol, and carteolol. Timolol has been shown to cause endothelial cell degeneration and breakdown in treated rabbit eyes in vivo [26]. These alterations are also markedly increased with the combined use of a contact

lens [26]. However, in healthy human corneas, topical timolol application had no significant effect on corneal endothelial cell morphology [9]. Pure timolol maleate also caused reversible contraction of bovine corneal endothelial cells in vivo, but did not affect cell viability, even after prolonged exposure to high concentrations of the compound [27]. Moreover, while timolol has been shown to not significantly alter endothelial cell density [28, 29], it has been reported to induce a statistically significant increase of percent cell hexagonality and decrease of coefficient variation of cell area in human corneal endothelium in vivo, findings that may be characterized as contradictory [29]. A trend of timolol to increase corneal thickness in human CECs in vivo has additionally been described [28], a fact that could possibly be attributed to the aforementioned increase in intracellular calcium concentration that timolol induces [21]. According to Whikehart et al., timolol maleate and betaxolol have also been found to significantly inhibit Na + /K + -ATPase in corneal endothelium [30], an effect that could account for corneal thickness changes as well.

Betaxolol was found to induce multiple negative effects on the corneal endothelium and even cytotoxicity. Wu et al. investigated the effects of a variety of drugs, including β-adrenergic blocking agents betaxolol, timolol, levobunolol, and carteolol, on cellular cytotoxicity in bovine CECs in vitro [31]. The cytotoxic effect was confirmed with assay of lactate dehydrogenase (LDH), a stable cytosolic enzyme being released upon cell lysis. Betaxolol at 1/100 dilution significantly increased LDH release, whereas timolol, levobunolol, and carteolol did not affect LDH release at any dilution [31]. Apart from that, betaxolol has been shown to induce dose- and time-dependent morphological and structural changes in human CECs in vitro [32]. The apoptosis cascade is probably triggered by plasma membrane permeability increase, leading to DNA fragmentation in a dose-dependent manner. The same study also reported the toxic effects of betaxolol on cat CECs in vivo, which included cell density decrease, cell size increase, and apoptosis-like morphological and ultrastructural changes [32].

A few years later, a similar effect of carteolol on human CECs in vitro was reported [33]. Morphological abnormalities, as well as dose- and time-dependent cell viability decrease, PM permeability increase, and cytotoxicity, were observed. Su et al. managed to further elucidate the mechanisms that are implicated in the cytotoxic effect. Carteolol in high doses (0.5–2%) was found to induce necroptosis via increased protein expression levels of RIPK1, RIPK3, MLKL, and pMLKL and downregulation of the activity of caspase-2 and caspase-8 (RIPK/MLKL pathway). Conversely, carteolol in low doses (< 0.25%) induced G1 phase arrest and apoptosis in human CECs via caspase activation, dampened expression of the anti-apoptotic protein Bcl-2 and Bcl-xL, enhanced expression of the pro-apoptotic proteins Bax and Bad, and mitochondrial-released pro-apoptotic proteins Cyt.c and AIF. Furthermore, carteolol was found to cause time-dependent endothelial cell density decrease, cell size increase, and apoptosis-inducing effect on feline CECs in vivo [33].

In conclusion, β-adrenergic blockers may affect various physiologic functions in corneal endothelium, possibly via the protein kinase A signaling pathway. Most significantly, they could regulate cell proliferation and migration, facilitating Na–K ATPase activity. There is strong evidence that timolol could have an additional protective role against oxidative stress. Furthermore, betaxolol and carteolol have been found to cause cytotoxicity in human corneal endothelial cells in vitro, stimulating different cell death pathways at different concentrations. Whether these changes may be clinically significant remains to be proven, since at the moment there are not enough data supporting compromised endothelial function to the level of impaired corneal deturgescence. The studies that examine the effects of β-adrenergic blockers are summarized in Tables 1, 2, and 3.

Carbonic Anhydrase Inhibitors

Carbonic anhydrases (CAs) are a family of enzymes that facilitate the bidirectional

Table 1 Summary of studies that investigate various β-blockers and show beneficial effects on corneal endothelium

β-blockers	Study	Type of study	Effect
Timolol	Wang et al. (2000) [19]	In vitro	0.025% to 0.00025% timolol induced cAMP concentration increase in cultured porcine CECs
Timolol	Wu et al. (2006) [21]	In vitro	58 µM and 5.8 µM timolol for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs
Timolol	Izzotti et al. (2008) [25]	In vitro	Pre-treatment with 0.5 mg timolol/ml complete medium/well for 20 h demonstrated protective activity of timolol against oxidative stress induced by exposure to iron/ ascorbic acid in cultured HCECs
Betaxolol	Wang et al. (2000) [19]	In vitro	0.05% to 0.0005% betaxolol induced cAMP concentration increase in cultured porcine CECs
Betaxolol	Wu et al. (2006) [21]	In vitro	162 µM, 16.2 µM, and 1.62 µM betaxolol for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs
Carteolol	Wu et al. (2006) [21]	In vitro	680 µM and 68 µM carteolol for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs
Levobunolol	Wang et al. (2000) [19]	In vitro	0.05% to 0.0005% levobunolol induced cAMP concentration increase in cultured porcine CECs
Levobunolol	Wu et al. (2006) [21]	In vitro	171 µM, 17.1 µM, and 1.71 µM levobunolol for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs
Propanolol	Wang et al. (2000) [19]	In vitro	10^{-5} M propanolol causes intracellular calcium increase in cultured porcine CECs

CECs corneal endothelial cells, *HCECs* human corneal endothelial cells, *Ca^{2+}* intracellular calcium, *cAMP* cyclic adenosine monophosphate

conversion of CO_2 and H_2O to H^+ and $HCO3^-$ [34, 35]. Inhibition of principally CA-II, CA-IV, and CA XII isoenzymes in the ciliary processes of the eye results in decreased aqueous humor secretion with subsequent reduction in intraocular pressure [34–38]. The idea of administering carbonic anhydrase inhibitors (CAIs) topically had already been addressed by Becker et al. since 1955 [39]. However, due to their physicochemical properties, the topical administration for the treatment of glaucoma was established years later, in the 1990s, with dorzolamide being the first commercial agent approved by the Food and Drug Administration (FDA) [40]. Topical instillation of sulfonamide CAIs counteracted the critical systematic adverse effects of oral administration, limiting them to allergic dermatitis/conjunctivitis, corneal edema, keratitis, and metallic taste [38, 41].

Besides the ciliary processes, corneal endothelium also possesses the CA-II as well as the CA-I isoenzyme, both of which facilitate and regulate fluid transport through ATPase pump mechanisms to maintain corneal stromal deturgescence [34, 36, 42]. The presence of carbonic anhydrase isoform IV in the human corneal endothelium has also been reported [34], however, its role seems to differ from that of other isoforms [43, 44]. Apparently, inhibition of these enzymes in corneal endothelium could compromise corneal hydration control and lead to corneal edema [45]. Therefore, it has been crucial to assess the possible effects of topically instilled CAIs on corneal endothelium.

Table 2 Summary of studies that investigate various β-blockers and show negative effects on corneal endothelium

β-blockers	Study	Type of study	Effect
Timolol	Staatz et al. (1981) [27]	In vivo	Pure timolol malate caused reversible contraction of bovine corneal endothelial cells in vivo, but did not affect cell viability, even after prolonged exposure to high concentrations of the compound
Timolol	Arthur et al. (1983) [26]	In vivo	Timolol caused small areas of endothelial cell degeneration and breakdown in treated rabbit eyes
Timolol	Whikehart et al. (1991) [30]	In vitro	In cultured corneal endothelial and epithelial cells, a decrease in activity is indicated at just under 10–15 M (fM) for Na/K-ATPase; however, the loss of activity does not become significant until 0.42 pM ($p < 0.001$)
Timolol	Lass et al. (1998) [36]	In vivo	The mean percent change in CCT at 12 months from baseline was a decrease of 0.25% for timolol group. The mean percent endothelial cell loss (4.5%) and percent change in corneal thickness was similar in timolol, betaxolol, and dorzolamide groups at both 6 and 12 months from baseline
Timolol	Grüb et al. (2006) [28]	In vivo	Timolol 0.5% eye drops (BID) increased corneal thickness from 537 to 557 µm after 4 days in HCECs
Timolol	Mundorf et al. (2020) [29]	In vivo	Timolol 0.5% (BID) caused a statistically significant CV decrease from baseline to month 3 in HCECs in vivo. Timolol 0.5% (BID) caused a statistically significant percent HEX increase from baseline to month 3 in HCECs in vivo
Betaxolol	Whikehart et al. (1991) [30]	In vitro	In cultured corneal endothelial and epithelial cells, a decrease in activity of Na/K-ATPase becomes significant after 5 nM level is reached
Betaxolol	Lass et al. (1998) [36]	In vivo	The mean percent change in CCT at 12 months from baseline was an increase of 0.39% for betaxolol group
			The mean percent endothelial cell loss (4.5%) and percent change in corneal thickness was similar in timolol, betaxolol, and dorzolamide groups at both 6 and 12 months from baseline
Betaxolol	Wu et al. (2007) [31]	In vitro	Betaxolol 162 µM (1/100 dilution) increased LDH release compared with controls in cultured bovine CECs
Betaxolol	Miao et al. (2014) [32]	In vivo	Betaxolol 2.8 g/L induced apoptosis-like morphological and ultrastructural changes in cat CECs
Betaxolol	Miao et al. (2014) [32]	In vitro	Betaxolol 0.0875 g/L to 2.8 g/L causes dose- and time- dependent morphological, ultrastructural changes, and decreases viability of cultured HCECs in vitro
Carteolol	Su et al. (2020) [33]	In vivo	Carteolol 2% caused time-dependent ECD decrease, average cell size increase, and apoptosis-inducing effect on feline CECs

Table 2 continued

β-blockers	Study	Type of study	Effect
Carteolol	Su et al. (2020) [33]	In vitro	Carteolol 0.015625 to 2% caused morphological abnormalities, decreased cell viability, and induced dose- and time-dependent cytotoxicity in HCECs

CECs corneal endothelial cells, *HCECs* human corneal endothelial cells, Ca^{2+} intracellular calcium, *cAMP* cyclic adenosine monophosphate

Table 3 Summary of studies that investigate various β-blockers and show no effect on corneal endothelium

β-blockers	Study	Type of study	Effect
Timolol	Alanko et al. (1983) [9]	In vivo	Topical timolol for 2 weeks had no significant effect on corneal endothelial cell morphology in young, human, healthy eyes
Timolol	Lass et al. (1998) [36]	In vivo	The mean percent endothelial cell loss and percent change in corneal thickness was similar in timolol, betaxolol, and dorzolamide groups at both 6 and 12 months from baseline
Timolol	Wu et al. (2007) [31]	In vitro	Timolol did not increase LDH release in cultured bovine CECs at any dilution
Timolol	Miura et al. (2008) [52]	In vivo	0.5% timolol + latanoprost showed no significant changes in CD after treatment compared with baseline
Carteolol	Wu et al. (2007) [31]	In vitro	Carteolol did not increase LDH release in cultured bovine CECs at any dilution
Levobunolol	Wu et al. (2007) [31]	In vitro	Levobunolol did not increase LDH release in cultured bovine CECs at any dilution

CECs corneal endothelial cells, *LDH* lactate dehydrogenase, *CD* cell density

Several studies have investigated fluid pump activity of corneal endothelium and corneal thickness changes in association with the use of CAIs. Acetazolamide, ethoxzolamide, and benzolamide have been reported to produce a great decrease, about 40–60%, in the rate of fluid pumping in rabbit corneal endothelium in vitro [46, 47]. Wilkerson et al. conducted a 4-week, double-masked, randomized, placebo-controlled, three-center study on safety and efficacy of dorzolamide, finding that it caused a statistically significant increase in corneal thickness in patients with bilateral primary open angle glaucoma or ocular hypertension [48]. According to the authors, this change was small, 7 μm to 9 μm, and therefore not clinically significant. Several following studies reported a significant increase in central corneal thickness being observed with the topical administration of dorzolamide in patients with glaucoma. Once again, the reported difference in CCT was relatively small, and varied from 2.5 μm to 18 μm [12, 36, 49, 50]. In contrast to the above, a study that compared the effects of dorzolamide monotherapy with dorzolamide in combination with either timolol or pilocarpine revealed no significant alterations regarding corneal thickness in either group or between them during 3 months of follow-up [51].

As far as endothelial cell count and cell morphology, almost all of the aforementioned clinical investigations agreed that they were not significantly affected by using topical dorzolamide [12, 48, 50, 51]. Only Lass et al. contradicted this result, reporting a mean percent corneal endothelial cell loss of 3.6% after 1 year of topical dorzolamide administration in patients with primary open angle glaucoma or ocular hypertension [36]. Moreover, the study concluded that dorzolamide is equivalent to timolol and betaxolol regarding their effects on corneal endothelial cell density and central corneal thickness after 1 year of therapy. Although they presented a double-masked, randomized, multicenter study with a longer follow up than previous studies, an investigator effect was found to be statistically significant, and therefore could have possibly influenced the arisen outcomes.

Contradicting the variable outcomes of dorzolamide on corneal endothelium, brinzolamide has shown greater consistency, although fewer studies have investigated its effects. Its topical instillation has not been found to induce statistically significant changes in cell density and morphology either in the short [52] or long term [53]. An additional comparison of brinzolamide and latanoprost with timolol and latanoprost revealed no significant differences between the two combinations in terms of the parameters mentioned above [52]. Even though a few case reports have described the development of corneal edema in association with brinzolamide use, in all cases, the edema resolved shortly after drug administration had ceased [54, 55].

Interestingly, although dorzolamide at 1/100 dilution significantly increased LDH release in cultured bovine CECs in vitro, indicating cytotoxicity and endothelial cell lysis, brinzolamide did not significantly affect dilution [31]. Furthermore, both of them have been reported to increase intracellular Ca^{2+} concentration, favoring the significant cellular responses that calcium mediates [21].

A more recent study of Malikowski et al. showed that all CAIs used in their experiment, namely acetazolamide, dorzolamide, brinzolamide, and ethoxzolamide, produced a decrease in short-circuit current (Isc) in bovine corneal endothelium, whereas in human corneal endothelial cells no change was noted with any of them [34]. Nonetheless, according to a previous report, even if the potential difference across the endothelium remains unaffected, fluid pump activity can be decreased to a significant level after application of a carbonic anhydrase inhibitor [46].

These outcomes highlight the need for additional investigations to further elucidate the precise pathophysiologic mechanism of action of carbonic anhydrases and their inhibitors in corneal endothelial cells. Since the exact effect on corneal deturgescence also remains unclear, concerns have been raised for their prolonged use or administration on compromised corneas [45, 56]. These concerns are additionally supported taking into account the decreased cell density being observed in eyes with glaucoma compared with normal eyes [56, 57], as well as normal annual cell loss that occurs with aging [58].

In this direction, researchers have tried to assess the safety of CAIs for corneal endothelium after technically induced, short-term hypoxia. Topically administered dorzolamide hydrochloride had no significant effect on swelling after hypoxic stress, as well as on open eye steady state thickness and corneal deswelling rate of glaucoma compared with normal eyes [10, 56]. Even more importantly, 1 year of dorzolamide use did not significantly affect either these parameters or corneal endothelial cell density [56], an outcome that is in agreement with previous investigations [12, 48, 50, 51]. Dorzolamide, however, was noticed to increase corneal endothelial permeability to fluorescein after induced short-term hypoxia, although this did not result in increased corneal thickness [10].

In 1999, nine cases of corneal swelling were reported after dorzolamide use in eyes with diminished or borderline corneal endothelial function [14]. However, dorzolamide could not indisputably account for the irreversible corneal decompensation that occurred in the reported cases, since other ophthalmological pathologies coexisted in all included patients and could have caused or contributed to the corneal

Table 4 Studies that investigate various carbonic anhydrase inhibitors and show beneficial effect on corneal endothelium

Carbonic anhydrase inhibitors (CAIs)	Study	Type of study	Effect
Dorzolamide	Wu et al. (2006) [21]	In vitro	616 μM and 61.6 μM dorzolamide for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs
Brinzolamide	Wu et al. (2006) [21]	In vitro	260 μM brinzolamide for 200 s increased intracellular Ca^{2+} concentration in cultured bovine CECs

CECs corneal endothelial cells, Ca^{2+} intracellular calcium

edema [8]. A few years later, Wirtitsch et al. conducted two double-masked randomized, placebo-controlled clinical trials, demonstrating that CCT was significantly increased in eyes with corneal guttata compared with normal ones after topical instillation of dorzolamide hydrochloride. Consequently, the authors suggested close monitoring of patients with diminished corneal endothelium density or function when receiving a carbonic anhydrase inhibitor [59, 60].

While CAIs were expected to burden normal corneal hydration control, their effects remain arguable. Even when their use was associated with an increase in central corneal thickness, the reported change seemed to be of low clinical significance. CAIs should be carefully considered and administered in corneas with reduced functional reserve on account of the reported swelling in such eyes. The studies that examine the effects of CAIs are summarized in Tables 4, 5, and 6.

α_2-Agonists

α_2-Agonists function as ocular hypotensives by affecting both production and outflow of aqueous humor [3, 61]. They are found to cause vasoconstriction of blood vessels and reduced blood flow in ciliary body, resulting in decreased production of aqueous humor [62, 63]. They have additionally been reported to increase its drainage through the uveoscleral tract with extended usage [61, 63].

Only a few studies exist that report the effects of α_2-agonists on corneal endothelium. Grueb et al. managed to support, with data, that brimonidine mediates an inhibitory effect on cAMP and protein kinase A pathway through stimulation of α_{2A}-adrenoreceptors on bovine corneal endothelium [64]. Cyclic AMP possesses a key role in crucial physiologic functions, such as cell proliferation and migration and wound healing and ion transport [18, 19, 64]. Further investigations are needed to assess how long-term administration of α_2-agonists could influence these functions.

Brimonidine was also shown to be the only anti-glaucoma medication that decreased intracellular Ca^{2+} concentration in bovine CECs in vitro [21]. Furthermore, it caused an increase in LDH release at 1/100 dilution, indicating possible toxicity of the drug in high administrative doses [31].

More importantly, a few years later, Grueb et al. reported effects of brimonidine on central corneal thickness, investigating how each corneal layer was influenced [62]. They found that topical instillation of brimonidine caused a significant increase in CCT on day 2 of follow-up. Apart from the effect of α_2-agonists on corneal endothelium, α_2-adrenergic stimulation on corneal epithelial cells has also been reported to contribute to corneal swelling by inhibiting transepithelial Cl transport. However, CCT returned at almost baseline levels 2 days after. While corneal epithelium and stroma thicknesses followed the same pattern, corneal endothelial thickness did not change significantly with brimonidine administration [62].

On the basis of the current literature, it is difficult to determine the effects of α_2-agonists

Table 5 Studies that investigate various carbonic anhydrase inhibitors and show negative effect on corneal endothelium

Carbonic anhydrase inhibitors (CAIs)	Study	Type of study	Effect
Acetazolamide	Fischbarg et al. (1974) [46]	In vitro	Arrest of fluid transport was seen at high concentrations (10 mm) in rabbit corneal endothelium
Acetazolamide	Kuang et al. (1990) [46]	In vitro	Greatly decreasing the rate of fluid transport 3 h after installation at high concentration (5 mM) in rabbit corneal endothelium
Acetazolamide	Malikowski et al. (2014) [34]	In vitro	500 lM acetazolamide produced a decrease in bovine corneal endothelial short-circuit current (Isc)
Dorzolamide	Wilkerson et al. (1993) [48]	In vivo	2% dorzolamide treatment significantly increased CCT on days 15 and 28 ($p < 0.05$) in right eye and on day 28 in the left eye. However, this increase of 0.007 mm to 0.009 mm was not clinically significant
Dorzolamide	Herndon et al. (1997) [49]	In vivo	The mean CCT of the 46 eyes being treated with dorzolamide (0.551 ± 0.020) was significantly greater than that of the 28 eyes with glaucoma not receiving dorzolamide (0.560 ± 0.025) ($P = 0.02$)
Dorzolamide	Uva et al. (1997) [50]	In vivo	2% dorzolamide t.i.d caused a significant increase in CCT at months 3 and 6 from baseline in human patients in vivo, whereas no significant changes were observed in the control group
Dorzolamide	Egan et al. (1998) [10]	In vivo	Treatment with dorzolamide 2% increased endothelial permeability to fluorescein at a statistically significant level
Dorzolamide	Lass et al. (1998) [36]	In vivo	(1) Mean percent endothelial cell loss at 12 months from baseline was 3.6% (2) Mean percent change in CCT at 12 months from baseline was an increase of 0.47% for dorzolamide group Note: There was a significant investigator effect, effect on age, iris color, and corneal thickness in this study
Dorzolamide	Inoue et al. (2003) [12]	In vivo	1% dorzolamide treatment for 3 months demonstrated increase in CCT compared with baseline (mean increase 7 mm)
Dorzolamide	Wu et al. (2007) [31]	In vitro	616 µM dorzolamide increased LDH release compared with controls in cultured bovine CECs
Dorzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM dorzolamide produced a decrease in bovine corneal endothelial short-circuit current (Isc)

Table 5 continued

Carbonic anhydrase inhibitors (CAIs)	Study	Type of study	Effect
Brinzolamide	Zhao and Chen (2005) [55]	Case report	Mild corneal edema, Descemet's folds, and whitish fleck-like debris on the corneal endothelium of one eye after 2 years use of brinzolamide 1% in combination with other antiglaucoma drugs in both eyes. The cornea was clear 1 week after discontinuation of brinzolamide in both eyes
Brinzolamide	Tanimura et al. (2005) [54]	Case report	Bilateral corneal stromal edema and SPK were noted in two patients after the switch of dorzolamide 1% to brinzolamide 1%
Brinzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM brinzolamide produced a decrease in bovine corneal endothelial short-circuit current (Isc)
Ethoxzolamide	Fischbarg et al. (1974) [46]	In vitro	Ethoxzolamide 10^{-5} or 10^{-4} M produced a dose-dependent 40–60% decrease in the rate of fluid pumping in rabbit corneal endothelium
Ethoxzolamide	Kuang et al. (1990) [46]	In vitro	Ethoxzolamide (0.1, 0.2, and 0.3 mM) greatly decreased the rate of fluid transport to one-third of its control level in about 3 h in rabbit corneal endothelium
Ethoxzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM ethoxzolamide produced a decrease in bovine corneal endothelial short-circuit current (Isc)
Benzolamide	Fischbarg et al. (1974) [46]	In vitro	Benzolamide 10^{-3} M produced a dose-dependent 40–60% decrease in the rate of fluid pumping in rabbit corneal endothelium

CECs corneal endothelial cells, *HCECs* human corneal endothelial cells, *LDH* lactate dehydrogenase, *CD* cell density, *CV* coefficient of variation of cell area, *CCT* central corneal thickness, Ca^{2+} intracellular calcium, *cAMP* cyclic adenosine monophosphate

on corneal endothelial layer. Further studies both in vivo and in vitro are needed to fully understand the mechanisms triggered by α_2-agonists and whether clinical significance is reached. The studies that examine the effects of α_2-agonists are summarized in Table 7.

Prostaglandin Analogues

Prostaglandin analogues (PGAs) were first introduced into clinical practice in 1996 with topical drops of latanoprost, followed by bimatoprost, travoprost in 2001, and finally tafluprost in 2008 [65]. Topical PGAs have become the first-line treatment in glaucoma as they reduce IOP more effectively than other classes of topical hypotensive drugs. In addition, they are systemically safer and generally well tolerated [66]. PGAs are hydrolyzed by esterases in the cornea to their active free acid form [67]. Aqueous outflow is increased by PGAs via the uveoscleral pathway, while bimatoprost has been reported to involve the trabecular meshwork pathway as well, thus achieving lower IOP [68]. At a molecular level, PGAs involve matrix metalloproteinases (MMPs) degrading the extracellular matrix (ECM) components, mainly collagen, in the uveoscleral and trabecular meshwork aqueous outflow pathways, leading to reduced resistance

Table 6 Studies that investigate various carbonic anhydrase inhibitors and show no significant effect on corneal endothelium

Carbonic anhydrase inhibitors (CAIs)	Study	Type of study	Effect
Acetazolamide	Fischbarg et al. (1974) [46]	In vitro	Acetazolamide 10^{-3} M did not produce any change in the potential difference in rabbit corneal endothelium
Acetazolamide	Malikowski et al. (2014) [34]	In vitro	500 lM acetazolamide did not produce a rapid decrease in human corneal endothelial short-circuit current (Isc)
Dorzolamide	Wilkerson et al. (1993) [48]	In vivo	(1) 2% dorzolamide treatment did not significantly alter corneal endothelial cell count on days 15 and 28. (2) No statistically significant changes in CEC count were seen between dorzolamide-treated and placebo groups
Dorzolamide	Uva et al. (1997) [50]	In vivo	2% dorzolamide t.i.d caused no significant changes in endothelial cell count from baseline at any time in human patients
Dorzolamide	Egan et al. (1998) [10]	In vivo	Corneal deswelling after short-term hypoxia, induced by application of a contact lens for 2 h, was not significantly different between dorzolamide 2% treatment group and placebo group in normal human eyes
Dorzolamide	Kaminski et al. (1998) [51]	In vivo	2% Dorzolamide monotherapy t.i.d. and dorzolamide b.i.d. in combination with either timolol 0.5% b.i.d. or pilocarpine 1% t.i.d. No significant alterations regarding endothelial cell density, cell morphology, and corneal thickness during the 3 month follow-up were observed
Dorzolamide	Giasson et al. (2000) [56]	In vivo	Hypoxic corneal edema was induced by applying a thick contact lens for 2 h after pretreatment with dorzolamide 2% (treated group) or instillation of saline (control eyes) in human patients. No significant difference between measurements for endothelial cell density, induced corneal swelling, percentage recovery per hour, nor time to 95% elimination of swelling was observed
Dorzolamide	Inoue et al. (2003) [12]	In vivo	1% dorzolamide treatment for 3 months did not demonstrated any significant changes in CD, CV, and HEX compared with baseline in vivo
Dorzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM dorzolamide did not produce a rapid decrease in human corneal endothelial short-circuit current (Isc)
Brinzolamide	Wu et al. (2007) [31]	In vitro	Brinzolamide did not increase LDH release in cultured bovine CECs in vitro at any dilution

Table 6 continued

Carbonic anhydrase inhibitors (CAIs)	Study	Type of study	Effect
Brinzolamide	Miura et al. (2008) [52]	In vivo	(1) 1% brinzolamide + latanoprost showed no significant changes in CD after treatment compared with baseline (before starting brinzolamide); (2) 1% brinzolamide + latanoprost compared with 0.5% timolol + latanoprost showed no significant differences in CD at 12 weeks
Brinzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM brinzolamide did not produce a rapid decrease in human corneal endothelial short-circuit current (Isc)
Brinzolamide	Nakano et al. (2016) [53]	In vivo	1% brinzolamide in addition to 0.005% latanoprost demonstrated no significant changes in CD, CV, or %Hex at 4, 12, 24, or 48 weeks compared with the baseline
Ethoxzolamide	Fischbarg et al. (1974) [46]	In vitro	Ethoxzolamide 10–4 M did not produce any change in the potential difference in rabbit corneal endothelium
Ethoxzolamide	Malikowski et al. (2014) [34]	In vitro	100 lM ethoxzolamide did not produce a rapid decrease in human corneal endothelial short-circuit current (Isc)

CECs corneal endothelial cells, *HCECs* human corneal endothelial cells, *LDH* lactate dehydrogenase, *CD* cell density, *CV* coefficient of variation of cell area, *CCT* central corneal thickness, *BID* twice daily, *QD* once daily, *%HEX* percentage of hexagonality

[69]. Apart from the intended activation of MMPs in targeted ocular tissues, non-targeted tissues such as cornea undergo upregulation of MMPs [69]. Since cornea is rich in collagen, studies have shown that use of PGAs causes lowering of central corneal thickness (CCT) [11, 40, 69–71].

Very few studies have been conducted examining the effect of prostaglandins on corneal endothelial cells. In 2001, P. Rieck et al. measured in vitro whether prostaglandin F2a (PG-F2a) stimulation modulates the proliferation of corneal endothelial cells [72]. Bovine CEC (BCEC) cultures were incubated with different concentrations of latanoprost ranging from 0.05 μg/ml to 5 μg/ml. The number of cells were measured every 24 h for 5 days and compared with control cultures without PG-F2 stimulation. At the highest concentration, a twofold increase in BCEC number was found on day 5 compared with control cultures. Such concentrations cannot be reached with commercially available topical drops; therefore, such results cannot be extrapolated in vivo [72]. In a single-blind, 1-year study using preservative-free tafluprost, the effect on corneal structures in vivo using confocal microscopy was examined. A total of 75 patients were enrolled, and after 12 months no effect on corneal endothelium was recorded in any study group [73]. Another study evaluating corneal structures using confocal microscopy showed difference in endothelial cell density (ECD) [74]. Specifically, the study included 22 control subjects, 24 glaucoma subjects with glaucoma under treatment for at least 2 years with β-blocker or PGAs, and 16 subjects with glaumcoma without any treatment. ECD was statistically lower in the glaucoma group under treatment with 2826 cells/mm2 compared with 3124 cells/mm2 in the untreated glaucoma group. There was no statistical significance in

Table 7 Summary of studies that investigate available α_2-agonists and show various effects on corneal endothelium

α_2-agonists	Study	Type of study	Effect
Brimonidine	Wu et al. (2006) [21]	In vitro	Brimonidine at 68 μM and 6.8 μM for 200 s decreased intracellular Ca^{2+} concentration in cultured bovine CECs
Brimonidine	Wu et al. (2007) [31]	In vitro	Brimonidine at 68.0 μM increased LDH release compared with controls in cultured bovine CECs
Brimonidine	Grueb et al. (2008) [64]	In vitro	Brimonidine at 10^{-8} and 10^{-6} mg/ml revealed a dose-dependent decrease in intracellular cAMP from 0.206 pmol/ml to 0.089 pmol/ml in bovine corneal endothelial cells
Brimonidine	Grueb et al. (2011) [62]	In vivo	0.1% brimonidine significantly increased CCT from baseline and returned to almost the same level of baseline 2 days later. ($p = 0.276$). Corneal endothelium showed no signs of increased thickness

CECs corneal endothelial cells, *LDH* lactate dehydrogenase, *CCT* central corneal thickness, Ca^{2+} intracellular calcium, *cAMP* cyclic adenosine monophosphate

the ECD between untreated glaucoma subjects and control subjects. The authors did not mention any specific pathophysiological mechanism for this result apart from the possible use of preservatives in the treated group. An additional similar study evaluating corneal structures for 3 years using confocal microscopy showed changes in ECD [75]. The study compared 44 patients in preservative-free tafluprost (PF group) versus 35 patients in preservative bimatoprost or travoprost (P group) versus 14 patients in which a switch was needed in therapy (S group). ECD was significantly different at baseline ($p = 0.007$) at the end of 3 years; both in PF group and in S group ECD tended to decrease ($p = 0.048$ and $p = 0.001$, respectively), while in P group they increased ($p = 0.0065$). These outcomes are in accordance with the previous study showing that treated patients tend to have lower ECD, probably independently from the use of preservatives [75]. I.B. Güneş et al. demonstrated that a 3-year use of latanoprost or latanoprost fixed with timolol did not show any ECD statistical change [11]. Finally, a meta-analysis of the long-term effect of latanoprost fixed combination with timolol versus latanoprost monotherapy reported no difference between the two groups after 12 months of use [76].

Latanoprostene bunod (LBN) is a novel drug in the PGAs group that has been FDA approved for lowering IOP in patients with glaucoma [77]. It is also hydrolyzed by corneal esterases into latanoprost acid (active metabolite) and butanediol mononitrate, which is further metabolized to 1,4-butanediol (inactive metabolite) and nitric oxide (active metabolite). Therefore, not only is the uveoscleral outflow enhanced via classical PGA activation of PGF2a, but trabecular outflow is also increased owing to relaxation of the trabecular meshwork and Schlemm's canal, induced by nitric oxide (NO) [77, 78].

Regarding NO production, a study measured electrochemical direct real-time production in ex vivo cultured human corneoscleral segments [79]. This was carried out by placing an electrode on the segment and measuring NO production after infusion with LBN. The study reported that ocular tissues such as trabecular meshwork, corneal endothelium, and Schlemm's canal endothelium can metabolize donor compounds, that is, LBN and produce NO currents. Therefore, although the main mechanism is trabecular meshwork relaxation, corneal endothelial cells may play a role in the total intracameral NO production.

Table 8 Summary of studies that investigate available prostaglandins and show various effects on corneal endothelium

Prostaglandins	Study	Type of study	Effect
Latanoprost	Honda et al. (2010) [70]	In vivo	Expression of MMP-9 and MMP-1 was increased and TIMP-1 expression was decreased after 8 weeks of latanoprost use in mice
Latanoprost	Lee et al. (2015) [80]	In vivo	Latanoprost significantly reduced CCT in patients with NTG after 5 years of treatment
Latanoprost	Güneş et al. (2021) [11]	In vivo	Latanoprost appeared to reduce CCT at 3-year follow-up, though this 8–10 µm difference in CCT was not clinically significant
Tafluprost	Panos et al. (2013) [81]	In vivo	Decrease of CCT in 93% of eyes treated with tafluprost. CCT reduction was more significant within the first 6 months of the treatment period in patients with glaucoma
Tafluprost	Rossi et al. (2013) [73]	In vivo	No changes of CCT in patients with glaucoma after 12-month treatment

MMP-9 metalloproteinase-9, *MMP-1* metalloproteinase-1, *CCT* central corneal thickness, *NTG* normal tension glaucoma

Overall, more studies are needed to evaluate any effect of PGAs on corneal endothelial cells. The studies that examine the effects of prostaglandins are summarized in Table 8.

Rho-kinase Inhibitors

Rho-kinase inhibitors (ROCK-Is) such as ripasudil and netarsudil are a novel class of drugs clinically approved by the FDA for glaucoma management [82]. Multiple cellular events are modulated via ROCK signaling pathway ranging from cell cycle, cell adhesion and migration, mesenchymal transformation, and cell function [6, 83]. ROCK-Is reduce IOP via relaxation of the trabecular meshwork, therefore increasing the conventional drainage pathway and offering a novel target for the treatment of glaucoma [83, 84]. Apart from their reduction in IOP, both of the clinically approved ROCK-Is also affect the corneal endothelial cells [83]. Corneal physiology is highly regulated by corneal endothelial cells (CEC) and ROCK-Is have been shown to stimulate their cell cycle regulation and proliferation in vitro by downregulating cyclin-dependent kinase inhibitor p27Kip1 and upregulating cyclin D1 And D3 via PI3K/AKT signaling. Cumulatively, these mechanisms promote the transition from G1 to S phase on the CEC [85, 86]. Furthermore, ROCK-Is provoke CEC migration by coordinated effects on actin stress fiber depolymerization, cell detachment/cell–matrix re-adhesion, and regulation of the pericellular proteolytic microenvironment [87]. Such effects are expressed by the upregulation of several integral subunits, together with their extracellular ligands laminin α5 and β1, as well as the downregulation of multiple other molecules, including β-actin, intercellular adhesion molecule-1 (ICAM-1), focal adhesion kinase 1 (FAK1), Paxillin, and plasminogen activator inhibitor-1 (PAI-1), which is an integral part for cell movement and migration [6]. Notably, the administration of ROCK-Is has been reported both in vivo [13, 88] and in vitro [6] to be beneficiary in patients with Fuchs' endothelial corneal dystrophy (FECD) by decreasing central corneal thickness. Furthermore, it has been reported that healthy patients receiving either ripasudil or netarsudil, although without clinical symptoms, presented with guttae-like transformation of CEC [86, 89, 90], and the previously described inducement of CEC migration may be responsible [86].

In addition to the previous two important effects on CEC, ROCK-Is can inhibit mesenchymal transformation of CEC [91]. When a

Table 9 Studies that investigate available rho-kinase inhibitors, showing various effects on corneal endothelium

Rho-kinase inhibitors	Study	Type of study	Effect
Ripasudil	Koizumi et al. (2013) [13]	Case report	Lowering CCT in Fuchs' endothelial dystrophy
Ripasudil	Okumura et al. (2015) [95]	In vitro and in vivo	Promotion of CE wound healing, promotion of endothelial cell proliferation and migration, and suppression of apoptosis
Ripasudil	Okumura et al. (2016) [96]	In vitro and in vivo	Transient guttata-like morphology of CEC caused by reduced cellular actomyosin contractility
Ripasudil	Moloney et al. (2017) [88]	In vivo	Lowering CCT in conjunction with descemetorhexis in Fuchs' endothelial dystrophy
Ripasudil	Tanna et al. (2020) [90]	Case report	Transient guttata-like morphology of CEC
Ripasudil	Matsumura et al. (2020) [89]	In vivo	Transient guttata-like morphology of CEC caused by reduced cellular actomyosin contractility
Ripasudil	Maruyama et al. (2021) [89]	In vivo	Morphological changes that recovered 6 h after drop by modulating the actin cytoskeleton in various types of cells
Ripasudil	Schlötzer-Schrehardt et al. (2021) [6]	In vivo	Promotion of CE wound healing, promotion of endothelial cell proliferation and migration, and suppression of apoptosis
Nitarsudil	Tanna et al. (2020) [90]	In vivo	Transient guttata-like morphology of CEC caused by reduced cellular actomyosin contractility
Nitarsudil	Wisely et al. (2020) [97]	In vivo	Decreasing CCT without affecting cell density or morphology
Nitarsudil	Ganesh et al. (2022) [98]	Case report	Thinning of corneal stroma and flattening of cornea curvature

CCT central corneal thickness, *CE* corneal endothelium, *CEC* corneal endothelial cells

static non-proliferative cell starts to proliferate and migrate, a phenotypic switch from an endothelial to a fibroblastic phenotype can occur, known as mesenchymal transformation; this has been shown to be involved in the pathophysiology of FECD [92]. Such transformation can be detected via specialized markers such as vimentin and α-SMA as well as transcription factors such as ZEB and Snail. ROCK-Is downregulate the expression of all those mesenchymal markers, highlighting their potential to inhibit the mesenchymal transformation of the CEC in patients with FECD [6].

Apart from the above mechanisms, functional characteristics such as barrier integrity and pump function are equally important for normal corneal physiology [92]. Barrier integrity is maintained via intercellular junctions including adherents and tight junctions formed mainly by N-cadherin and ZO-1, whereas pump functions are maintained mainly by Na^+/K^+-ATPase pump sites, supported by ion transporters such as bicarbonate transporters, monocarboxylate transporters, and aquaporin water channels [92]. ROCK-Is have shown to upregulate all the aforementioned parameters

for barrier and pump integrity of the CEC both in patients with FECD as well as in healthy patients [6]. Recently, another beneficial effect of rho-kinase inhibitors to corneal endothelium has been described in patients with FECD undergoing descemetorhexis without endothelial keratoplasty (DWEK) [93, 94]. These studies suggest reduced corneal clearance time in patients receiving netarsudil, as well as increased endothelial cell counts [93], effects that could be attributed to the above-described regulating mechanisms (see Table 9).

Therefore, summing all the effects of ROCK-Is, they can stimulate cell cycle progression, enhance cell migration, and augment barrier and pump function, as well as prevent mesenchymal transformation of CEC in patients with FECD, resulting in thinner CCTs and clearer corneas. The studies that examine the effects of ROCK-Is are summarized in Table 8.

CONCLUSIONS

Investigation of glaucoma medications can provide great insight into their effects on corneal endothelial cells. An important point that can be concluded from this literature review is to further consider or closely monitor using carbonic anhydrase inhibitor in patients with preexisting corneal pathology, specifically those with compromised endothelial function. Moreover, the β-adrenergic blockers betaxolol and carteolol could preferably be avoided in high administrative doses, whereas rho-kinase inhibitors should be preferred in patients with Fuchs' endothelial corneal dystrophy.

The existing literature consists of a small number of studies, most of which have been conducted in vitro and do not take into account all the medications used clinically. Moreover, since most of the included analyses did not study preservative-free and preservative-containing hypotensive solutions separately, the investigated medications might include preservatives, which have also been reported to result in corneal endothelial toxicity [99, 100], a fact that should be taken into consideration. In addition, it is crucial to distinguish the healthy state of corneal endothelium in patients with primary open angle glaucoma and the compromised endothelium that may be present in patients with secondary glaucomas, such as pseudoexfoliation glaucoma, uveitic glaucoma, and other types of secondary glaucomas [5].

Furthermore, whether the reported findings are clinically significant remains arguable. Baratz et al. presented a very interesting and important study in 2006 that showed that corneal endothelial cell density, percent hexagonal cells, and coefficient of variation of cell area were similar between participants treated with glaucoma medications and observation group after 6 years of follow-up [7]. Both the number and kind of medications used were not universal for the participants in medication group, rendering it difficult to draw a specific conclusion for any glaucoma drug category. However, this study highlights the need for further investigations and a larger follow-up of administration to prove whether the demonstrated effects of glaucoma drugs on corneal endothelium could reach clinical significance.

Consequently, a validated treatment algorithm could not yet be discussed. Additionally and more significantly, pharmacotherapy mostly depends on other parameters, such as the stage of glaucoma, intraocular pressure, risk factors, and the overall health profile of the patient, and therefore should be personalized.

Further research studies, both in vivo and in vitro, should be implemented to elucidate the exact mechanisms that determine the relationship of corneal endothelium with not only glaucoma medications, but also surgical and laser management of glaucoma. Light should also be shed on the contributing effect of preservatives. As a result, future clinical practice could benefit from an optimized treatment regimen, considering, among other parameters, corneal endothelial cell condition of each patient.

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