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REVIEW

Endothelin antagonism as an active principle for glaucoma therapy

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Endothelin, the most potent vasoactive peptide known to date, has been suggested to play a potential role in the pathogenesis of open-angle glaucoma. Open-angle glaucoma is the most common optic nerve head neuropathy and is associated with a loss of retinal ganglion cells and visual field damage. Although an increased intraocular pressure is a major risk factor for glaucomatous optic neuropathy, other factors such as a reduced ocular blood flow play an important role for appearance of the disease. Thus, treatment of glaucoma is focused on lowering of intraocular pressure and preventing the occurrence or progression of glaucomatous optic neuropathy. Endothelin participates in the regulation of intraocular pressure by an effect on trabecular outflow, the main route for aqueous humour outflow from the eye. Trabecular outflow is modulated by trabecular meshwork contractility which is affected by endothelin. In addition to the effects of endothelin in the anterior part of the eye, the vasoconstrictor causes a decrease in ocular blood flow followed by pathological changes in the retina and the optic nerve head which is assumed to contribute to the degeneration of retinal ganglion cells. In sum, inhibition of endothelin signalling leads to lowering of intraocular pressure and exerts neuroprotective effects. Thus, endothelin antagonism in the eye represents a promising approach for pharmacological treatment of glaucoma.

Abbreviations

ECE, endothelin-converting enzyme; ET-1, endothelin-1; ET_A receptor, endothelin receptor A; ET_B receptor, endothelin receptor B; FP receptor, prostaglandin F receptor; IOP, intraocular pressure; MLCK, myosin light chain kinase; MMP, matrix-metalloproteinase; OBF, ocular blood flow; PG, prostaglandin; ROCK, Rho kinase; RGC, retinal ganglion cell

Introduction

Endothelin-1 (ET-1), the most potent vasoconstrictor known to date, is expressed in many organs and tissues and has been shown to play an important role in vascular homeostasis (Yanagisawa *et al.*, 1988) as well as in a variety of pathological processes (Levin, 1995).

In the human eye endothelin is detectable in the posterior part, especially in the choroid, retinal blood vessels, retinal pigment epithelium and optic nerve (Wollensak *et al.*, 1998; Narayan *et al.*, 2004). The precise source of endothelin in the posterior segment of the eye remains unclear. One possible source is the retinal pigment epithelium, which secretes ET-1 towards the basolateral side, suggesting an involvement in the regulation of choroidal blood flow (Narayan *et al.*, 2003, 2004; Dibas *et al.*, 2005a). The ET-1 synthesis and release from optic nerve head astrocytes as seen in cultured human cells may contribute to the signal in the optic nerve (Desai *et al.*, 2004).

In the anterior part of the eye, ET-1 was found in the iris, non-pigmented ciliary epithelium, ciliary muscle, as well as in the trabecular meshwork, endothelial cells lining Schlemm's canal and corneal epithelium (Wollensak *et al.*, 1998; Fernandez-Durango *et al.*, 2003). Apart from its localization in ocular tissues, ET-1 is present in aqueous humour. One possible source of ET-1 in aqueous humour is the

non-pigmented ciliary epithelium, because cultured cells synthesize and secrete ET-1 (Lepple-Wienhues *et al.*, 1992; Prasanna *et al.*, 1998). The local synthesis of ET-1 suggests a physiological role in the eye; however, the impact of ET-1 in the healthy eye is not completely understood yet and therefore a subject of intense research.

There is accumulating evidence for a role of ET-1 in the pathogenesis of primary open-angle glaucoma (POAG) (Sugiyama *et al.*, 1995b; Orgul *et al.*, 1996a; Tezel *et al.*, 1997; Yorio *et al.*, 2002; Prasanna *et al.*, 2003). POAG is the most common optic nerve head neuropathy which is associated with a loss of retinal ganglion cells (RGCs) and visual field damage. The main risk factor for causing glaucomatous damage is an elevated intraocular pressure (IOP). Although an elevated IOP is a major risk factor for glaucomatous optic neuropathy, ocular hypertension (increased IOP without glaucomatous damage) and normal tension glaucoma (glaucomatous damage despite normal IOP) indicate that other factors such as an insufficient blood supply due to either increased IOP or reduced ocular blood flow (OBF) play an important role (Flammer *et al.*, 1999, 2002). Today, all therapies applied in glaucoma are focused on lowering of IOP, but there are strong efforts to evaluate the relevance of targeting OBF in glaucoma therapy (Orgul *et al.*, 2005). Both IOP and OBF are affected by ET-1 (Figure 1) which is elevated in aqueous humour of glaucoma patients compared to normal subjects (Noske *et al.*, 1997; Tezel *et al.*, 1997; Koliakos *et al.*, 2004). Also, in animal models of glaucoma increased ET-1 concentrations in aqueous humour were detected (Kallberg *et al.*, 2002; Prasanna *et al.*, 2005). In contrast, no increase in the ET-1 plasma levels were observed in patients with POAG versus controls of similar age (Tezel *et al.*, 1997; Hollo *et al.*, 1998; Henry *et al.*, 2006; Kunimatsu *et al.*, 2006). Another study reported increased plasma ET-1 levels in patients with progressive open-angle glaucoma compared to glaucoma patients with stable visual fields (Emre *et al.*, 2005). Increased ET-1 plasma levels have been found in various diseases (Levin, 1995). In contrast to aqueous humour ET-1, increased plasma ET-1 has no effect on IOP which may be due to the blood-aqueous barrier (Karadag *et al.*, 2009). Increased ET-1 plasma levels were found in patients with multiple sclerosis. This has no effect on brain and retinal circulation when the blood-retina barrier is intact, but the blood flow in the fenestrated choroid and therewith at the ocular nerve head is reduced (Pache *et al.*, 2003; Flammer and Mozaffarieh, 2008). Thus, plasma ET-1 levels do not necessarily reflect local ET-1 concentration in the eye.

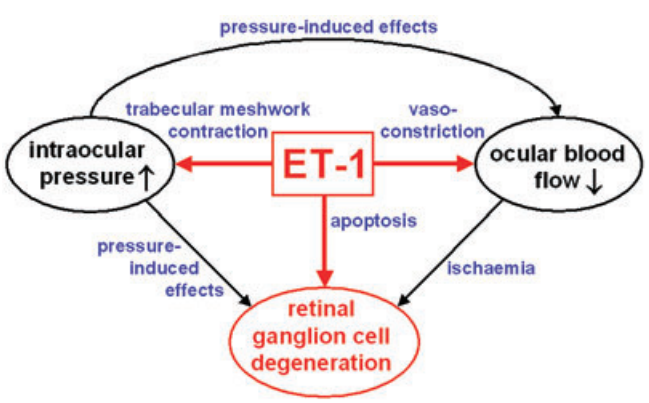

Figure 1

Involvement of endothelin-1 (ET-1) in the pathogenesis of glaucoma. ET-1 causes an increase in intraocular pressure, which directly and via reduced ocular blood flow leads to degeneration of retinal ganglion cells (RGCs). Furthermore, ET-1-induced vasoconstriction generates a decrease in ocular blood flow affecting RGCs. In addition, ET-1 evokes apoptosis of RGCs.

Studies on various animal models have shown that ocular application of ET-1 influences aqueous humour production and outflow (Erickson-Lamy *et al.*, 1991; Taniguchi *et al.*, 1994, 1996; Wiederholt *et al.*, 1995). Several studies with different experimental approaches have established an increase in IOP caused by an elevation of ET-1 in aqueous humour (Granstam *et al.*, 1991; Okada *et al.*, 1995; Sugiyama *et al.*, 1995a; Hollo *et al.*, 2000). It was further observed that intravitreal injection of endothelin decreased retinal and optic nerve head blood flow (Granstam *et al.*, 1992; Sugiyama *et al.*, 2009) which causes ischemic conditions leading to injury of RGCs and pathological changes of the optic nerve head (Haefliger *et al.*, 1993; Sugiyama *et al.*, 1996). Reduced optic nerve blood flow caused by exogenous application of ET-1 resulted in RGC death in the absence of elevated IOP (Orgul *et al.*, 1996b; Chauhan *et al.*, 2004). This may be caused by ischemic conditions and also by a direct apoptotic effect of ET-1 as shown in a study of Krishnamoorthy *et al.* (Krishnamoorthy *et al.*, 2008). This study revealed that ET-1 treatment directly mediates apoptosis of virally transformed rat RGCs.

Moreover, it has been shown that increased ET-1 levels in glaucoma is involved in astrocyte proliferation that occurs in glaucomatous optic neuropathy in human and also in animals with experimentally increased IOP (Hernandez, 2000). Additionally, an effect of ET-1 on the regulation of anterograde axonal transport in the optic nerve was described (Stokely *et al.*, 2002). Possibly, ET-1 is indirectly linked to the loss of RGCs in glaucoma by these effects.

In accordance with these findings, it was demonstrated that intravitreal injection or perfusion of ET-1 into eyes of different animal models such as monkeys, rabbits and rats generate optic neuropathy similar to glaucomatous optic nerve head damage including axon loss (Cioffi *et al.*, 1995; Orgul *et al.*, 1996a,b; Cioffi and Sullivan, 1999; Oku *et al.*, 1999).

In conclusion, ET-1 exerts effects on aqueous humour dynamics in the anterior chamber, on optic nerve head blood circulation, and on viability of RGCs. Consequently, the ocular effects of endothelin may provide a possible target for the pharmacological treatment of glaucoma which includes lowering of IOP and consequently the risk for glaucomatous damage and diminishing the probability of formation or progression of glaucomatous optic neuropathy. The sites and mechanisms of ET-1 antagonism described in the following are illustrated in Figure 2 and drugs with anti-endothelin effects are listed in Table 1.

Endothelin effect on intraocular pressure

The IOP is regulated by the balance of aqueous humour production in the non-pigmented ciliary epithelium and the

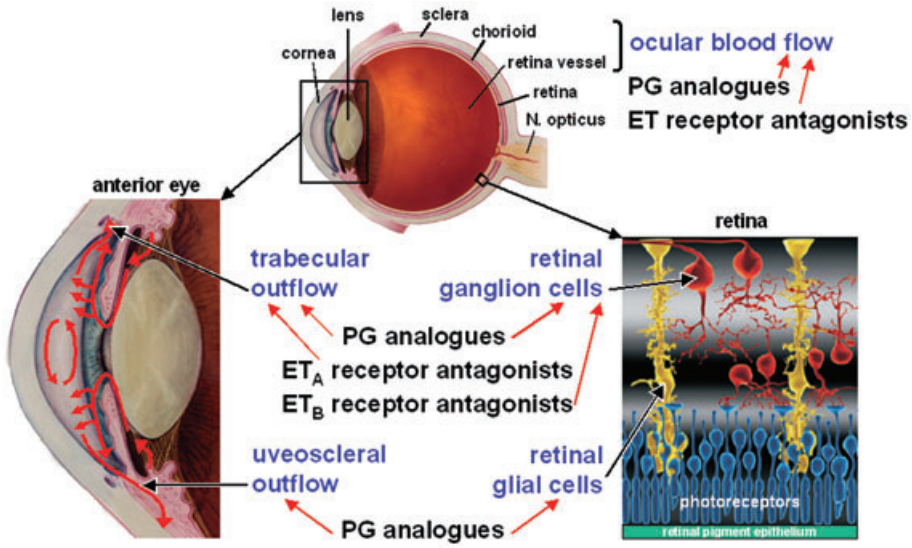

Figure 2

Sites and mechanisms of endothelin-1 (ET-1) antagonism in the eye. The ET-1 effects on ocular blood flow, trabecular outflow and viability of retinal ganglion cells could be inhibited by prostaglandin (PG) analogues, especially by prostaglandin F receptor agonists, and by endothelin receptor antagonists. In addition, PG analogues exert positive effects on uveoscleral outflow and retinal glial cells [retina part modified from Lu *et al.*, 2006; Copyright (2010) National Academy of Sciences, USA].

Table 1

Drugs with anti-endothelin effects as a possible approach for glaucoma therapy

Drug classes	Human studies IOP	Human studies Blood flow	Animal studies IOP	Animal studies Blood flow	RGC
ET receptor blocker					
ET_A blocker					
BQ-123	Ø	↑	↓	↑	
ET_B blocker					
BQ-788					+
$ET_{A/B}$ blocker					
Bosentan	Ø	↑			
PG F analogues					
Travoprost	↓	↑	↓	↑	
Latanoprost	↓	↑	↓	↑	+
Bimatoprost	↓	↑	↓		
Tafluprost	↓		↓	↑	+
Unoprostone	↓	↑	↓	↑	+

(↑) increase, (↓) decrease, (+) positive effect, (Ø) no effect.
ET, endothelin, IOP, intraocular pressure; PG, prostaglandin, RGC, retinal ganglion cell.

aqueous humour outflow. Aqueous humour leaves the eye in part via the uveoscleral and mainly via the trabecular outflow route. An increase in IOP is caused by a decrease in aqueous humour outflow from the eye. Aqueous humour has to pass the trabecular meshwork, a sponge-like tissue with contractile properties, before entering the venous system via Schlemm's canal and collector channels. Contraction of the trabecular meshwork leads to decreased aqueous humour outflow and increased IOP, whereas relaxation exerts the converse effect (Wiederholt *et al.*, 1995, 2000).

Different effects of endothelins on IOP are described in various animal models. MacCumber *et al.* found a decrease in IOP after injection of ET-1 or ET-3 in rabbit eyes which was not due to an increased aqueous outflow (MacCumber *et al.*,

1991). The authors assumed a decreased production of aqueous humour as the most likely mechanism for the IOP reduction.

In contrast, other studies on rabbit eyes presented a dose-dependent rise in IOP after intracameral injection of ET-1, ET-2 or ET-3 (Granstam *et al.*, 1991) or a biphasic IOP response, an early transient IOP rise followed by a subsequent prolonged decrease after intravitreal injection of ET-1 (Sugiyama *et al.*, 1995a).

In the monkey eye, intracameral injection of ET-1 increased the outflow facility which is expected to be, at least in part, mediated through an ET-1 effect on the ciliary muscle (Erickson-Lamy *et al.*, 1991).

In the bovine eye, it was shown that ET-1 induced a reduction of the aqueous humour outflow (Wiederholt *et al.*, 1995). This effect is caused by the ET-1-mediated contraction of trabecular meshwork (Choritz *et al.*, 2005; Rosenthal *et al.*, 2005; Thieme *et al.*, 2006). According to this model, it is assumed that drugs which inhibit the ET-1-induced contraction in the trabecular meshwork are suitable for lowering IOP. The ET-1-induced contraction could be blocked by inhibition of myosin light chain kinase (MLCK) or Rho kinase (ROCK) (Rosenthal *et al.*, 2005; Renieri *et al.*, 2008). Studies on animal models showed that inhibition of MLCK (Tian *et al.*, 2000; Honjo *et al.*, 2002) or ROCK (Honjo *et al.*, 2001; Rao *et al.*, 2001; Tokushige *et al.*, 2007) resulted in an increase in trabecular outflow facility and reduction in IOP.

Although MLCK and ROCK inhibitors, ML-7, M-9 or Y27632, lower the IOP, they are not suitable for glaucoma drug therapy because of side effects on accommodation and pupil size, due to an influence on the parasympathetically innervated muscles in the anterior chamber of the eye. A specific endothelin antagonism on the trabecular meshwork is described for endothelin receptor blockers and prostaglandin (PG) analogues.

Endothelin receptor antagonists

The effect of ET-1 is mediated by two G-protein-coupled receptors, endothelin receptor A (ET_A receptor) and endothelin receptor B (ET_B receptor). The ET_A receptor predominates in vasculature smooth-muscle cells and other smooth-muscle organs whereas ET_B receptor is additionally found on other tissues including brain and many epithelia [for review, see (Davenport, 2002; Pinet, 2004)]. Both receptors induce contraction when expressed in smooth muscle cells (Clozel and Gray, 1995; Lüscher and Wenzel, 1995).

For both receptors specific and mixed antagonists have been developed (Figure 3). The competitive antagonists ambrisentan and bosentan have been proved to be promising therapeutic agents.

Most antagonists are either ET_A receptor selective such as BQ-123, BQ-485 and ambrisentan, or mixed antagonists, such as bosentan. Only a limited number of ET_B receptor selective antagonists such as BQ-788 have been developed so far. The endothelin receptor antagonists can be divided into two groups, peptide (BQ-123, BQ-485, BQ-788) and non-peptide antagonists (ambrisentan and bosentan). However, the peptide compounds do not penetrate the blood-brain barrier when given systemically (Benigni and Remuzzi, 1999).

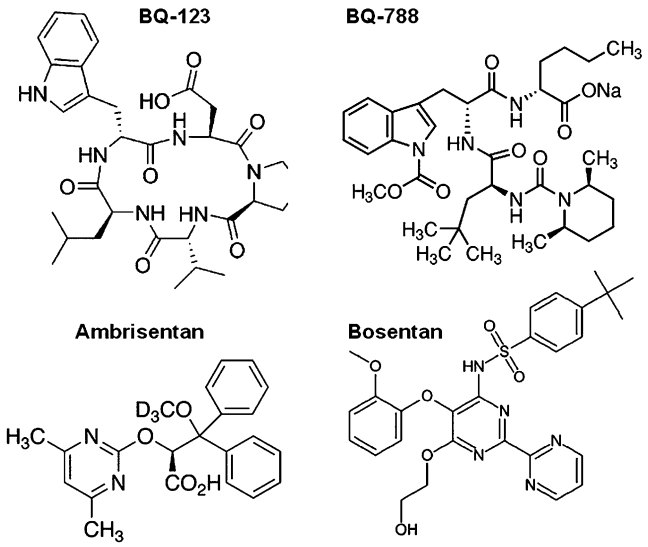

Figure 3

Endothelin receptor antagonists. ET_A receptor antagonists: BQ-123 (peptide), ambrisentan (non-peptide); ET_B receptor antagonist: BQ-788 (peptide); mixed antagonist: bosentan (non-peptide).

Hence, several non-peptide antagonists have been developed, but no non-peptide ET_B receptor antagonist is available so far. Unlike peptide antagonists, many non-peptide receptor-selective antagonists have oral bioavailability and some may cross the blood-brain barrier (Benigni *et al.*, 2004).

Endothelin receptor antagonists are approved for the management of pulmonary arterial hypertension. The mixed antagonist bosentan was the first drug of this class, now specific ET_A receptor antagonists, ambrisentan and sitaxsentan, are available (Rubin and Roux, 2002; Kingman *et al.*, 2009). The most significant adverse effects associated with the use of these drugs are major birth defects, thus they are contraindicated in pregnant women. Furthermore, a dose-dependent incidence of liver toxicity, headache, nasopharyngitis and peripheral oedema is described. These strong side effects may prevent the development of endothelin receptor antagonists for glaucoma therapy.

Effect of endothelin receptor inhibition on intraocular pressure

The ET-1 effect on IOP seems be mediated predominantly via the ET_A receptor as contractility studies on the trabecular meshwork and different animal studies reveal.

Although both endothelin receptors are expressed in the trabecular meshwork, ET-1 induces contraction of this tissue predominantly by activation of ET_A receptor, as shown in studies with specific endothelin receptor antagonists (Choritz *et al.*, 2005) (Figure 4). Because this contraction contributes to the increase in IOP in glaucoma, an inhibition by specific ET_A receptor antagonists seems to be a target for IOP-lowering drugs.

Convincing evidence for this assumption is provided by several studies on the rabbit model. In this model, argon laser treatment of one eye causes an increase in ET-1 concentration

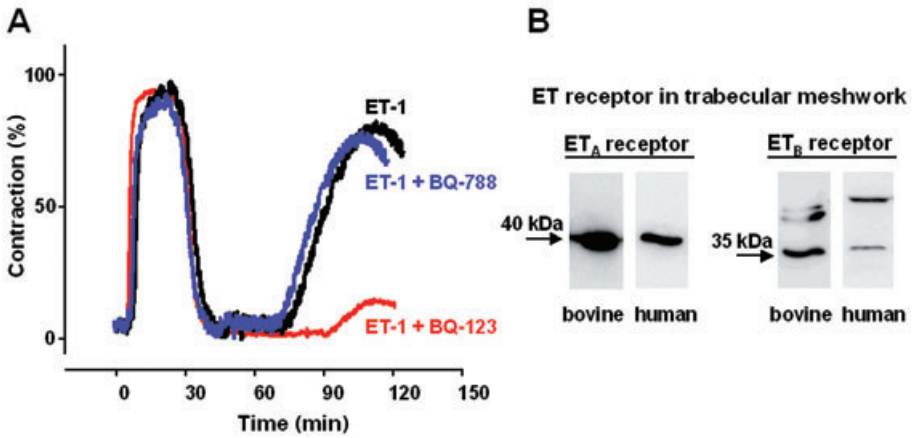

Figure 4

Effect of endothelin receptor blockers on endothelin-1 (ET-1)-induced contraction in trabecular meshwork. (A) Original recording of isometric force in bovine trabecular meshwork which was a well-established model for glaucoma research. After a carbachol (10^{-6} mol·L^{-1})-induced peak which was set to 100% contraction obtainable, the contraction was elicited by ET-1 (10^{-8} mol·L^{-1}) or ET-1 after pre-incubation with either the ET$_A$ receptor antagonist BQ-123 or the ET$_B$ receptor antagonist BQ-788 (both 10^{-6} mol·L^{-1}). The following ET-1-induced contraction in the presence of BQ-123 was strongly reduced, whereas BQ-788 has no effect. (B) Expression of endothelin receptors verified with Western blot analysis in cultured cells of bovine and human trabecular meshwork (modified from Choritz *et al.*, 2005; Copyright S. Karger AG, Basel; Rosenthal *et al.*, 2007).

in the aqueous humour and an increase in IOP in comparison to the contralateral eye without laser treatment (Hollo *et al.*, 2000, 209). Pretreatment of the eye with the ET$_A$ receptor antagonist BQ-485 protected against the laser treatment-induced increase in IOP, but did not influence the laser-induced ET-1 release. This suggests that ET$_A$ receptor antagonists may prevent the effect of ET-1 on IOP (Hollo *et al.*, 2002).

The above assumption is confirmed by another study that reveals an increased IOP after intraocular injection of the serine protease chymase, which converts big endothelin to endothelin. This chymase-induced ocular hypertension was inhibited by the ET$_A$ receptor antagonist BQ-123 (Haque *et al.*, 1996; Konno *et al.*, 2005).

A further study showed that intravitreally administered ET-1 induced a biphasic IOP response, an early transient IOP rise followed by a subsequent prolonged decrease (Sugiyama *et al.*, 1995a). The transient rise in IOP was probably due to contraction of the trabecular meshwork, whereas the prolonged IOP reduction was caused by an indirect ET-1 effect. It came out that intraocular ET-1 injection activates the release of endogenous PGs into aqueous humour which in turn antagonize the ET-1 effect on IOP. The ET$_A$ receptor selective antagonists 97–139 and BQ-123 had no effect on IOP when used alone but significantly inhibited both, the IOP rise and the IOP reduction caused by ET-1. These results indicate that both, the IOP response and the elevation of PGs in aqueous humour following ET-1 injection, are at least partially mediated by ET$_A$ receptors.

Taken together, animal studies revealed that the ocular hypertensive effect of ET-1 can be blocked by intraocularly administered antagonists of the ET$_A$ receptor. In contrast, patient studies which analyse the effects of orally or intravenously applied endothelin receptor antagonists (bosentan or BQ-123) on OBF showed no effect on IOP neither in glaucoma patients nor in healthy subjects (Fuchsjager-Mayrl *et al.*, 2003; Polak *et al.*, 2003; Resch *et al.*, 2009). Possibly, an intraocular administration of endothelin receptor antagonists would exert IOP-lowering effects in glaucoma patients as seen in animal models. Until now, it is not tested whether local application of ET receptor antagonists could affect the IOP and also, no endothelin receptor antagonist for topical application is available.

Effect of endothelin receptor inhibition on ocular blood flow

In addition to the endothelin receptors on the trabecular meshwork, ET$_A$ receptor and ET$_B$ receptor were detected in the vascular smooth muscle of choroidal and retinal vessels, with the former being predominant (Stitt *et al.*, 1996). Several studies indicate that the endothelin system is involved in the processes that lead to reduced OBF in glaucoma. In rabbits intravitreal injection of ET-1 caused a decrease in OBF (Sugiyama *et al.*, 1995b, 2009).

Also in healthy volunteers intravenous injection of ET-1 induced a decrease in optic nerve head, choroidal and retinal blood flow (Polak *et al.*, 2001, 2003). These effects were significantly inhibited when the ET$_A$ receptor inhibitor BQ-123 was co-administered. Since application of BQ-123 alone did not affect optic nerve head, choroidal, and retinal blood flow, ET-1 does not seem to contribute substantially to the regulation of basal vascular tone in these tissues. Another study presented, that ET-1 contributes to the hyperoxia-induced retinal vasoconstriction in humans and this effect is attenuated by BQ-123 (Dallinger *et al.*, 2000).

Additionally, administration of the ET$_A$ receptor/ET$_A$ receptor blocker bosentan increased choroidal and optic nerve head blood flow in patients with POAG and sex- and age-matched healthy volunteers. The effect of bosentan on OBF parameters was comparable between the two groups (Resch *et al.*, 2009).

However, endothelin receptor antagonists improve OBF and may be taken into consideration as a new approach for the treatment of glaucoma.

Effect on retinal ganglion cells

Additionally to the indirect damaging effects of ET-1 on RGCs caused by an increased IOP and decreased OBF, ET-1 also exerts a direct apoptotic effect on RGCs. This effect is mediated exclusively via the ET_B receptor, although both receptors are expressed in RGCs (MacCumber and D'Anna, 1994). Ocular ET-1 administration in rats has been shown to cause apoptosis of RGCs which was markedly attenuated in ET_B receptor-deficient rats, suggesting a key role for ET_B receptor in apoptosis of RGCs. In virally transformed rat RGCs (RGC-5 cells), ET-1 treatment produced apoptotic changes which were associated with ET_B receptor activation and accompanied by a significant up-regulation of ET_B receptor expression. Pretreatment of the cells with the ET_B receptor antagonist BQ788 attenuated ET-1-mediated apoptosis (Krishnamoorthy *et al.*, 2008).

Additionally, an effect of ET-1 on the regulation of anterograde axonal transport in the optic nerve was described which seems to be mediated by activation of the ET_B receptor (Stokely *et al.*, 2002). Intravitreous injection of ET-1 in rats caused a biphasic change of anterograde axonal transport, after an initial, rapid enhancement a prolonged reduction of anterograde axonal transport into the optic nerve occurs. This dysregulation of axonal transport might contribute to RGC loss. These findings indicate positive effects of ET_B receptor inhibition on the survival of RGCs.

Furthermore, ET-1 might be indirectly linked to the loss of RGCs by astroglial proliferation which is associated with glaucomatous optic nerve neuropathy. ET-1 induces astroglial proliferation in cultured human optic nerve head astrocytes through $ET_{A/B}$ receptor activation (Prasanna *et al.*, 2002). This effect could be blocked by a mixed receptor antagonist.

Taken together, the neuroprotective effect of mixed ET receptor antagonists or specific ET_B receptor antagonists may be used in glaucoma therapy for preventing or delaying RGC loss.

Prostaglandin analogues

Today, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogues (Figure 5) are the first-line drugs in the medical treatment of glaucoma and ocular hypertension (Al-Jazzaf *et al.*, 2003; Hylton and Robin, 2003; Perry *et al.*, 2003; Nguyen, 2004) because these agents are the most effective drugs for lowering IOP.

The $PGF_{2\alpha}$ analogues latanoprost, travoprost and bimatoprost are approved for glaucoma therapy in the USA and Europe, tafluprost is approved in Germany since 2008 and in Switzerland since 2010. Travoprost, latanoprost, and tafluprost are ester prodrugs of $PGF_{2\alpha}$. Bimatoprost is the amide prodrug of 17-phenyl-$PGF_{2\alpha}$ and has been classified as a prostamide. The docosanoid unoprostone that is often included in the group of PG analogues is approved for glaucoma therapy in Japan (unoprostone isopropyl ester, Rescula®).

Several studies performed on animal models revealed that these drugs attenuate the ET-1-induced smooth muscle

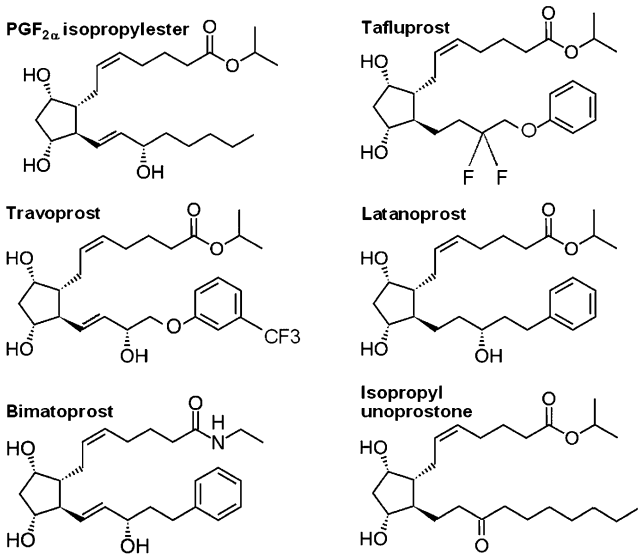

Figure 5

Prostaglandin F receptor agonists. Chemical structure of the prostaglandin analogues travoprost, latanoprost, and bimatoprost and the docosanoid isopropyl unoprostone which are applied in glaucoma therapy in comparison to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) isopropylester.

contraction (Thieme *et al.*, 2001, 2006). It is assumed that this effect contributes to the reduction of IOP and the improvement of OBF during treatment with PG analogues. Additionally, neuroprotective effects against ET-1-induced neuronal injury are described (Munemasa *et al.*, 2008).

Effect of prostaglandin F analogues on intraocular pressure

Prostaglandin analogues provoke a strong reduction of IOP which is caused by an enhancement of both, the uveoscleral and trabecular outflow of aqueous humour. The increase of uveoscleral outflow is due to an increased matrix-metalloproteinase (MMP) production in tissues of the uveoscleral outflow pathway, such as ciliary muscle (Weinreb *et al.*, 1997, 2002; Gaton *et al.*, 2001) and sclera (Kim *et al.*, 2001; Weinreb *et al.*, 2004). Additionally, an increased trabecular outflow was observed in patients with ocular hypertension or POAG (Toris *et al.*, 2007) and in human anterior segments in organ culture (Wan *et al.*, 2007) which might be due to histological changes in the trabecular outflow pathway (Bahler *et al.*, 2008).

Furthermore, in animal studies the ET-1-induced contraction of the trabecular meshwork was found to be reduced by unoprostone, $PGF_{2\alpha}$ and fluprostenol (fluprostenol-Isopropylester = travoprost) (Figure 6) (Thieme *et al.*, 2001, 2006). This effect is mediated via the prostaglandin F receptor (FP receptor). The IOP-lowering effect of PG analogues used in glaucoma therapy is also mediated via the FP receptor as studies on FP receptor knock-out mice revealed. In FP receptor knock-out mice application of latanoprost, travoprost, bimatoprost or unoprostone had no effect on IOP (Ota *et al.*, 2005).

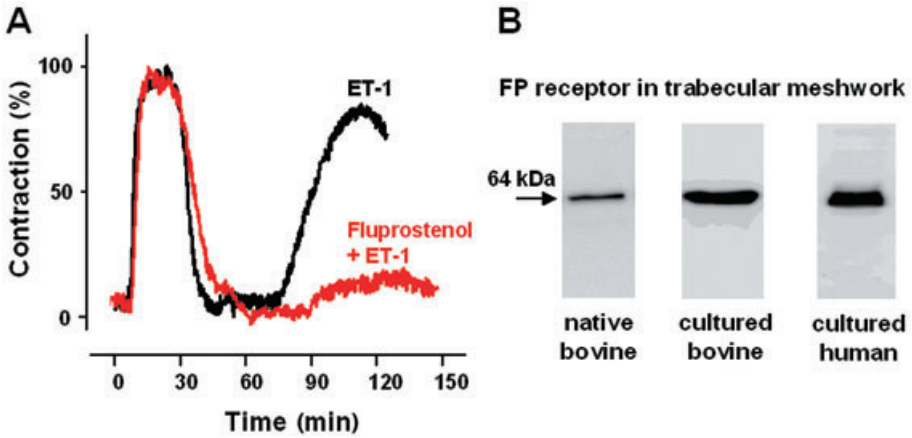

Figure 6

Effect of fluprostenol on endothelin-1 (ET-1)-induced contraction in trabecular meshwork. (A) Original recording of isometric force in bovine trabecular meshwork which serves as a well-established model for glaucoma research. After a carbachol (10^{-6} mol·L^{-1})-induced peak which was set to 100% contraction obtainable, the contraction was elicited by ET-1 (10^{-8} mol·L^{-1}) or ET-1 after pre-incubation with fluprostenol (10^{-6} mol·L^{-1}). The ET-1-induced contraction was strongly diminished in presence of the prostaglandin F receptor (FP receptor) agonist fluprostenol. (B) Expression of FP receptors verified with Western blot analysis in native bovine trabecular meshwork and cultured cells of bovine and human trabecular meshwork (modified from Thieme *et al.*, 2006).

In contrast, the contraction elicited by muscarinic agonists is not affected by the PG analogues. This is in accordance with the finding that glaucoma treatment with PG analogues is not associated with side effects on accommodation and pupil size. It is probable that the anti-endothelin effect on trabecular meshwork contractility accounts for the IOP-lowering effect of PG analogues for two reasons. First, the strong reduction of IOP could not be explained by an exclusive increase in the comparatively low uveoscleral outflow (Nilsson, 1997); and second, the rapid IOP reduction within hours after application of the drugs could not be explained by ECM degradation. Therefore, endothelin-antagonism on trabecular meshwork contractility decisively contributes to the IOP-lowering effect of PG analogues.

Improvement of ocular blood flow by prostaglandin F analogues

Several studies on glaucoma patients showed an increase in OBF after treatment with PG analogues which was assumed to correlate with the IOP-lowering effect of the drugs (Sponsel *et al.*, 2002a,b; Erkin *et al.*, 2004). Application of bimatoprost and travoprost resulted in an improvement in the central retinal artery blood flow in newly diagnosed open-angle glaucoma patients (Alagoz *et al.*, 2008). An improved OBF after treatment with latanoprost was also found in patients with POAG, normal tension glaucoma and ocular hypertension (Vetrugno *et al.*, 1998; McKibbin and Menage, 1999; Georgopoulos *et al.*, 2002; Sponsel *et al.*, 2002b; Erkin *et al.*, 2004; Gherghel *et al.*, 2008). Also, unoprostone caused an increase in OBF, a comparison of latanoprost and unoprostone revealed, that latanoprost once daily produced a OBF increase nearly twofold greater than those obtained with unoprostone twice daily (Sponsel *et al.*, 2002a). In this study, latanoprost caused a larger IOP reduction than unoprostone. A direct anti-endothelin effect of unoprostone on OBF was verified in a study with healthy individuals. In this placebo-controlled study, it was shown that intravenous injection of ET-1 decreased choroidal blood flow. This effect was significantly blunted when topical unoprostone was coadministered (Polska *et al.*, 2002).

Different animal studies revealed a direct, IOP-independent effect of PG analogues on blood flow. In rabbits ET-1 decreased optic nerve head blood flow. Pretreatment with intravitreal injection of unoprostone did not affect IOP, but partly inhibited the blood flow-decreasing effect of ET-1 (Sugiyama and Azuma, 1995). Another study showed that the ET-1-induced decrease in OBF was almost completely prevented by tafluprost and significantly inhibited by latanoprost and travoprost. These drugs are shown to relax the ciliary artery contraction induced by ET-1 (Kurashima *et al.*, 2010). Moreover, the vasoconstrictive effect of ET-1 on perfused porcine retinal arterioles was slightly inhibited by $PGF_{2\alpha}$, a pronounced inhibition was induced by unoprostone (Yu *et al.*, 2001).

Assuming a similar mode of action for all PG analogues, the improvement of OBF after treatment with these drugs is due to an IOP-dependent effect and additionally to a direct anti-endothelin effect on vascular smooth muscle.

In summary, the improvement of OBF induced by application of PG analogues could be beneficial for glaucoma patients suffering from impaired ocular perfusion, either due to vasoconstriction caused by ET-1 or to abnormal vascular autoregulation.

Neuroprotective effect of prostaglandin F analogues

In addition to the indirect neuroprotective effects of PG analogues due to an increased OBF, some direct neuroprotective effects of these drugs are described in animal studies, data on

humans are not available so far. A morphometric study in the rat showed that intravitreal injection of ET-1 led to cell loss in the RGC layer. This is accompanied by a decrease in neurofilament protein in the optic nerve. Simultaneous injection of the unoprostone metabolite M1 attenuated RGC loss and the decrease in neurofilament protein induced by ET-1 compared with ET-1 injection alone. These results suggest that unoprostone exerts neuroprotective effects against ET-1-induced neuronal injury (Munemasa *et al.*, 2008). A direct anti-apoptotic effect of latanoprost and tafluprost on rat RGCs *in vivo* and *in vitro* was also described (Kudo *et al.*, 2006; Kanamori *et al.*, 2009).

Furthermore, an anti-apoptotic effect of latanoprost (Nakanishi *et al.*, 2006) and the unoprostone metabolite M1 (Mukuno *et al.*, 2004) on rat retinal glial cells which ensure the maintaining of retinal homeostasis and trophic support for the neurons was observed. In contrast, in an experimental rat model with increased IOP and resulting gliosis in the retina, latanoprost attenuates the retinal glial reaction and may afford neuroprotection to the ganglion cells by this effect (Vidal *et al.*, 2010).

Inhibition of endothelin synthesis

A quite different way to antagonize the endothelin-induced glaucomatous damage in the eye would be the inhibition of endothelin synthesis. ET-1 is produced from its biologically almost inactive precursor Big ET-1 (38 amino acids) by a membrane-bound Zn-dependent metalloprotease, endothelin-converting enzyme (ECE). The ECE is expressed in the blood vessels of the retina, optic nerve and choroids (Wollensak *et al.*, 2002; Dibas *et al.*, 2005b). Additionally, ECE activity was found in ciliary epithelium and retinal pigment epithelium (Prasanna *et al.*, 1999; Dibas *et al.*, 2005a). Plasma membrane ECE activity could be inhibited by phosphoramidon (potent inhibitor of ECE), thiorphan (metalloprotease inhibitor) and phenanthroline (inhibitor of zinc-dependent proteases).

The ECE activity may emerge as a possible target in preventing ET-1-induced increase in IOP and ET-1-induced damage of RGCs and the optic nerve.

General conclusions

The potent vasoconstrictor ET-1 has been found to be increased in the aqueous humour of glaucoma patients compared to control and is suspected to be involved in the pathogenesis of the disease by effects on IOP, OBF and RGCs. An antagonism of endothelin effects provides a promising approach for medical treatment of glaucoma, by three mechanisms: (i) lowering the IOP due to relaxation of the trabecular meshwork; (ii) promotion of blood flow caused by IOP-dependent and -independent effects on ocular vessels; and (iii) increasing the survival of RGCs.

Hence, antagonism of endothelin signalling through both, pressure-dependent and -independent pathways, stands for a promising therapeutic principle in the pharmacological treatment of glaucoma.

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Conflict of interest

The authors declare no conflict of interest.

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