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A novel D2-dopaminergic and α2-adrenoceptor receptor agonist induces substantial and prolonged IOP decrease in normotensive rabbits

Jouko Savolainen, Jarkko Rautio, Roberta Razzetti, Tomi Järvinen

Abstract

The effects of a novel and selective D2-dopaminergic/α2-adrenoceptor agonist, CHF1035, and its metabolite CHF1024 on intraocular pressure (IOP) were determined in rabbits. Because CHF1035 is a mixture of two enantiomers, CHF1800 (+) and CHF1810 (−), pure enantiomers were also studied to determine possible differences in IOP-decreasing ability depending on the stereochemistry of the molecule. CHF1035, CHF1800 (+), CHF1810 (−), CHF1024, brimonidine and 0.9% NaCl were administered topically to rabbits and IOP was then measured at fixed time intervals. The dose–response profile (0.01–1.0% w/v) was determined for CHF1035. CHF1035 and its metabolite CHF1024 significantly lowered IOP in the treated eyes. CHF1035 showed a maximum IOP decrease (7.6 ± 1.5 mmHg) 5 h post-dosing, whereas the metabolite CHF1024 showed a maximum decrease in IOP (7.0 ± 0.8 mmHg) 3 h post-dosing. The maximum IOP decrease produced by CHF1035 in the treated eye was comparable with that produced by brimonidine (7.8 ± 0.9 mmHg), but CHF1035 had a significantly longer duration of action. Unlike brimonidine, CHF1035 and CHF1024 did not decrease IOP in the untreated eye. CHF1810 (−) lowered the IOP more than CHF1800 (+). No irritation, evaluated as eyelid closure, was observed after topical administration of any of the compounds. Only in the case of CHF1035 1% solution, two rabbits out of six closed the eye for 30–45 s. In conclusion, CHF1035 and its metabolite CHF1024 significantly decreased the IOP in rabbits, and are potential novel IOP lowering agents. Especially, CHF1035 produced a substantial decrease in IOP for a prolonged period of time, and thus may prove useful in glaucoma therapy.

Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211, Kuopio, Finland

Jouko Savolainen, Jarkko Rautio, Tomi Järvinen

Pharmacology and Toxicology Department, Chiesi Farmaceutici S.p.A. Via Palermo, Parma, Italy

Roberta Razzetti

Finncovery Ltd, Kuopio, Finland

Tomi Järvinen

Correspondence: Jouko Savolainen, University of Kuopio, Department of Pharmaceutical Chemistry, P.O. Box 1627, FIN-70211 Kuopio, Finland. E-mail: jouko.savolainen@uku.fi

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Introduction

α2-Adrenoceptor agonists (α2-agonists) play a significant role in the medical management of glaucoma by lowering intraocular pressure (IOP) (Harrison & Kaufmann 1977; Burke & Potter 1986; Vartiainen et al 1992; Greenfield et al 1997). Clonidine was the first α2-agonist to be approved for the treatment of glaucoma, but its clinical use is limited by significant systemic side effects, such as the lowering of systemic blood pressure after topical administration (Harrison & Kaufmann 1977; Burke & Schwartz 1996). Apraclonidine, a hydrophilic analogue of clonidine, efficiently decreases IOP and is currently used for the treatment of glaucoma (Wallace & Alward 1998). Apraclonidine, however, has quite a high affinity for α1-adrenoreceptors, resulting in ocular side effects (e.g., ocular allergy). Brimonidine (Burke & Potter 1986) is a fairly new and selective α2-agonist. It is currently used in treating glaucoma, and is fairly well tolerated with a low incidence of adverse effects (Wallace & Alward 1998; Lee et al 2000). Brimonidine, however, crosses the blood–brain barrier, potentially causing central nervous system (CNS) toxicity, and thus brimonidine should be used with caution (Enyedi & Freedman 2001).

Although many glaucoma drugs are considered to be α2-agonists, they often have appreciable affinity towards D2-dopaminergic receptors. Since dopamine receptors are present in ciliary processes, α2-agonists may also act via D2-receptors in the eye (Kaufman & Mittag 1994). Moreover, the topical administration of D2-receptor agonist, including the apomorphine derivative TNPA, has been shown to be associated with a reduction in IOP (Karnezis & Murphy 1988; Ogidigben et al 1993; Prunte et al

1997; Surgue 1997). In particular, it has been demonstrated that TNPA exerts suppressive action on aqueous humour flow, predominantly through prejunctional D2-receptors of peripheral sympathetic nerves (Ogidigben et al 1993). CHF1035 ((±) 5,6-diisobutyroyloxy-1,2,3,4-tetrahydro-2-methylamino-naphtalene hydrochloride) is rapidly transformed by plasma and tissue esterases into its active metabolite CHF1024 ((±) 5,6-dihydroxy-2-methylamino-1,2,3,4-tetrahydronaphtalene). CHF1035 has been characterised as a potent and selective agonist for the prejunctional D2- and α2-receptors (Pastore et al 2000; Razzetti et al 2000), whose stimulation leads to diminished noradrenaline (norepinephrine) release from sympathetic nerve endings. Due to this mechanism of action CHF1035 shows beneficial effects in treating chronic heart failure and it is currently undergoing clinical trials for this indication (McMurray & Pfeffer 2002).

The potency and selectivity of CHF1035 towards D2- and α2-receptors make it a potentially interesting chemical entity for treating illnesses where down-regulation of sympathetic drive may have a role. The aim of this study was to determine the effects on IOP of topically administered CHF1035 and its metabolite CHF1024, compared with a clinically used α2-agonist, brimonidine. As CHF1035, due to the presence of a chiral carbon in its molecule, is a 50:50 mixture of two enantiomers, CHF1800 (+) and CHF1810 (−), the effect of the pure enantiomers on IOP was also determined. The two enantiomeric forms have been shown to have different receptor profiles (i.e., the D2-dopaminergic receptor activity is essentially linked to the (−) form, while that for α2-adrenoceptors is shared by both) (Pastore et al 2001).

Materials and Methods

Materials

CHF1035, CHF1024, CHF1800 (+) and CHF1810 (−) were obtained from Chiesi Farmaceutici S.p.A. (Parma, Italy). Brimonidine was purchased from Sigma-RBI (St Louis, MO). Ethylenediaminetetraacetic acid (EDTA) and 1-octanesulfonic acid sodium salt were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany).

Animals

Normotensive Dutch Belted rabbits (National Laboratory Centre, University of Kuopio) of either gender (2.3–4.0 kg, n = 6) were used. The rabbits were housed singly in cages under standard laboratory conditions with a 12-h dark–light cycle, at an ambient temperature of 20.0 ± 0.5 °C and 55–75% relative humidity. Water and food were given freely, except during the tests. All rabbits were treated in accordance with the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research, and all experimental procedures were reviewed and approved by the Animal Ethics Committee at the University of Kuopio.

Preparation of eyedrop solutions

An appropriate amount of CHF1035, CHF1800 (+), CHF1810 (−), CHF1024 or brimonidine were dissolved in deionized water, and the solution was adjusted to pH 4.5 with NaOH solution. The pH of the eyedrop solutions was selected according to the chemical stability of CHF1035, which is good at pH 4.5 (data not shown). The solutions were made isotonic (288–300 mOsm) with sodium chloride. Final drug concentrations in eyedrop solutions were determined by high-pressure liquid chromatography (HPLC).

Analytical procedure

A Beckman System Gold Programmable Solvent Module 126, Beckman System Gold Detector Module 166 with variable wavelength UV detector (set at 230 nm) and Beckman System Gold Autosampler 507e were used for HPLC analysis. Separations were performed on a Purospher RP-18e reverse-phase column (12.5 cm × 4.0 mm, i.d. 5 μm), purchased from Merck (Darmstadt, Germany). The chromatographic conditions were as follows: injection volume, 50 μL; column temperature, 27 °C; flow rate, isocratic or gradient at 1.0 mL min^{-1}.

The mobile phase consisted of 37% or 10% of an acetonitrile–water mixture (80:20) and 63% or 90% of 20 mM phosphate buffer (pH 3.5) for the analysis of CHF1035 and brimonidine, respectively. The retention times were 7.5 and 2.6 min for CHF1035 and brimonidine, respectively.

In the case of CHF1024, CHF1800 (+) and CHF1810 (−), a 20 mM phosphate buffer (pH 2.15) was used, and 600 mg L^{-1} of 1-octanesulfonic acid (sodium salt) and 50 mg L^{-1} of EDTA was added to the buffer phase to obtain satisfactory resolution and retention time. The organic phase was acetonitrile–water (80:20), and the ratio of buffer phase to organic phase was 57:43 for CHF1800 (+) and CHF1810 (−). For CHF1024, a gradient HPLC method was used with the same mobile phase components. The retention times were 7, 6 and 5.9 min for CHF1024, CHF1800 and CHF1810, respectively.

All analyses were performed in triplicate.

Intraocular pressure (IOP) measurements

To perform an IOP test, the rabbits were placed in plastic restraining boxes located in a quiet room. A single drop (25 μL) of the test solution was instilled unilaterally into the left eye of each rabbit, on the upper corneoscleral limbus. During installation, the upper eyelid was pulled slightly away from the globe. IOP was measured using a BioRad (Cambridge, MA) Digilab Modular One Pneumatonometer. Before each measurement, one or two drops of topical anaesthetic (0.06% oxybuprocaine) was applied to the cornea before tonometry to eliminate discomfort. The upper and lower eyelids were then gently retracted, and the applanation sensor was brought into contact with the centre of the cornea. For each determination, at least two readings were taken from each treated (ipsilateral) and untreated (contralateral) eye, and the

mean of these readings was used. The IOP was measured at 1 and 0 h before, and then 0.5, 1, 2, 3, 4, 5, 6 and 7 h after, eyedrop administration. IOP at the time of eyedrop administration (0 h) was used as the baseline value. All studies were set up using a masked and randomized crossover design. At least 72 h washout time was allowed for each rabbit between dosings. The irritation caused by an instilled eyedrop was evaluated by recording the extent of eyelid closure (closed or half-closed) after topical administration. Recording of the eyelid closure behaviour was discontinued once the rabbit opened its eye fully.

Statistical analysis

All IOP results are presented as a change in IOP (mmHg), and expressed as mean ± s.e.m. (standard error of the mean). A one-factor analysis of variance for repeated measurements was used to test the statistical significance of any differences between groups. Significance in differences of the means was tested using Fisher's Protected Least Significance Difference (PLSD) method in which $P < 0.05$ denoted significance.

Results and Discussion

Treated eyes

The chemical structures of CHF1035 and CHF1024 are presented in Figure 1. Topical administration of both CHF1035 and CHF1024 significantly decreased the IOP in the treated eyes (Figure 2A). A maximum IOP decrease of 7.6 ± 1.5 mmHg was observed at 5 h after topical administration of 0.2% CHF1035. The onset of IOP decrease was faster after topical administration of metabolite CHF1024; the maximum ocular hypotension (7.0 ± 0.8 mmHg) was obtained at 3 h post-dosing, and the IOP decrease started to decline sooner when compared with CHF1035 (Figure 2A). The maximum effect on IOP of CHF1035 and CHF1024 in the treated eyes was comparable with an equivalent dose of brimonidine.

The different IOP decrease profiles for CHF1035 and CHF1024 are most probably due to differences in their chemical structures, as CHF1035 is a isobutyroyl ester prodrug of CHF1024 (Figure 1). After topical absorption, CHF1035 must first be biotransformed to the monosubstituted derivative, and then to CHF1024 before the onset of action can begin, as CHF1024 is the only pharmacologically active structure (Tjeerdsma et al 2001). Besides, it is possible that partition of lipophilic CHF1035 in ocular tissues is different compared with the more hydrophilic CHF1024.

The dose–response study shows that 0.2%, 0.5% and 1.0% of CHF1035 have similar IOP decrease profiles (Figure 3). These doses caused a minor initial IOP increase, followed by a subsequent IOP decrease in the treated eye. In contrast, the lowest dose (0.01% CHF1035) decreased IOP without any initial IOP increase.

CHF1035 is a racemate. Thus, it is reasonable to consider a difference in the IOP lowering ability of the isomers (Carabaza et al 1996). The IOP effects of the pure

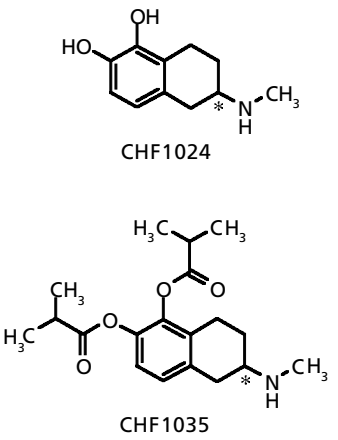

Figure 1 Chemical structures of CHF1035 and CHF1024, with chiral carbons marked by an asterisk.

CHF1035 enantiomers, CHF1800 (+) and CHF1810 (−), are shown in Figure 4. CHF1810 (−) had a greater effect on IOP than CHF1800 (+), although a statistical difference between these enantiomers was only observed at 6 h after administration. Based on this preliminary data, it is very possible that there is lack of stereospecificity between the enantiomers. If there were real differences in IOP-lowering ability between the enantiomers, it could also be due to different ocular pharmacokinetics, since enantiomers may have different pharmacodynamic (Carabaza et al 1996) or pharmacokinetic properties (Kroemer et al 1996; Tarjányi et al 1998; Hongmei & Lewander 1999). Pharmacokinetic profiles of the enantiomers from aqueous humour, however, were not measured in this study.

No irritation, evaluated as an eyelid closure, was observed after topical administration of any of the compounds. Only in the case of 1% CHF1035, two rabbits out of six closed or half-closed the eye for 30–45 s.

Untreated eyes

CNS penetration is a major drawback for many antiglaucoma agents (Kaufman & Mittag 1994; Enyedi & Freedman 2001). Centrally acting α2-agonists, such as apraclonidine and its parent compound clonidine, enter the CNS and cause systemic arterial hypotension. Topical administration of 0.2% CHF1035 and 0.124% CHF1024 did not have a significant effect on IOP in the untreated eye (Figure 2B). These results suggest that neither of them enter the CNS to a significant degree. In contrast, topical administration of 0.2% brimonidine significantly decreased IOP in the untreated eye where the maximum decrease in IOP was 8.7 ± 0.8 mmHg at 1 h after administration (Figure 2B). Moreover, brimonidine has been shown to cross the blood–brain barrier and cause CNS toxicity in patients (Enyedi & Freedman 2001).

Duration of action

CHF1035 and CHF1024 have long durations of action. In this study, the compounds produced significant decreases

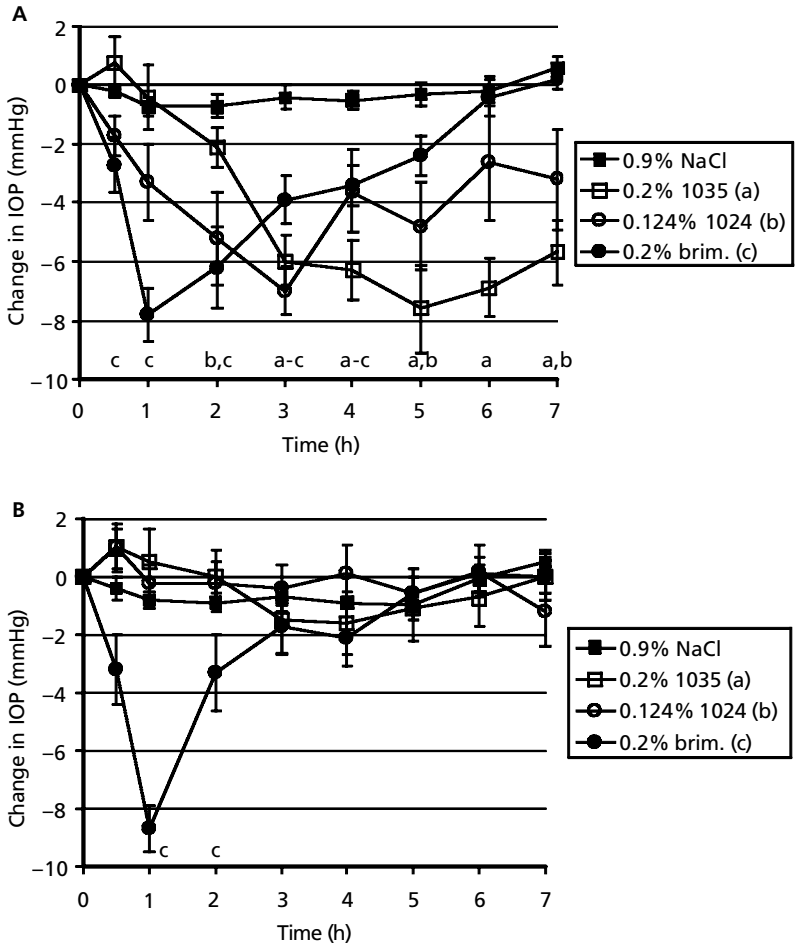

Figure 2 The effects (mean ± s.e.m., n = 6) of CHF1024, CHF1035, brimonidine (brim.) and 0.9% NaCl on IOP in treated (A) and untreated (B) eyes of normotensive rabbits after unilateral ocular administration. Letters (a, b, c in the legend) indicate data significantly different from values for 0.9% NaCl. CHF1024 0.124% is molarly equal to CHF1035 0.2%.

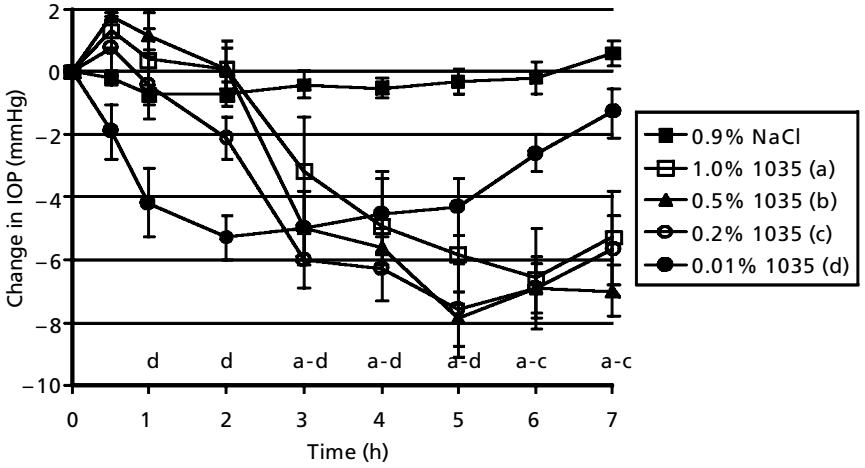

Figure 3 Dose–response (IOP) profile for CHF1035 in treated eyes of normotensive rabbits. Letters (a, b, c, d in the legend) indicate data significantly different from values for 0.9% NaCl (mean ± s.e.m., n = 6).

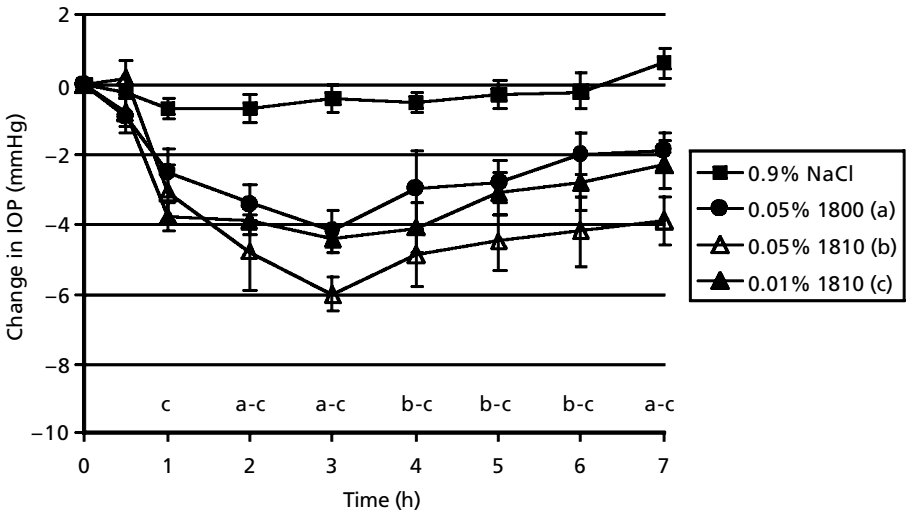

Figure 4 The effects (mean ± s.e.m., n = 6) of CHF1035 enantiomers, CHF1800 (+) and CHF1810 (−), and 0.9% NaCl on IOP in the treated eye of normotensive rabbits. Letters (a, b, c in the legend) indicate data significantly different from values for 0.9% NaCl.

in IOP, even 7 h post-dosing, whereas an equivalent dosage of brimonidine significantly lowered IOP only 4 h post-dosing (Figure 2A). The prodrug CHF1035 seems to be an especially long-lasting and potent IOP-lowering agent. The IOP levels, however, returned back to their base-line values after 24 h (data not shown). Thus, the action of CHF1035 and CHF1024 is reversible. The longer duration of action offers clear benefits in ocular drug treatment, particularly with regard to patient compliance in the treatment of a chronic illnesses such as glaucoma. Several formulation approaches, such as high-viscosity gels, polymer matrices, mucoadhesive particles, nanoparticles and liposomes have been studied and used to reduce the frequency of administration in the medicinal management of glaucoma (Greaves et al 1992; Le Bourlais et al 1995; Davies 2000). CHF1035, as a prodrug, offers a clear benefit in this regard because it is long lasting as a chemical entity itself.

Conclusion

The results of this study demonstrate that topical administration of CHF1035 and its metabolite CHF1024 significantly decreases IOP in normotensive rabbits. CHF1035, especially, produces a substantial decrease in IOP for prolonged periods of time, and thus may prove useful in the development of glaucoma therapy. The findings that both enantiomers are effective and that the (−) enantiomer, endowed with both D2- and α2-receptor-agonist activity, is somewhat more effective than the (+) enantiomer, suggest that both receptors are involved in the action of CHF1035 on IOP. This, however, needs to be confirmed by using α2-adrenoceptor antagonists, since it is possible that the enantiomers differ in their ocular pharmacokinetic profiles (e.g., absorption).

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