JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 191–195

Received September 6, 2004
Accepted October 15, 2004
DOI 10.1211/0022357055308
ISSN 0022-3573

Fadolmidine-induced ocular hypotension in normotensive rabbits

Jouko Savolainen, Riku Niemi, Antti Mäntylä, Juhani Huuskonen and Tomi Järvinen

Abstract

Fadolmidine, a novel selective α_2-adrenoceptor agonist, was evaluated for its efficacy to lower intraocular pressure in normotensive rabbits (n = 5–6). The dose–response profile between 0.004 μg and 12.5 μg of fadolmidine was determined. The effect of pH on the partition of fadolmidine was studied in order to select an optimal pH for topical fadolmidine administration. After topical administration, fadolmidine significantly lowered the intraocular pressure in normotensive rabbits. The onset of action was immediate, with no initial increase in intraocular pressure. A significant decrease in intraocular pressure was already observed at 1 h after dosing. The maximum decrease in intraocular pressure was observed after a 2.5 μg dose of fadolmidine in both eyes at 2 h after dosing. The mean maximum decrease in the treated and untreated eye was 6.4 mmHg and 3.9 mmHg, respectively. In conclusion, fadolmidine is a potent intraocular pressure lowering agent. In addition, fadolmidine does not cause a significant initial increase in intraocular pressure. Because of the strong dependence of the distribution coefficient on pH, the pH of the administered solution is important, with physiological pH being optimal in this respect.

Introduction

α_2-Adrenoceptor 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 α_2-adrenoceptor agonist to be approved for the treatment of glaucoma, but its clinical use is limited due to significant systemic side-effects, such as lowering of systemic blood pressure after topical administration (Harrison & Kaufmann 1977; Burke & Schwartz 1996). Apraclonidine, a hydrophilic analogue of clonidine, efficiently lowers IOP and is currently used for the treatment of glaucoma (Alward 1998). Apraclonidine, however, has quite high affinity towards α_1-adrenoceptors, which results in ocular side-effects, such as ocular allergy. Brimonidine (Burke & Potter 1986) is a fairly new selective α_2-agonist currently used in treating glaucoma: it is fairly well tolerated and has a low rate of adverse effects (Alward 1998; Lee et al 2000). However, brimonidine crosses the blood–brain barrier, potentially causing central nervous system toxicity, and therefore should be used with caution, especially in the case of children (Enyedi & Freedman 2001).

Fadolmidine [2,3-dihydro-3-(1*H*-imidazol-4-ylmethyl)-1*H*-indan-5-ol] (Figure 1) is a novel, selective α_2-adrenergic agonist with a low nanomolar affinity (K_i values of 1–2.1 nM) towards the α_2-adrenergic receptor (Eisenach et al 1999; Lehtimäki et al 1999). Fadolmidine has been shown to produce an antinociceptive effect in animals, with little effect on systemic blood pressure (Eisenach et al 1999; Onttonen & Pertovaara 2000; Pertovaara & Wei 2000; Xu et al 2000). Haemodynamic side-effects limit the wider use of clonidine for example, which is approved for epidural use in neuropathic pain.

The aim of the present study was to evaluate the potential of using fadolmidine for the treatment of glaucoma. The effect of fadolmidine to lower IOP in normotensive rabbits was evaluated; the dose–effect profile studied ranged from 0.004 to 12.5 μg of fadolmidine. The partitioning behaviour of fadolmidine was studied as a function of pH in order

Department of Pharmaceutical Chemistry, University of Kuopio, PO Box 1627, FIN 70211, Kuopio, Finland

Jouko Savolainen, Riku Niemi, Antti Mäntylä, Juhani Huuskonen, Tomi Järvinen

Current address: Department of Chemistry, University of Jyväskylä, PO Box 35, FIN 40014, Jyväskylä, Finland

Juhani Huuskonen

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

Acknowledgments: We would like to thank Ms Pirjo Hakkarainen and Mrs Helly Rissanen for their skilful technical assistance.

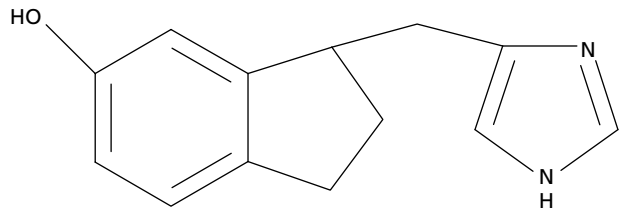

Figure 1 Molecular structure of fadolmidine.

to determine the optimum pH for the topical administration of fadolmidine. Partitioning was studied with the traditional shake-flask method and also by titration.

Materials and Methods

Chemicals

Fadolmidine hydrochloride was obtained from Orion Pharma (Espoo, Finland), and oxybuprocaine was obtained from Santen Oy (Tampere, Finland). The titrants used in the pH-metric pK_a and logD determinations were Titrisol (0.5 M KOH; Merck, Darmstadt, Germany) and hydrochloride acid volumetric standard (0.5 M; Aldrich Milwaukee, WI). All other materials and solvents were of reagent grade and were used as received.

Animals

The experimental animals used were normotensive Dutch Belted pigmented rabbits (National Laboratory Centre, University of Kuopio, Finland) of either gender (2.3–4.0 kg, $n = 5–6$). The rabbits were housed singly in cages under standard laboratory conditions with a 12-h dark/light cycle, ambient temperature of $20.0 \pm 0.5°C$, and 55–75% relative humidity. Water and food were given *ad libitum* except during the tests. All the animals were treated in accordance with the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research, and all procedures with animals were reviewed and approved by the Animal Ethics Committee at the University of Kuopio.

Determination of distribution coefficients by the shake-flask method

The distribution coefficient (D) of fadolmidine (pH 5.00, 6.00, 7.00, 7.40, 7.65 and 8.00) was determined from the distribution of the compound between 1-octanol and phosphate buffer (0.16 M). Before use, 1-octanol was saturated with phosphate buffer by vigorously shaking for 24 h. A known concentration of the compound in phosphate buffer solution was shaken 60 min with a suitable volume of saturated 1-octanol. The phases were separated by centrifugation (3 min at 1500 rev min^{-1}) and the concentration of the compound in the buffer phase before and after partitioning was determined by high-performance liquid chromatography (HPLC). The distribution coefficients (D) were calculated as follows:

$$D = ((C_b - C_a)/C_a) \times (V_w/V_o)$$

where C_b and C_a represent the initial and equilibrium solute concentration of the aqueous phase, V_w denotes the volume of the buffer and V_o the volume of the 1-octanol phase.

Determination of ionization constants and distribution coefficients by the pH-metric technique

Titrations were performed on a PCA200 (Control200 software Revision 1.000) semi-automatic titrator (Sirius Analytical Instrument Ltd, Forest Row, UK).

Ionization constant (pK_a) titration was carried out over the pH range 1.8 to 12.2 under argon atmosphere at 25.2°C (average temperature). A known amount of the compound was dissolved in 20 mL 0.15 M KCl solution. The pH was then adjusted automatically with 0.5 M HCl to the start pH of the titration at pH 1.8. The maximum titrant (0.5 M KOH) volume increment for one titration step was limited to 0.25 mL. The pH change per titrant addition was limited to 0.2 pH units. A total of 42 pH readings were collected from the titration. The pH values were recorded when the pH drift was lower than 0.002 pH min^{-1}. Processing of titration data was carried out using the Sirius Refine200 Revision 1.000 (Sirius Analytical Instrument Ltd). The detailed procedure for the determination of ionization constants has been described elsewhere (Takacs-Novak et al 1997).

For the determination of the distribution coefficient, 1-octanol was saturated with 0.15 M KCl solution by vigorously shaking for 24 h. A known amount of the compound was dissolved in 7.5 mL 0.15 M KCl solution and 0.5 mL 1-octanol was added to the solution. The first titration was carried out from pH 2.7 to 8.3. The second titration of the same sample was carried out from pH 8.3 to 2.7, and the third titration was carried out from pH 2.7 to 8.3. Additional 1-octanol was added manually before the second (2.0 mL) and the third (10.0 mL) titrations. This procedure is called MultiTitrations. Processing of titration data was carried out using the Sirius Refine200 Revision 1.000. A detailed description of the pH-metric logD method can be found elsewhere (Avdeef 1996).

Preparation of the eyedrop formulations

An appropriate amount of fadolmidine was dissolved in 20 mM phosphate buffer (pH 5.0 or 7.4). In all solutions, the pH was adjusted with phosphoric acid or sodium hydroxide, if necessary, and the solutions were made isotonic with sodium chloride. The final fadolmidine concentrations of the solutions were determined by HPLC.

Analytical procedure

The HPLC system used consisted of a Merck Hitachi Model L-7100 HPLC pump, a Merck Hitachi Model

L-7400 variable wavelength UV detector (set at 206 nm) and a Merck Hitachi Model L-7250 programmable autosampler. Separations were performed with a Purospher RP-18e reversed-phase column (125 × 4.6 mm, i.d. 5 μm). The chromatographic conditions were as follows: injection volume, 20 μL; flow rate, 1.2 mL min^{-1}. The gradient elution system consisted of 20 mM phosphate buffer (pH 7.0) and 80% acetonitrile in water. All separations were performed in triplicate.

In-vivo IOP measurements

To perform an IOP test, a rabbit was placed in a plastic restraining box located in a quiet room. A single drop (25 μL) of the test solution was instilled unilaterally into the left eye on the upper corneoscleral limbus. During instillation, the upper eyelid was pulled slightly away from the globe. IOP was measured using a BioRad Digilab Modular One pneumatonometer (BioRad, Cambridge, MA, USA) . Before each measurement, one or two drops of oxybuprocaine (0.06%) were 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. IOP of the rabbits was measured at 2, 1 and 0 h before, and at 0.5, 1, 2, 3, 4 and 5 h after eyedrop administration. IOP at the time of eyedrop administration (0 h) was used as a baseline value. All studies were set up using a masked and randomized crossover design. At least 72 h wash-out time was allowed for each rabbit between dosings. Based on a previous pharmacokinetic study (Eisenach et al 1999), a shorter wash-out period would have been sufficient. However, for the well-being of the animals, a 3-day resting period was used.

In order to eliminate the influence of possible circadian effects, all IOP studies were conducted at similar times of the day.

Analysis of the data

All IOP results are presented as a mean ± s.e.m. change in IOP (mmHg). A one-factor analysis of variance for repeated measurements was used to test the statistical significance of differences between groups. The significance of differences in the means was tested using Fisher's protected least significance difference method in which $P < 0.05$ denoted significance.

Results and Discussion

Distribution coefficient of fadolmidine

The distribution coefficient, D (expressed as logD), of fadolmidine increased with increasing pH over the pH range 2.7–8.3 (Figure 2). The distribution coefficients determined by the shake-flask method correlated well

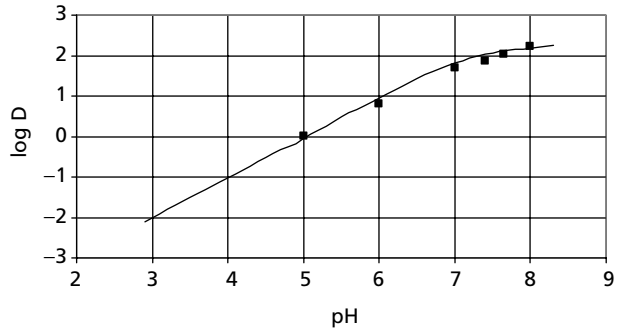

Figure 2 Lipophilicity profile of fadolmidine. The squares in the plot represent the distribution coefficients of fadolmidine determined by the shake-flask method (mean ± s.d., n = 3); the error bars are smaller than symbols. Fitted data present fadolmidine's lipophilicity determined by pH-metric titration.

with the values determined by pH-metric determination. For example, $logD^{5.0}$ was 0.01 and −0.06, and $logD^{7.4}$ was 1.87 and 2.02 when determined by the shake-flask and pH-metric techniques, respectively. The marked increase in lipophilicity when going up the pH scale is due to the increased portion of the un-ionized form of the imidazole ring NH group (pK_a 7.3) in the more basic environment. When going even further up the pH scale (>8.3), it is likely that the log P_{app} of fadolmidine starts to decline due to the ionization of the phenolic OH group (pK_a 10.1). It has been stated that an apparent partition coefficient in the order of 100–1000 (log P_{app} 2–3) is optimal for efficient permeability across the cornea (Florence & Attwood 1988), suggesting the optimal administration pH of fadolmidine is above 7.0. This was confirmed in a preliminary IOP study in normotensive rabbits, where fadolmidine was administered both at pH 5.0 and at pH 7.4 (Table 1). It was clear that at pH 7.4 the decrease in IOP was significantly greater than at pH 5.0 (Figure 3), and thus pH 7.4 was chosen for the actual IOP studies.

Dose–response profile of fadolmidine in the treated eye

In our studies, no ocular irritation was observed after topical administration of fadolmidine, as evaluated by eyelid closure. In addition, no visible signs of irritation or discomfort were observed after fadolmidine administration. The dose–response profile of fadolmidine is given in Table 1. The effect of fadolmidine on IOP in the treated eye increased with increasing dose. However, when the dose exceeded 2.5 μg (i.e. 12.5 μg dose), the decrease in IOP was systematically lower when compared with the IOP decrease after the administration of a 2.5-μg dose. Doses equal to or below 0.1 μg did not have significant effects on IOP compared with the vehicle. The exact reason for the decline in IOP-lowering capability of fadolmidine after exceeding the 2.5-μg dose could not be clarified in this study.

The onset of ocular hypotension after topical fadolmidine administration was immediate, with no initial

Table 1 Intraocular pressure (IOP) changes (mean mmHg ± s.e.m.) at pre-determined times in the treated and untreated eyes of normotensive rabbits (n = 5–6) after unilateral administration of 25 μL of fadolmidine solutions and vehicles

Dose	Time (h)						
	0	0.5	1	2	3	4	5
Treated eyes							
pH 7.4 buffer	0.0 ± 0.0	0.8 ± 0.4	−1.0 ± 1.0	−0.2 ± 1.0	−0.4 ± 1.4	−0.2 ± 1.3	0.1 ± 1.2
12.5 μg (pH 7.4)	0.0 ± 0.0	0.6 ± 1.4	−1.9 ± 1.7	−4.6 ± 1.9*	−6.3 ± 1.9*	−3.3 ± 2.1	−1.5 ± 1.7
2.5 μg (pH 7.4)	0.0 ± 0.0	−0.5 ± 0.7	−4.2 ± 1.0*	−6.4 ± 0.6*	−5.4 ± 1.5*	−4.4 ± 0.6*	−2.6 ± 1.7
0.5 μg (pH 7.4)	0.0 ± 0.0	0.2 ± 0.5	−2.4 ± 1.3	−4.9 ± 1.2*	−4.4 ± 0.6*	−5.0 ± 1.9*	−2.3 ± 1.8
0.1 μg (pH 7.4)	0.0 ± 0.0	−0.8 ± 0.8	−2.1 ± 1.6	−2.1 ± 1.6	−1.9 ± 1.8	−1.5 ± 1.3	0.6 ± 0.9
0.02 μg (pH 7.4)	0.0 ± 0.0	−1.0 ± 0.8	−3.8 ± 0.9	−2.7 ± 0.6	−1.5 ± 0.8	−1.9 ± 1.0	−0.9 ± 1.6
0.004 μg (pH 7.4)	0.0 ± 0.0	−0.5 ± 0.7	−0.2 ± 0.5	−0.7 ± 0.9	1.3 ± 1.1	1.5 ± 0.6	1.2 ± 0.9
pH 5.0 buffer	0.0 ± 0.0	0.7 ± 0.6	−0.9 ± 1.3	1.2 ± 0.7	1.5 ± 1.1	−1.1 ± 0.6	1.7 ± 0.7
12.5 μg (pH 5.0)	0.0 ± 0.0	−0.1 ± 1.0	−2.4 ± 1.3	−2.9 ± 2.1	−3.5 ± 1.5	−2.0 ± 1.9	−0.2 ± 1.4
2.5 μg (pH 5.0)	0.0 ± 0.0	1.3 ± 0.9	−1.6 ± 1.1	−1.1 ± 1.3	−0.5 ± 0.8	−2.0 ± 1.1	−0.2 ± 0.7
0.5 μg (pH 5.0)	0.0 ± 0.0	0.9 ± 0.8	−0.7 ± 0.8	−2.0 ± 0.7	−1.3 ± 0.8	0.4 ± 0.5	2.0 ± 0.9
Untreated eyes							
pH 7.4 buffer	0.0 ± 0.0	0.0 ± 0.6	−0.6 ± 0.8	0.2 ± 0.8	−0.1 ± 1.0	−0.6 ± 1.3	0.2 ± 1.6
12.5 μg (pH 7.4)	0.0 ± 0.0	−1.9 ± 1.3	−2.6 ± 1.8	−2.1 ± 1.1	−2.5 ± 1.0	−1.0 ± 1.2	0.6 ± 1.5
2.5 μg (pH 7.4)	0.0 ± 0.0	−1.8 ± 0.8	−3.1 ± 0.8	−3.9 ± 0.4*	−1.5 ± 1.1	−1.4 ± 1.2	−2.5 ± 1.0
0.5 μg (pH 7.4)	0.0 ± 0.0	−0.6 ± 0.7	0.1 ± 1.4	−2.8 ± 0.8	−3.5 ± 0.8	−0.6 ± 1.8	−1.1 ± 1.3
0.1 μg (pH 7.4)	0.0 ± 0.0	−1.9 ± 0.6	−1.5 ± 0.3	−2.9 ± 0.8	−3.5 ± 0.7	−1.6 ± 0.7	1.6 ± 0.8
0.02 μg (pH 7.4)	0.0 ± 0.0	−0.6 ± 1.1	−2.5 ± 1.1	−2.0 ± 1.1	−1.1 ± 1.0	−2.0 ± 1.0	−0.5 ± 0.9
0.004 μg (pH 7.4)	0.0 ± 0.0	−0.8 ± 0.3	−1.3 ± 0.5	−0.1 ± 0.8	−1.0 ± 0.9	0.7 ± 0.7	0.1 ± 0.8
pH 5.0 buffer	0.0 ± 0.0	0.1 ± 0.9	−1.8 ± 1.1	1.5 ± 0.8	0.5 ± 0.8	−0.5 ± 0.5	0.5 ± 1.4
12.5 μg (pH 5.0)	0.0 ± 0.0	−2.5 ± 0.5	−3.4 ± 0.8	−3.4 ± 0.8	−3.8 ± 1.0	−2.3 ± 1.2	−1.4 ± 1.5
2.5 μg (pH 5.0)	0.0 ± 0.0	0.6 ± 0.9	−1.0 ± 1.1	0.3 ± 1.1	−0.5 ± 0.8	−0.6 ± 1.1	−0.9 ± 0.7
0.5 μg (pH 5.0)	0.0 ± 0.0	1.2 ± 0.8	1.9 ± 1.2	−0.5 ± 1.3	−0.2 ± 0.9	2.0 ± 0.6	1.9 ± 1.3

*Significantly different compared with values for the vehicle (buffer solution at either pH 7.4 or 5.0).

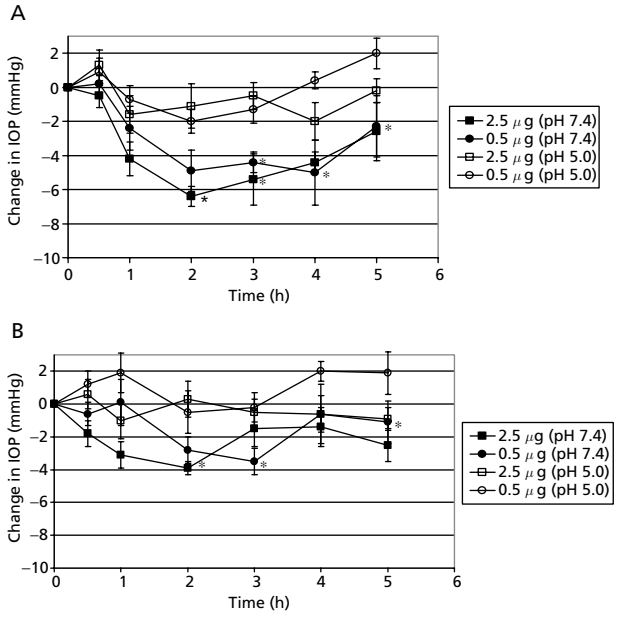

Figure 3 Intraocular pressure (IOP) changes (mean ± s.d., n = 5–6) in treated (A) and untreated eyes (B) of normotensive rabbits after ocular administration of fadolmidine at pH 5.0 and 7.4. *Significantly different compared with values for the matching dose at pH 5.0.

increase in IOP, and a significant decrease in IOP was already observed 1 h after dosing (Table 1). Some other α_2-agonists, such as dexmedetomidine and *p*-aminoclonidine (Vartiainen et al 1992), medetomidine (Potter & Ogidigben 1991) and brimonidine (Burke & Potter 1986), have been reported to cause an initial increase in IOP, which is then followed by an IOP decrease. The lack of the initial raise in IOP after topical fadolmidine administration may be due to a highly specific α_2-agonism of fadolmidine over α_1-agonism compared with other α_2-agonists. It is also possible that high concentrations of an α_2-agonist produces an excitatory effect on the extraocular muscles, which causes the initial increase in IOP (Burke & Potter 1986); this was not observed with the fadolmidine doses that were used in this study.

Dose–response profile of fadolmidine in the untreated eye

A slight decrease in IOP in the untreated eye was observed (Table 1). It is possible that a portion of the drug that does not penetrate the cornea drains through the nasolacrimal duct into the nose and is absorbed into the systemic circulation, as systemic absorption of drugs is usually much higher than that of ocular absorption (Urtti & Salminen 1993;

Järvinen et al 1995). Although the concentration of a drug may be substantially lower in the untreated eye than in the treated eye, it often enables a slight reduction in IOP. Fadolmidine is quite lipophilic at physiological pH (Figure 2), and thus may be partly absorbed into the central nervous system, leading to ocular hypotension in the untreated eye. Eisenach et al (1999) reported poor penetration of fadolmidine into the central nervous system (0.17% bioavailability in cerebrospinal fluid). This suggests that the IOP decrease in the untreated eye is probably due to systemic transfer of the drug to α_2-adrenoceptors in the untreated eye through the blood circulation. However, the set-up in this study does not rule out the possibility of IOP-lowering effects mediated through the central nervous system. In addition, the study by Eisenach et al (1999) did not determine the concentrations of fadolmidine in the brain parenchyma; in many cases the cerebrospinal fluid drug levels do not correlate well with those in brain tissue (Pardridge 1995). Possible systemic transfer of fadolmidine allows potential adverse effects not associated at the ocular level.

Conclusions

Based on this preliminary IOP study in rabbits ($n = 5–6$), fadolmidine seems to be a potent IOP-lowering agent. In addition, at the doses studied, fadolmidine does not cause an initial increase in IOP, which is common with other α_2-agonists. Fadolmidine also has lower risks of other adverse effects, such as hypotension, than other α_2-agonists, making it a good candidate for further development as an IOP-lowering agent. Although fadolmidine contains both an acidic and a basic moiety, it shows good lipophilicity (i.e. permeability) at physiological pH, and thus easily penetrates the cornea.

References

Alward, W. L. M. (1998) Medical management of glaucoma. *N. Engl. J. Med.* **339**: 1298–1307

Avdeef, A. (1996) Assessment of distribution-pH profiles. In: Pliska, V., Testa, B., van de Waterbeemd, H. (eds) *Lipophilicity in drug action and toxicology*. VCH, Weinhem, pp 109–139

Burke, J. A., Potter, D. E. (1986) Ocular effects of a relatively selective α_2 agonist (UK-14, 304-18) in cats, rabbits and monkeys. *Curr. Eye Res.* **5**: 665–676

Burke, J., Schwartz, M. (1996) Preclinical evaluation of brimonidine. *Surv. Ophthalmol.* **41** (Suppl. 1): 9–18

Eisenach, J. C., Lavand'homme, P., Tong, C., Cheng, J.-K., Pan, H.-L., Virtanen, R., Nikkanen, H., James, R. (1999) Antinociceptive and hemodynamic effects of a novel α_2-adrenergic agonist, MPV-2426, in sheep. *Anesthesiology* **91**: 1425–1436

Enyedi, L. B., Freedman, S. F. (2001) Safety and efficacy of brimonidine in children with glaucoma. *J. AAPOS* **5**: 281–284

Florence, A. T., Attwood, D. (1988) *Physicochemical principles of pharmacy*. Macmillan Press, London

Greenfield, D. S., Liebmann, J. M., Ritch, R. (1997) Brimonidine: a new alpha2-adrenoreceptor agonist for glaucoma treatment. *J. Glaucoma* **6**: 250–258

Harrison, R., Kaufmann, C. S. (1977) Clonidine: effects of a topically administered solution on intraocular pressure and blood pressure in open-angle glaucoma. *Arch. Ophthalmol.* **95**: 1368–1373

Järvinen, K., Järvinen, T., Urtti, A. (1995) Ocular absorption following topical delivery. *Adv. Drug Del. Rev.* **16**: 3–19

Lee, D. A., Gornbein, J., Abrams, C. (2000) The effectiveness and safety of brimonidine as mono, combination, or replacement therapy for patients with primary open-angle glaucoma or ocular hypertension: a post hoc analysis of an open-label community trial. *J. Ocul. Pharmacol. Ther.* **16**: 3–18

Lehtimäki, J., Haapalinna, A., Korhonen, T., Leino, T., Viitamaa, T., Wurster, S., Savola, J.-M., Virtanen, R. (1999) MPV-2426, a novel alpha2-adrenergic agonist for spinal analgesia. *Fundam. Clin. Pharmacol.* **59** (Suppl. 1): 380

Onttonen, T., Pertovaara, A. (2000) The mechanical antihyperalgesic effect of intrathecally administered MPV-2426, a novel α_2-adrenoceptor agonist, in a rat model of post-operative pain. *Anesthesiology* **92**: 1740–1745

Pardridge, W. M. (1995) Transport of small molecules through the blood-brain barrier: biology and methodology. *Adv. Drug Del. Rev.* **15**: 5–36

Pertovaara, A., Wei, H. (2000) Attenuation of ascending nociceptive signals to the rostroventromedial medulla induced by a novel α2-adrenoceptor agonist, MPV-2426, following intrathecal application in neuropathic rats. *Anesthesiology* **92**: 1082–1092

Potter, D. E., Ogidigben, M. J. (1991) Medetomedine-induced alterations of intraocular pressure and contraction of the nictitating membrane. *Invest. Ophthalmol. Vis. Sci.* **32**: 2799–2805

Takacs-Novak, K., Box, K. J., Avdeef, A. (1997) Potentiometric pK_a determination of water-insoluble compounds: validation study in methanol/water mixtures. *Int. J. Pharm.* **151**: 235–248

Urtti, A., Salminen, L. (1993) Minimizing systemic absorption of topically administered opthalmic drugs. *Surv. Ophthalmol.* **37**: 435–456

Vartiainen, J., MacDonald, E., Urtti, A., Rouhiainen, H., Virtanen, R. (1992) Dexmedetomidine-induced ocular hypotension in rabbits with normal or elevated intraocular pressures. *Invest. Ophthalmol. Vis. Sci.* **33**: 2019–2023

Xu, M., Wei, H., Konttinen, V. K., Kalso, E., Pertovaara, A. (2000) The dissociation of sedative from spinal antinociceptive effects following administration of a novel alpha-2-adrenoceptor agonist, MPV-2426, in the locus coeruleus in the rat. *Acta Anaesthesiol. Scand.* **44**: 638–655