Notes

Ocular Delivery of the β-Adrenergic Antagonist Alprenolol by Sequential Bioactivation of Its Methoxime Analogue

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Ocular delivery of alprenolol, a β-adrenergic antagonist, by site-specific bioactivation of its methoxime analogue results in significant and prolonged decrease of the intraocular pressure in rabbits after topical administration. Alprenolone methoxime is stable in isotonic phosphate vehicle but undergoes enzymatic hydrolysis to the corresponding ketone in the eye. The ketone is then converted to alprenolol by a carbonyl reductase present in the iris-ciliary body. The benefit of this chemical delivery system approach includes the facile release of a potential antiglaucoma agent only at the site of the action; thus, unwanted systemic effects of the drug can be avoided.

The site-specific action involving the reduction of intraocular pressure (IOP), a beneficial effect in glaucoma treatment, and the lack of systemic activity has recently been reported after the topical administration of the ketoxime analogues of several β-adrenergic receptor antagonists.[2,3]. This chemical delivery system (CDS) approach[4] is based on an enzymatic hydrolysis–stereospecific reduction sequence occurring only in the eye, and the corresponding amino alcohols [the potent (*S*)-(−)-forms[5]] have been identified.[6] Intravenous administration of the ketoximes did not yield the corresponding amino alcohols and, thus, did not produce significant cardiovascular effects.[2,3]

Alprenolone oxime (1), the oxime analogue of the β-adrenergic antagonist alprenolol [1-(isopropylamino)-3-(2-allylphenoxy)-2-propanol, **2**], exhibited remarkable ocular hypotensive activity following both uni- and bilateral topical administration to rabbits.[3] However, its stability in isotonic buffer solutions was discouragingly low, demanding storage in lyophilized form that should be reconstituted before use. Ocular administration of 1 (especially as an oxalate salt) also induces mild irritation to the eye of New Zealand albino rabbits involving lacrimation, conjunctiva hyperemia, mucous formation, and iris redness symptoms. To improve solution stability and reduce irritation, we suggest the methoxime analogue (**3**) as a potential ocular CDS for alprenolol. Upon replacement of the hydroxyimino group with a methoxyimino function for the transient protection of the chemically unstable ketone,[2] intramolecular H-bonding[7] that possibly contributes to the hydrolysis in the oxime analogue will be absent. In this paper, the evaluation of **3** as a possible ocular CDS for alprenolol is reported.

Results and Discussion

The methoxime analogue **3** [1-(isopropylamino)-3-(2-allylphenoxy)-2-propanone methoxime] was prepared by a multistep synthetic sequence, similarly to the corresponding oximes.[2] 3-Chloro-1-(2-allylphenoxy)-2-propanone, prepared from 3-chloro-1-(2-allylphenoxy)-2-propanol by oxidation with activated dimethyl sulfoxide (Pfitzner–Moffat method[8]), was reacted with methoxylamine hydrochloride to obtain 3-chloro-1-(2-allylphenoxy)-2-propanone oxime whose reaction with isopropylamine yielded **3**. Its oxalate salt was obtained as a mixture of approximately 75% *Z*- and 25% *E*-isomer, and the isomer composition varied within 10% among batches. No further purification has been attempted to isolate pure isomers, since previous studies involving 1 have shown that a pH- and temperature-dependent equilibrium is reached in solution around this isomer composition,[7] and the IOP reducing activity of the isomers is nearly equal.[3]

An important criterion for the evaluation of **3** was whether solution stability was improved compared to the oxime analogue (1). In ophthalmic vehicle solutions appropriate for therapy,[9] the hydrolysis rate of 1 decreased steadily with the pH, but the $t_{0.9}$ (the time within which only 10% of the drug was degraded) of a 1% (w/v) solution was 44 ± 5 days at room temperature (ca. 20 °C) even at pH 4.50. (This pH value may be considered the lower limit for ophthalmic vehicle solutions.) Although **3** (as an oxalate in 1% w/v solution) had $t_{0.9}$ of 75 ± 8 days at pH 4.50 and room temperature (a moderate improvement compared to the oxime, 1), raising the pH to or above 6.50 has resulted in an increase of solution stability, so that significant decomposition has not been detected for more than one year during storage in room temperature.

In ocular pharmacological experiments (Figure 1), the methoxime analogue (**3**) showed an early onset of IOP reduction and reached the maximum effect (3.8 ± 0.8 mmHg decrease of the IOP, about 18% reduction of the control 21.4 ± 0.3 mmHg) by the same time (approximately after an hour) as the unmanipulated β-adrenergic antagonist (**2**). Compound **3** had a prolonged ocular hypotensive activity; statistically significant decrease in IOP from the vehicle control was observed for 4–5 h after administration. Alprenolol (**2**) only produced statistically significant IOP reduction for 2–3 h. Although the duration of action for the methoxime

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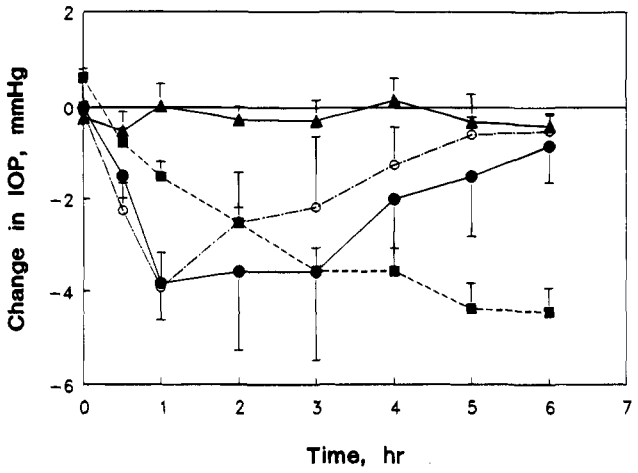


Figure 1. Intraocular pressure (IOP) reducing activity of the β-adrenergic antagonist **2** (alprenolol, ○), its oxime analogue **1** (■), and methoxime analogue **3** (●) in New Zealand Albino rabbits. (Topical administration of 100 μL doses of 60 mM solutions in pH 6.50 isotonic phosphate vehicle.) Isotonic saline solution was used as a control (▲). Data points represent average IOP differences (between the treated and untreated eye) of 12 animals for the solutions administered. Error bars give standard deviations.

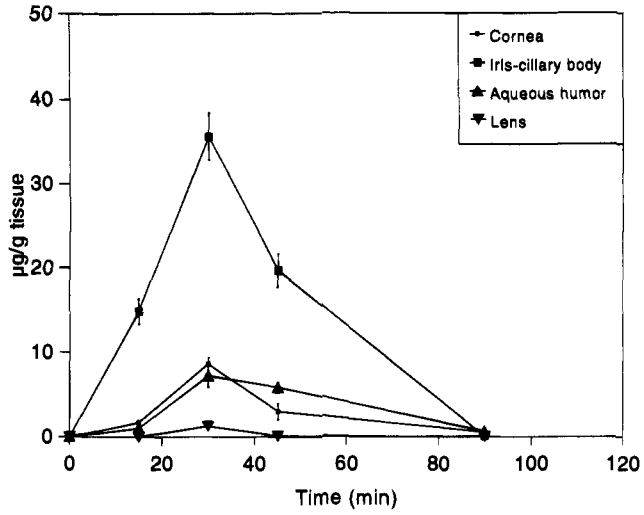


Figure 2. Concentration of alprenolol (**2**) in eye tissues after topical administration of 2% (w/v) solution (pH 6.50 isotonic phosphate vehicle) of the oxalate salt of **3** to rabbits. (Error bars represent standard error of the mean, based on four measurements per data point.)

analogue appears to be shorter than that of the oxime chemical delivery system (**1**), the ocular response of the latter took 3 h to fully develop and reach the maximum IOP reduction observed for **2** and **3**. Also, irritation noticed with compound **1** was practically absent upon and after the topical administration of the oxalate salt of **3** in a 60 mM, pH 6.50 isotonic phosphate vehicle solution.[7]

We have also confirmed by high-performance liquid chromatography that **3** is transformed to alprenolol (**2**) in the eye. The enzymatic hydrolysis of the methoxime to ketone and the subsequent reduction of the ketone to alcohol (**2**) is shown in Scheme 1.

The overall rate of converting the methoxime (**3**) to the β-adrenergic antagonist (**2**) is mainly determined by the slower hydrolysis to ketone. The subsequent reduction is fast, hence 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanone (**4**) can be found in relatively low concentrations. The rate of formation of **4** from the precursors by a hydrolase enzyme is possibly higher from **3** than from **1**. This has been indicated by the earlier onset and shorter duration of the IOP reducing activity after the administration of alprenolone methoxime (**3**) compared to the corresponding oxime (**1**).

The time profiles of alprenolol (**2**) in various eye compartments also indicate that the conversion probably occurs in the iris-ciliary body (Figure 2). The maximum concentration of **2** was achieved in this tissue, and the time to reach the maximum concentration (C_{max}) also was the shortest in the iris-ciliary body, followed by the cornea, aqueous humor, lens, and the vitreous body (not shown), indicating a postformation redistribution in the anterior segment of the eye.

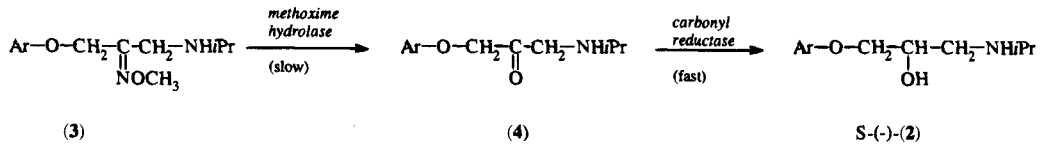
In conclusion, ocular delivery of β-adrenergic antagonists by sequential bioactivation of the methoxime analogues represents an important addition to site-specific chemical delivery systems. The advantage of this approach includes the release of a potential antiglaucoma agent only at the site of the action. Similarly to the oxime (**1**), the methoxime (**3**) also did not significantly (more than 6% of the baseline values) alter cardiac electrophysiological parameters in anesthetized closed-chest dogs.[10] The pharmacological effects of alprenolol (**2**) by using its ocular CDS **3** are localized since sequential hydrolysis–stereospecific reduction is only carried out by enzymes present in the iris-ciliary body, and thus, unwanted systemic effects of the drug are avoided.

Experimental Section

Chemistry. Melting point was determined with a Fisher-Johns apparatus and is uncorrected. Elemental analysis was performed by Atlantic Microlab, Inc., Norcross, GA. The 300-MHz ^{1}H-NMR spectra were taken on a General Electric (Schenectady, NY) QE-300 instrument. Mass spectra were obtained by a Kratos Analytical (Manchester, U.K.) MS80RFA instrument using 70 eV electron ionization and direct introduction.

1-(Isopropylamino)-3-(2-allylphenoxy)-2-propanone Methoxime Oxalic Acid Salt (3). To 10.96 g (48.8 mmol) of 3-chloro-1-(2-allylphenoxy)-2-propanone dissolved in 50 mL of ethanol, 5.0 g (60 mmol) of methoxylamine hydrochloride and 5 mL of water. The reaction was allowed to proceed until the solid was completely dissolved (30–40 min). The solution was diluted with water until slight turbulence appeared, and then the ethanol was removed *in vacuo*. The residue was taken up in water, and the solution was extracted with diethyl ether (50 mL). The organic layer was separated, washed with water, and dried over magnesium sulfate, and the solvent was evaporated *in vacuo*. The residue was dissolved in 100 mL of tetrahydrofuran, the solution was cooled to 3 °C, and 16 g (271

Scheme 1



mmol) of isopropylamine in 60 mL of tetrahydrofuran was added dropwise so that the temperature of the solution remained at 3–8 °C. The reaction mixture was stirred for 4 h while the temperature was allowed to rise to ambient, and then 100 mL of diethyl ether was added. The precipitate was filtered off and discarded, and the filtrate was evaporated to an oily residue that was dissolved in acetone. After the addition of ethereal oxalic acid (8.0 g, 89 mmol), the solution was concentrated. On cooling, a white solid was obtained. Recrystallyzation from 20 mL of ethyl acetate yielded 5.1 g (29%) of product. Mp: 103–105 °C. Anal. ($C_{18}H_{26}N_2O_6$) C, H, N. ^{1}H-NMR (DMSO-d_6 + tetramethylsilane): 7.25–6.85 (m, 4H, Ar-H), 6.05–5.35 (m, 1H, CH=), 5.08–4.98 (m, 2H, =CH_2), 4.90 (s, 1.5H, OCH_2, *Z*-isomer), 4.78 (s, 0.5H, OCH_2, *E*-isomer), 3.93 (s, 3H, $NOCH_3$), 3.90 (s, 1.5H, CH_2N, *Z*-isomer), 3.81 (s, 0.5H, CH_2N, *E*-isomer), 3.45–3.20 (m, 3H, Ar-CH_2 and NCH), 1.26 (d, J = 8 Hz, 4.5H, CH_3, *Z*-isomer), 1.24 (d, J = 8 Hz, 1.5H, CH_3, *E*-isomer). MS *m/z* (rel abundance) 262 (2, $M^{\cdot+}$), 173 (37), 133 (37), 131 (32), 115 (33), 105 (100), 91 (31), 79 (34), 78 (34), 77 (56), 72 (30), 63 (22), 56 (79).

Stability Studies in Isotonic Phosphate Vehicle. Alprenolone oxime oxalate and alprenolone methoxime oxalate, respectively, were dissolved in 25 mL of isotonic phosphate vehicle (pH 4.5–6.5)[9] by sonicating for 5 min to obtain 35 mM solutions. The solutions were filtered, distributed in 3.0 mL portions in borosilicate glass vials (15 mm o.d. × 48 mm height, 4 mL total volume with 13-425 GPI thread finish) that protected against the change of pH (Fisher Scientific, Pittsburgh, PA). The vials were screw-capped with open-top phenolic closures using Teflon-faced silicon rubber septa. The dead volume of the sample vials was purged with nitrogen stream before closing. The solutions were stored at room temperature (20 °C). During storage, 25 μL aliquots were withdrawn from the vials and admixed to 500 μL of aqueous bupranolol (2.5 mg/100 mL, internal standard) solution and analyzed by high-performance liquid chromatography (HPLC, see Analytical Method below).

***In Vivo* Ocular Distribution Studies.** Adult New Zealand albino rabbits weighing 2.5–3.0 kg were used. Standard doses (100 μL) of 60 mM solution of alprenolone methoxime oxalate in aqueous solution were administered topically in both eyes of four groups of rabbits (four animals per group). After appropriate intervals (15, 30, 45, and 90 min), each animals in a group was sacrificed and the eyes were removed surgically. Aqueous humor was obtained by making a single puncture at the limbus using a 25 gauge × $^5/_8$ in. needle attached to a 1 mL syringe. Then the cornea, the iris-ciliary body, and the lens from both eyes were isolated. These tissues were homogenized in a pH 9.8 alkaline buffer by a Tekmar STD tissuemizer to yield 20% (w/w) homogenates. Aqueous humor was only diluted with the alkaline buffer for analysis. The samples were extracted twice with equal volumes of a methylene chloride/diethyl ether mixture (1:4, v/v) by shaking for 15 min. The organic layer was then separated and reextracted with an equal volume of aqueous 0.01 M hydrochloric acid solution. The aqueous layer was withdrawn and made alkaline again, and the solvent extraction was repeated. The organic layer was transferred into a deactivated glass vial, and the solvent was evaporated under a nitrogen stream. The residue was dissolved in 0.01 M hydrochloric acid solution and analyzed by HPLC. Quantitation of **2** was done by a calibration curve obtained from samples of known amount of compound added to tissue homogenates from untreated rabbit eyes.

Analytical Method. The HPLC system consisted of a Spectra Physics (San Jose, CA) Model SP8800 pump, a Rheodyne (Cotati, CA) Model 7125 injector with a 20 μL loop, a Spectra Physics SP8480 detector operated at 280 nm, and a Spectra Physics SP4270 integrator. A Spectra Physics Model SP8780 autosampler was used in the solution stability study. Separation was done on a 7.5 cm × 4.6 mm i.d. 3 μm Supelcosil LC-8-DB column (Supelco, Bellefonte, PA) with mobile phase consisting of 30% acetonitrile in 0.02 M monobasic potassium phosphate (pH 3.0) buffer containing 0.01% triethylamine.[11] The flow rate was 1.2 mL/min. Contrary to the isomers of **1**,[11] the *E* and *Z* forms of **3** were not separated by this method and eluted at 10.2 min. The ketone (**4**) was detected at 8.2 min retention time.[6] Alprenolol (**2**) eluted at 4.3 min, and its limit of quantitation in eye tissue was 100 ng/g.

Pharmacology. Twelve New-Zealand Albino rabbits (about 3 kg body weight, kept in individual cages with free access of food and water) were used. The animals were divided into two groups (six each) for cross-over testing. A Digilab (Cambridge, MA) Model 30 pneumatometer was used to measure the intraocular pressure (IOP). The rabbits were unrestrained during IOP determination. One droplet of propacain hydrochloride (0.5% w/v solution) instilled in the eye immediately before the measurement was used as a local anesthetic. The control IOP were determined in both eyes two or three times before administration of the CDSs. The mean value of IOP for the eyes selected for treatment was 20.8 ± 0.4 (SD) mmHg, based on the measurements including all (12) animals involved in the study, while the IOP average of the eyes serving as control was 20.7 ± 0.6 mmHg. The 60 mM test solutions or the isotonic saline were only administered into one eye in 100 μL doses, and the other eye served as a control. IOP was determined in both eyes 30 min to 8 h after topical application of the test solution, and the IOP difference between the treated eye and the untreated eye was calculated for each measurement. The average IOP values of the untreated (control) eyes measured after administration of the test solutions of alprenolol (**2**), alprenolone oxime (1), and alprenolone methoxime (**3**) were 20.6 ± 1.0, 21.8 ± 0.8, and 21.4 ± 0.3 mmHg, respectively. Data generated from studies were compared separately by using analysis of variance (ANOVA) to detect significant differences between treatment groups with $p < 0.05$ selected as the level of significance.

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