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Report

Site- and Stereospecific Ocular Drug Delivery by Sequential Enzymatic Bioactivation

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Intraocular enzymes convert the ketoxime analogues of some β-adrenergic blockers via a sequential bioactivation process involving hydrolysis to the corresponding ketones followed by reduction to the aryloxyaminoalcohols, which then exert known and predictable physiological and pharmacological effects only at the site of the action—i.e., in the eye—without any systemic side effects. The sequential nature of the process is highlighted by the observation that the administration of the ketone intermediate also leads to its conversion to the β-adrenergic antagonist, the active compound. The reduction is stereospecific resulting in the formation of the more potent *S*-(−)-form of the drug, thus providing prospect to glaucoma treatment. The same activation process of the ketoximes does not take place systemically, thus administration of these ketoximes does not produce cardiovascular effects.

KEY WORDS: glaucoma; reductase enzyme; oxime hydrolase; stereospecific reduction; β-blocker.

INTRODUCTION

Active site-specific drug delivery is one of the major possible routes to improve the therapeutic index of a drug. While physical methods of delivery have shown limited success, ideally the active drug component should be released at the site of action via enzymatic activation (1). This is particularly true for ophthalmic drugs, where about 98% of a topically applied compound finds its way to the general circulatory system, instead of the site of the action. Thus, introducing a pharmacologically inactive compound to the eye, which is activated *only* within the eye should provide the solution, particularly for drugs of known significant systemic cardiovascular effects, like the β-adrenergic antagonists. The use of the ketoximes was proposed (2), since the generated ketones show limited stability and, thus, have limited potential as drugs given systemically.

The site-specific action in the reduction of intraocular pressure, a beneficial effect in glaucoma treatment, has recently been achieved by the local administration of the ketoxime analogues of some β-adrenergic receptor antagonists. The proposed mechanism involves an enzymatic hydrolysis-reduction sequence and, indeed, the corresponding amino alcohols have been identified in the ocular tissues. Here we report that the *in vivo* biotransformation proceeds via a ketone intermediate to the stereoselective formation of the β-adrenergic blocker by the subsequent enzymatic reduction. These bioactivation processes occur only in ocular tissues, providing a site- and stereospecific drug and delivery. The ketoximes lack systemic activity.

The importance of this novel chemical delivery system may also be highlighted by the fact that the enantiomers of 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol (propranolol), a widely used β-blocker, differ in potency, pharmacological action and metabolism. The *S*-(−)-isomer is 100 times as potent as the *R*-(+)-enantiomer (3,4), which is, in turn, metabolized faster than the *S*-form (5–9). The therapeutic formulation, however, contains the racemic drug (10). Presumably, the β-adrenergic blocking feature of other related substances is, also, connected to the chirality of the compounds. Accordingly, one should use the pure active stereoisomer (11), particularly since simple transport-delivery competition reduces the chances of the active component to reach the site of action.

Enzymes capable of hydrolyzing oximes have been described in living organisms (12). It was assumed (2) that the enzymatically rich iris-ciliary body will be capable of effectively hydrolyzing an oxime. Carbonyl reductases are a well-known family of NADPH-dependent oxidoreductases with similar physical and chemical properties which catalyze the reduction of aldehydes and ketones to the corresponding alcohol products (13). The tissue distribution of carbonyl reductases appears to be widespread in mammals (liver, kidney, heart, brain, spleen, etc.), and they are expected to be present in ocular tissues. The iris-ciliary body is one of the major sites of the enzymatic activities in the eye (14), and the presence of an amino-ketone reductase activity was first reported not long ago (15,16).

MATERIALS AND METHODS

The syntheses of the compounds involved in these studies have been described in previous publications (2,17).

The iris-ciliary body and the cornea were isolated from the eyes of adult male New Zealand rabbits 0.5 hr after the

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topical administration (by instillation) of 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanone oxime hydrochloride salt in 1% (w/v) or 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanone oxime hydrochloride in 2% (w/v) aqueous solution. In another experiment, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanone in propylene glycol/ethanol/pH 5.9 ophthalmic buffer (25:25:50 mixture, v/v) solution (concentration, 0.5%, w/v) was applied. The tissue was homogenized in a pH 10.0 alkaline buffer to give 10% (w/w) homogenate and 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 separated, then reextracted with an equal volume of aqueous 0.1 *M* hydrochloric acid solution. The aqueous phase was made alkaline (pH 10.0) and extracted with methylene chloride/diethyl ether; the organic layer was transferred to a deactivated glass vial, and the solvent was evaporated at reduced pressure under nitrogen stream. The residue was dissolved in 0.01 *M* hydrochloric acid solution and analyzed by high-performance liquid chromatography (HPLC) using a Spectra Physics (San Jose, CA) SP8800 pump, Rheodyne 7125 injector, SP8480 detector, and SP4270 integrator. A Supelcosil LC-8-DB analytical column (7.5 cm × 4.6-cm i.d, 3-μm particle size) and mobile phase (1.5 ml/min flow rate) consisting of 30% acetonitrile in an aqueous buffer solution of 0.02 *M* monobasic potassium phosphate (adjusted to pH 3.0 with phosphoric acid) and 0.01% (v/v) triethylamine were used. UV detection at a 280-nm wavelength was applied. Authentic reference samples were used to identify the β-adrenergic blockers in the chromatograms, while the corresponding ketones were prepared by Pfitzner–Moffat oxidation of these amino alcohols (17) and were also characterized by gas chromatography–mass spectrometry [as trifluoroacetyl derivatives (18)].

Part of the extract residue from the iris-ciliary body of rabbit treated with 1-(isopropylamino)-3-(1-naphthyloxy)-2-propranolone oxime was reacted with the chiral reagent, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate, in acetonitrile and analyzed by HPLC (19) using a Supelcosil LC-18 reversed-phase column of 7.5 cm × 4.6 mm with 3-μm particles, with a mobile phase of 60% acetonitrile in 0.02 *M* aqueous monobasic ammonium phosphate solution, a 1-ml/min flow rate, and UV detection at 254 nm.

RESULTS AND DISCUSSION

Figures 1a and b show high-performance liquid chromatographic analyses of eye compartments after local administration of the ketoxime analogues of the β-blockers propranolol and 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanol (alprenolol) to rabbits. Both the proposed ketone intermediates and the active aminoalcohols have been identified. To increase the recovery in the above experiments, the cornea and the iris-ciliary body were not separated for respective analysis. Although the latter appears to be the major site of the related biotransformation of the ketoximes, the cornea should also contain a relatively high level of met-

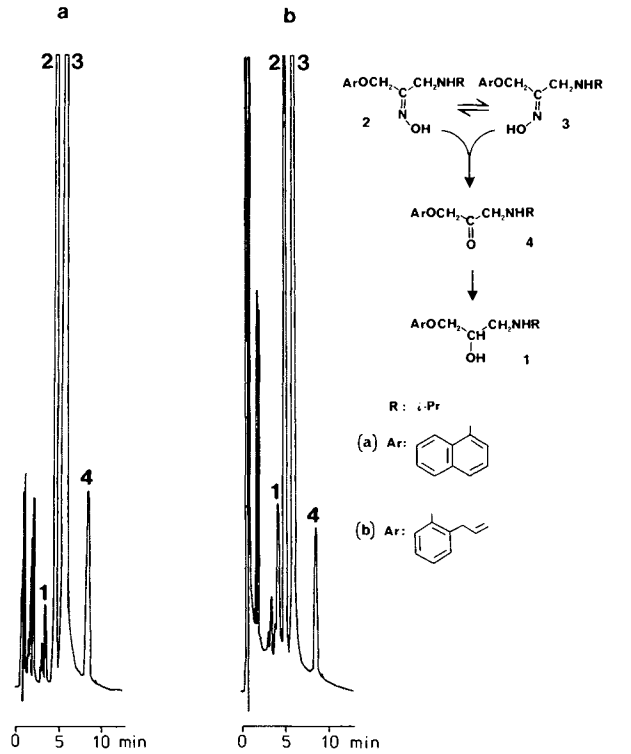


Fig. 1. HPLC analysis of the iris-ciliary body and cornea extracts of rabbits after administration of (a) 1-(isopropylamino)-3-(1-naphthyloxy)-2-propranone oxime and (b) 1-(isopropylamino-3-(2-allylphenoxy)-2-propanone oxime, which showed the presence of the two oxime isomers (2 and 3), 1-(isopropylamino-3-(1-naphthyloxy)-2-propranone and 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanone (4), and 1-(isopropylamino)-3-(naphthyloxy)-2-propanol (propranolol) and 1-(isopropylamino)-3-(2-allyphenoxy)-2-propanol (alprenolol) (1), respectively, indicating bioactivation via a hydrolysis-reduction sequence (scheme).

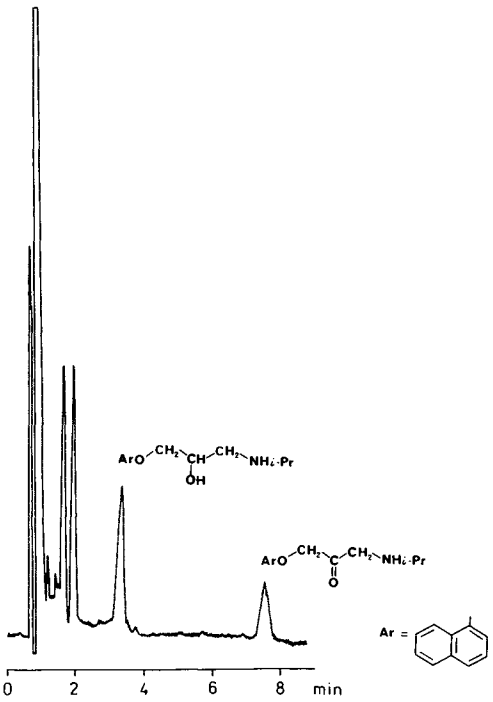
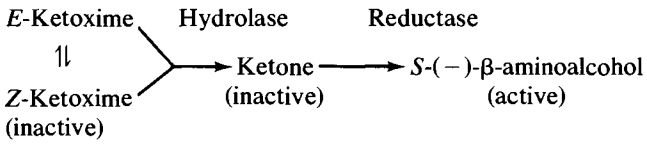


Fig. 2. HPLC analysis of iris-ciliary body extract from rabbit eye after administration of 1-(isopropylamino)-3-(1-naphthyloxy)-2-propranone.

abolic products because of their eventual redistribution after formation by enzymatic processes. The geometrical (*Z*- and *E*-) isomers of the oximes rapidly establish an equilibrium state during and after the distribution of the substance. This process appears to be also catalyzed by enzymes present in the eye as it is very slow in buffer solutions. Nevertheless, both oxime isomers yield the same hydrolysis product. The sequential nature of the formation of the active β-blocker is implied by the results shown in Fig. 2. Upon administering one of the ketones [1-(isopropylamino)-3-(1-naphthyloxy)-2-propanone], which has been prepared by Pfitzner–Moffatt oxidation of propranolol (17) and isolated as the hydrochloride salt, the product of its enzymatic reduction is readily detectable in the iris-ciliary body, the site of the process.

The final major finding was that after administration of the oximes or the ketones, only one enantiomer of the β-adrenergic blocker is present in the ocular tissues (viz., in the iris-ciliary body and cornea) at detectable levels, as shown for propranolol in Fig. 3. This has been assigned as the much more potent *S*-(−)-form, and this observation, together with the pharmacological data (2), supports the explanation that the effect of this ketoxime derivative is ultimately due to the biotransformation involving a hydrolysis-reduction sequence by the intraocular enzymes, and giving an optically active drug. The process is characteristic of the eye only; the systemic metabolism of these ketoximes proceeds via different routes. Intravenous administration of the ketoximes to rats, rabbits, or dogs, as a bolus or a 30-min infusion did not produce any cardiovascular effects (20). The oximes disappear fast from the blood, but *no* active β-blocker could be detected, in agreement with the lack of activity. The complex site-specific bioactivation can be summarized as follows:



as also shown in Fig. 1.

The overall result of this bioactivation is a very pronounced and prolonged intraocular pressure reducing effect (2) of the otherwise inactive ketoximes. In conclusion, a true site- and stereospecific drug delivery has been accomplished by using this approach. Similar approaches should be of general use for achieving site-specific drug delivery of other hydroxyl-containing drugs.

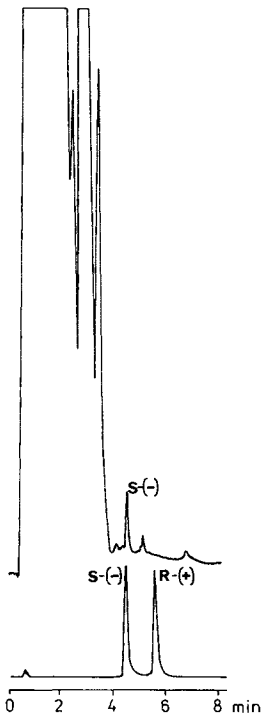


Fig. 3. HPLC analysis of the iris-ciliary body extract obtained from rabbit eyes after instillation with 1-(isopropylamino)-3-(1-naphthyloxy)-2-propranolone oxime after derivatization with the chiral reagent GITC (upper trace), in comparison with the analysis of the commercially available racemic drug (lower trace).

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