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# Formation of propranolol in the iris-ciliary body from its propranolol ketoxime precursor – a potential antiglaucoma drug

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## Summary

A new site-specific chemical delivery system (CDS) for propranolol was designed and investigated as a novel potential antiglaucoma agent. Effect of this compound, propranolone oxime (**1**), on the intraocular pressure (IOP) of rabbits was studied at two different concentrations (0.5% and 1.0%) by unilateral application to normal rabbits. Compound **1** effectively lowered the IOP at both dose levels, and its action was more prolonged and pronounced than that of propranolol (Bodor et al., 1988). Administration of **1** to the eye of rabbits was not associated with irritation, contrary to topical administration of propranolol. Propranolol (**3**) was detected in the different eye compartments for up to 2 h following administration of the oxime (**1**). Propranolol (**3**) was not, however, detected in the rat's blood following systemic administration of the oxime.

## Introduction

We have previously found that esters of adrenalone but not adrenalone itself can be converted via a reduction–hydrolysis sequence to the active adrenaline (epinephrine) only at the iris-ciliary body, the site of action (Bodor and Visor, 1984). This suggested that lipophilic ketones can be reduced in the iris–ciliary body. Accordingly, ketone precursors of β-blockers which are also β-hydroxylamines like adrenaline could then possibly be converted to the active β-blockers by a reductive process at the site of action, thus avoiding the various systemic side effects. Recently, a number of serious systemic adverse reactions secondary to topical ocular timolol administration has been reported. These include: cardiovascular (Mishra et al., 1983; Linkewich and Herling, 1981), respiratory (Ahmad, 1979; Richards and Tattersfield, 1985), CNS (Wilson et al., 1980) and ocular (Van Buskirk, 1980) side effects. Various attempts to synthesize the ketones corresponding to various β-blockers (propranolol, timolol, carteolol, etc.) have failed due to the chemical instability of these β-amino-ketone ethers (Bodor et al., unpublished results). In order to stabilize the ketone precursors, the hydrolytically sensitive oxime function was considered. Thus, a hydrolysis-reduction sequence could produce the active amino-alcohol at the iris–ciliary body, the site of action, as shown in Fig. 1.

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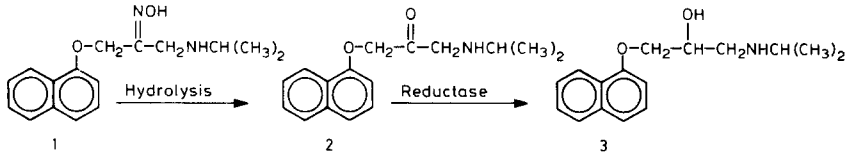



Fig. 1. Hydrolysis–reduction sequence from propranolone oxime for propranolol.

Here we report studies on the site-specific delivery of propranolol, based on the above concept, and in particular the potential use of the ketoxime precursor of propranolol in the treatment of glaucoma.

## Materials and Methods

The synthesis of the compounds used and related studies are published elsewhere (Bodor et al., 1988). The propranolone ketoxime used in these studies was synthesized in 10.8% overall yield according to Fig. 2. The compounds used were fully characterized by elemental analysis, NMR and HPLC. The oxime **1** as its HCl salt is a stable, water-soluble compound.

*Effect on the intraocular pressure of rabbits*

Adult male New Zealand albino rabbits weighing 2.5–3.5 kg were used. Animals were kept in individual cages with free access to food and water. Intraocular pressure (IOP) was measured using a Digilab model 30 R pneumatonometer. The pneumatonometer readings were checked at least twice a day using the Digilab calibration verifier. All measurements were obtained from unrestrained unanesthetized rabbits. One drop of 0.5% propacaine (Ophthetic-Allergan Pharmaceuticals, Inc.) diluted 1 : 2 with saline was instilled in each eye immediately prior to IOP measurement. Drugs were administered as 0.5 or 1.0% solution in saline in one of the eyes of a group of 5 rabbits. The untreated eye served as a control and received vehicle only. IOP was recorded after 30 and 60 min and then after 2, 3, 4, 5 and 6 h following the drug or carrier administration. All IOP measurements reported were carried out by the same operator using the same tonometer. Values are given as means ± S.E.M. Significance of the change was determined using the Student's *t*-test. Rabbits were also observed for any obvious manifestation of irritation caused by the drugs under investigation, such as congestion, redness and lacrimation.

*In vivo distribution – metabolism studies*

*In ocular tissues of rabbits.* Adult male New Zealand rabbits weighing 2.5–3.5 kg were used. Standard doses of 100 µl of a 1% solution of the drugs in saline solution were administered topically to both eyes of each rabbit. After appropriate time intervals (30, 60 and 120 min) animals were sacrificed. Aqueous humor was obtained by making a single puncture at the limbus using a 25 gauge × 1.6 cm needle attached to an 1 ml syringe. Then the cornea and the iris–ciliary body were isolated. The tissues were pooled and homogenized using a Tekmar SDT tissuemizer in

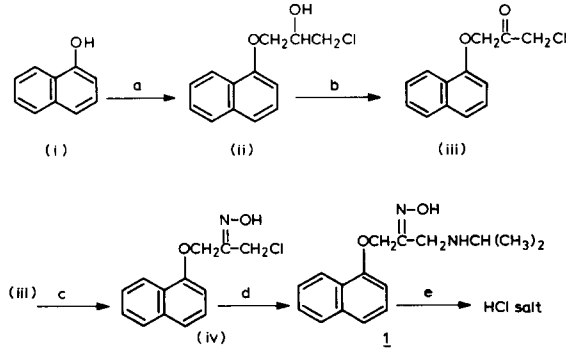
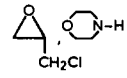



Fig. 2. **a**:  , 90–110 °C, 8–16 h. Yield 95–100%.

**b**: DCC, DMSO, $Et_2O$, pyridine. TFA, 5–20 °C, 2–12 h. Yield 46–56%.

**c**: $NH_2OH.HCl$, DMSO, 50 °C, 10 min, room temp. 2–3 h. Yield 75%.

**d**: $(CH_3)_2CHNH_2$, THF, 50 °C, 1.5 h. Yield 36%.

**e**: HCl gas, 10 °C, 0.5 h. Yield 84%.

ice-cold perchloric acid (0.05 M) which contained 0.05% sodium metabisulfite as antioxidant. Samples were then rehomogenized in $CH_3OH$ to prepare 10% homogenates, transferred to microfilters and centrifuged for 20 min at 10000 rpm to precipitate proteins. Aqueous humor was analyzed as such without any further dilution. Aliquots of 5–20 μl of the 10% tissue homogenate samples were analyzed by HPLC. Quantitation was done by using a calibration curve obtained by the addition of known amounts of the compound to aqueous humor, iris-ciliary body or cornea obtained from a control rabbit after topical administration of saline solution.

*In rat blood.* A group of 7 adult male Sprague–Dawley rats each weighing 150–250 g was used. Animals were intrajugularly injected with propranolone oxime at a dose of 6 mg/kg. After 1, 3, 5, 20, 40 and 60 min, 0.1 ml of blood was withdrawn from the jugular vein and dropped immediately into a tared tube containing 1 ml of ice-cold acetonitrile. The tubes were shaken vigorously, centrifuged, decanted and analyzed for propranolol and propranolone oxime by HPLC. Quantitation was done by using a calibration curve obtained by addition of known amounts of propranolone oxime to blood obtained from a control rat pretreated with saline solution.

*Analytical method*

A high-pressure liquid chromatography (HPLC) method was developed for the assay of propranolol and propranolone oxime in biological fluids. The chromatographic analysis was performed on a system consisting of Beckman Model 112 solvent delivery system, Model 340 Injector and Waters Model 481 variable wave length LC spectrophotometer. An ASI reverse-phase chrompack $C_{18}$ column, operated at ambient temperature, was used for all separations. The column was protected with a guard column packed with Whatman $C_{18}$ corasil packing material. The mobile phase used for separation consisted of water, 1-heptane sulfonic acid, 0.1 M acetic acid, 0.1 M triethanolamine and methanol (90 : 1 : 10 : 100 : 799). Flow rate was 1.5 ml/min with a column pressure of 1200–1500 p.s.i. at 25 °C. Retention time was 3.1 and 5.5 min for propranolone oxime and propranolol, respectively. Mixture of the two compounds has shown a very good separation with a linear concentration vs. peak height plots ($r =$ 0.9995–0.9998) for the range of 10–50 ng injected compound.

## Results

*Effect on IOP of rabbits*

We have recently reported (Bodor et al., 1988) that propranolol HCl (1%) reduces the IOP of rabbits for about 4 h. At this dose level, propranolol exerted a slight irritant activity on the rabbit's eye characterized by slight congestion, redness and increased eye secretion. The irritation was much more pronounced when propranolol was applied at the higher dose level (2.5%), and almost completely masked the IOP-reducing action.

Propranolone oxime HCl (**1**) (1%) administered bilaterally displayed an ocular hypotensive activity, which was more prolonged and in most cases more intense than that of propranolol HCl administered at the same concentration (Bodor et al., 1988). At this dose level the oxime (**1**) was completely devoid of any irritant action such as swelling, redness or lacrimation. Increasing the dose level of the oxime to 2.5% did not lead to a proportional increase in the ocular hypotensive activity of this compound, probably due to reaching a maximum in transport and enzymatic process rates. However, at the higher concentration (2.5%), propranolone oxime (**1**) was still completely devoid of any irritant action on the eye. In the present work, the unilateral administration of **1** was studied at two different concentrations, 0.5% and 1.0%. This regimen must lower IOP only in the treated eye if the proposed mechanism is correct. Indeed, significant decrease in the IOP was observed at both dose levels, *only* in the treated eye (Table 1).

*In vivo distribution and metabolism*

*In ocular tissues of rabbits.* In vivo studies revealed that propranolol concentrations are below detection limit in rabbit eye tissues examined 2 h following topical administration (Table 2). Its

TABLE 1

*Effect of the unilateral administration of propranolone oxime HCl (1) on the IOP of rabbits*

| Time after administration | Propranolone oxime HCl (0.5%) | | | | Propranolone oxime HCl (1%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | % Change | Treated | % Change after treatment | Control | % Change | Treated | % Change after treatment |
| Zero | 17.0 ± 0.6 | | 17.0 ± 0.4 | | 17.4 ± 1.1 | | 19.4 ± 1.1 | |
| 30 min | 16.8 ± 0.7 | −1.17 | 15.6 ± 0.7 | −8.2 | 18.2 ± 0.9 | +4.59 | 19.2 ± 1.0 | −1.0 |
| 60 min | 17.2 ± 0.6 | +1.17 | 14.4 ± 0.7 | −15.3 ** | 17.4 ± 0.8 | 0.00 | 18.2 ± 0.9 | −6.2 |
| 2 h | 16.4 ± 0.5 | −3.53 | 14.6 ± 0.8 | −14.1 ** | 17.2 ± 1.3 | +1.15 | 14.0 ± 0.4 | −27.8 ** |
| 3 h | 16.6 ± 0.7 | −1.17 | 14.2 ± 1.0 | −16.5 ** | 17.8 ± 1.4 | +2.29 | 14.0 ± 0.5 | −27.8 ** |
| 4 h | 16.6 ± 0.7 | −2.35 | 13.8 ± 0.9 | −18.8 ** | 18.2 ± 1.4 | +4.59 | 15.0 ± 0.4 | −22.7 ** |
| 5 h | 16.4 ± 0.5 | −3.53 | 14.4 ± 0.7 | −15.3 ** | 17.6 ± 1.3 | +1.15 | 16.6 ± 1.1 | −14.4 ** |
| 6 h | 17.2 ± 0.7 | +1.17 | 14.4 ± 0.4 | −15.3 ** | 17.4 ± 1.3 | +0.00 | 16.4 ± 1.2 | −15.5 ** |

Figures represent the IOP in mm Hg after administration of **1**. Values are means ± S.E.M. of the mean of 5 rabbits.
** Significant decrease in IOP ($P < 0.01$)

TABLE 2

*Tissue concentration of propranolol (μg/g) at various time intervals following topical administration of propranolol HCl (1% solution)*

| Tissue | 30 min | 60 min | 120 min |
|---|---|---|---|
| Cornea | 47.10 ± 5.57 | 14.54 ± 2.97 | 0.00 ± 0.00 |
| Iris–cilliary body | 8.05 ± 1.47 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Aqueous humor | 1.28 ± 0.19 | 0.26 ± 0.08 | 0.00 ± 0.00 |

Figures represent the mean ± S.E.M. of the mean of 4 rabbits ($n = 8$).

disappearance from the iris-ciliary body was even faster, 1 h following administration. When propranolone oxime HCl (**1**) was topically adminis-

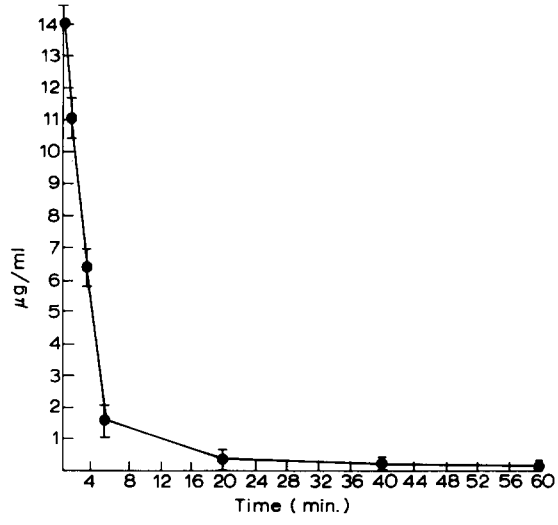



Fig. 3. Blood levels vs time of propranolone oxime (**1**) following its i.v. administration at a dose level of 6 mg/kg to rats.

TABLE 3

*Tissue concentrations of propranolol and propranolone oxime at various time intervals following topical administration of propranolone oxime HCl (1% solution)*

| Tissue | Propranolone oxime (μg/g) | | | Propranolol (μg/g) | | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 120 min | 30 min | 60 min | 120 min |
| Cornea | 23.75 ± 4.91 | 16.40 ± 5.80 | 0.00 ± 0.00 | 1.68 ± 0.75 | 1.14 ± 0.29 | 1.14 ± 0.22 |
| Iris–cilliary body | 7.79 ± 1.10 | 0.00 ± 0.00 | 0.00 ± 0.00 | 2.11 ± 0.29 | 1.79 ± 0.20 | 0.43 ± 0.11 |
| Aqueous humor | 0.82 ± 0.09 | 0.80 ± 0.06 | 0.00 ± 0.00 | 0.04 ± 0.02 | 0.71 ± 0.11 | 0.00 ± 0.00 |

Figures represent the mean ± S.E.M. of the mean of at least 4 rabbits ($n = 8$–12).

tered to the rabbit's eye, propranolol (**3**) was detected in measurable concentrations up to 2 h both in the cornea and the iris-ciliary body (Table 3). The oxime could not be detected in the iris-ciliary body after 1 h and from the cornea and aqueous humor after 2 h following its topical administration.

*In rat's blood.* Results of this set of experiments revealed that propranolol (**3**) was not detected as a biotransformation product of propranolone oxime (**1**) in blood. On the other hand, another degradation product which is more hydrophilic than the oxime itself was detected in the first few minutes after i.v. administration of the oxime. However, 5 min after injection this compound could not be seen any more. The oxime itself appeared to be metabolized rapidly in vivo (Fig. 3). The $t_{1/2}$ in blood was equivalent to $7.64 \pm 0.55$ min ($t_{1/2}\ \alpha = 1.49 \pm 0.1$ min).

## Discussion

The objective of this work was to develop a site-specific chemical delivery system (CDS) for propranolol for its possible use in the treatment of glaucoma. Propranolone oxime HCl (**1**) was synthesized for this purpose and was first tried at two different concentrations (0.5 and 1.0%) to see if it is effective in reducing the IOP of rabbits, when applied unilaterally. The results show that the oxime (**1**) has two major advantages over propranolol itself. First, at both concentrations, the ocular hypotensive activity of **1** gradually developed and was much more pronounced and prolonged than that of propranolol itself (Bodor et al., 1988). In addition, at both concentrations used, **1** was completely devoid of any irritant action on the rabbit's eye. On the other hand, as reported before (Bodor et al., 1988) propranolol HCl had a slight irritant activity on the rabbit's eye when administered as a 1% solution and a marked one at 2.5%, which appeared to contribute to its reduced action on the IOP.

The rapid disappearance of **3** from ocular tissues might explain the shorter duration of action of propranolol relative to that of propranolone oxime. Accordingly one could suggest that the ocular hypotensive activity of the oxime (**1**) is most probably due to its site-specific conversion to propranolol (**3**) in the ocular tissues of rabbits (Fig. 1).

The i.v. administration of **1** indicates that the metabolic pathway of the oxime in the blood is quite different from that in ocular tissues. Thus, propranolol was not detected in rat blood following the i.v. administration of the oxime and, instead, another more polar compound was detected as a degradation product of the oxime. These results indicate that even if some of the oxime is systemically absorbed after its topical administration, it is unlikely that it could be converted in the blood to propranolol at biologically active concentrations. This subsequently might lead us to expect less side effects after topical administration of the oxime than after administration of propranolol itself.

While these results would suggest that the propranolol formed in situ in the iris–ciliary body is responsible for the IOP reduction observed, the ketoxime itself might have some intrinsic activity. Further studies to clarify the extent of $\beta$-blocking activity of **1** and its possible metabolites are currently underway. Regardless of what the intimate mechanism of action is (bioactivation or intrinsic activity), the ketoxime **1** represents a novel, non-irritating and effective IOP reducing agent.

## Acknowledgements

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