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RESEARCH PAPER

Intraocular Pressure-Lowering Activity and In Vivo Disposition of Dipivalyl Terbutalone in Rabbits

Indra K. Reddy,[1,*] Siva R. Vaithiyalingam,[1] Mansoor A. Khan,[1] and Nicholas S. Bodor[2]

[1]*Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas 79106*
[2]*Center for Drug Discovery, J. Hillis Miller Health Center, College of Pharmacy, University of Florida, Gainesville, Florida 32610*

ABSTRACT

The ocular hypotensive activity and in vivo disposition of dipivalyl terbutalone, a chemical delivery system for terbutaline, were investigated in the albino rabbit model. The dose–response relationship was assessed at drug concentrations ranging from 0.5 to 4% in normal saline. Intraocular pressure (IOP)-lowering activity of dipivalyl terbutalone was compared with epinephrine in equal concentrations. The in vivo disposition was investigated after topical administration of dipivalyl terbutalone at 4% dose level for which two metabolic products, terbutaline, and terbutalone, were monitored in different anterior segment tissues/fluid of albino rabbits, including corneal and iris–ciliary body homogenates and aqueous humor. The instillation of 0.5, 1, 2, and 4% solutions (1 drop of 50 μL) significantly decreased the IOP of normotensive rabbits in a dose-dependant manner. At the highest dose, the maximum reduction (5.6 ± 0.65 mm Hg) was observed at 3 h. In a comparative efficacy study, dipivalyl terbutalone was found to be more effective than epinephrine. In the in vivo distribution after the topical administration of dipivalyl terbutalone, terbutaline was found only in the iris–ciliary body, whereas terbutalone was found in all parts of the eye studied. This work suggests the potential use of dipivalyl terbutalone as an antiglaucoma agent, representing a new chemical delivery system for terbutaline.

KEY WORDS: Intraocular pressure; Dipivalyl terbutalone; Site-specific; Antiglaucoma agent; Chemical delivery system.

*To whom correspondence should be addressed.

INTRODUCTION

Adrenergic agonists and antagonists have shown to affect intraocular pressure (IOP) in humans (1). Selective β_2 receptor agonists such as salbutamol and carbuterol were found to lower IOP of normotensive rabbits more than isoproterenol, and the ocular hypotensive effect of salbutamol was found to be antagonized by propranolol, a nonselective beta blocker (2–4). Selective β_2 stimulation with terbutaline, another β_2-selective drug, elicited both immediate and long-lasting hypotension, and it was found to be a more active ocular hypotensive drug compared with equivalent doses of epinephrine and isoproterenol (4). The IOP-lowering activity of terbutaline was associated with a reduction in aqueous humor formation (5). Ibuterol, a prodrug of terbutaline, was approximately 100-fold more potent than the parent species, terbutaline, in producing occular hypotension in the ipsilateral eye of normal as well as of sympathectomized rabbits (6). However, tachyphylaxis to the ocular hyppotensive effect of ibuterol develops fairly rapidly (6). Newer drug delivery strategies are continuously sought to better deliver drugs that have shown therapeutic potential in the eye.

Novel metabolism-based "retrometabolic" drug design strategies including the chemical delivery system (CDS) concept were proposed to design safe and effective opthalmic drug delivery systems (7–10). The CDS approach is based on the predictable multistep metabolic activation of bioreversible inactive agents at the site of action (9,11,12). The CDS concept was successfully applied to develop site-specific, mydriatic (13–16), and antiglaucoma agents (17,18). The CDS concept was extended to develop terbutaline and metaproterenol chemical delivery systems for the eye (9,19). According to the proposed concept, diacyl terbutalone derivatives such as dipivalyl terbutalone, **1** (Fig. 1) are capable of delivering the active drug terbutaline, **3** site-specifically to iris–ciliary body tissues via a reduction–hydrolysis sequence as shown in the case of diacyl adrenalone derivatives (14).

In this work, ocular hypotensive activity of dipivalyl terbutalone, a novel chemical delivery system for terbutaline, was investigated at different dose levels in the albino rabbit model. The IOP-lowering activity of dipivalyl terbutalone was compared with epinephrine at 4% concentration. The in vivo tissue distribution within the ocular tissues after topical administration of dipivalyl terbutalone was studied to assess the site specificity of the CDS to deliver terbutaline to iris–ciliary body tissues. In these investigations, two possible metabolites of dipivalyl terbutalone **1**, terbutalone, **2** (product of hydrolysis) and terbutaline, **3** (product of reductive-hydrolytic processes, Fig. 1).

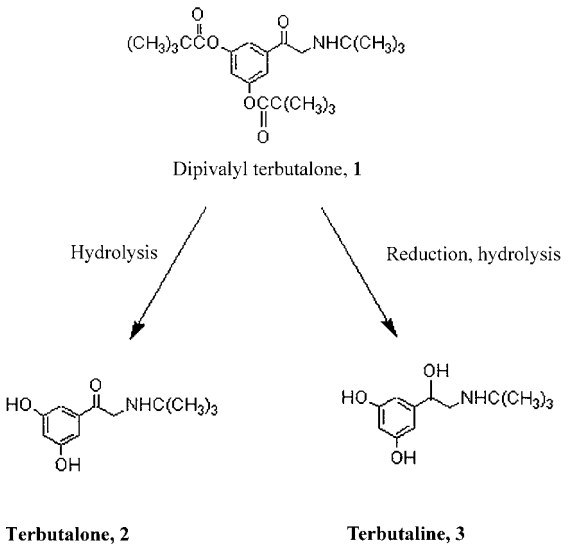

Figure 1. Chemical structures of dipivalyl terbutalone (**1**), terbutalone (**2**), and terbutaline (**3**).

MATERIALS AND METHODS

Materials

Adult male albino New Zealand rabbits weighing 2.0–3.0 kg each were used in these investigations. The dipivalyl terbutalone was synthesized according to the previously reported method (19). The terbutaline was supplied by Ciba-Geigy (Summit, NJ). All other chemicals used were of reagent grade and were purchased from Fisher Chemical Company (Pittsburg, PA), unless otherwise stated, and were used without additional purification.

Analytical Method

High-Performance Liquid Chromatographic (HPLC) analysis of dipivalyl terbutalone (**1**) and its possible metabolites (**2** and **3**) was performed using a Waters Corp. (Milford, MA) multicomponent system consisting of a model 6000A double-piston reciprocating chromatography pump; model U6K universal injector; and model 440 absorbance detector. A 25-cm reverse-phase analytical C_{18} column (Waters Corp., Milford, MA) and a Rainin guard column with a mean particle diameter of 5 μm were used at ambient temperature.

The mobile phase consisted of 50% each of acetonitrile and potassium dihydrogen phosphate monobasic (0.05 M)

with 0.005 *M* of tetrabutylammonium phosphate. At a flow rate of 2 mL/min and an absorbance detection at 254 nm, the retention times of dipivalyl terbutalone, terbutalone, and terbutaline were found to be 4.9, 4.0, and 3.5 min, respectively. The calibration curves were constructed from linear plots of peak height versus concentration. Quantitation was done using calibration curves obtained by the addition of known amounts of terbutalone and terbutaline to ocular tissues (cornea and iris–ciliary body) and aqueous humor obtained from a control rabbit.

Ocular Hypotensive Activity

IOP was measured using a pneumatonometer (Digilab, model 3 OR Hanover, Germany), which utilizes a pneumatic sensor. The tonometer was calibrated at the beginning of the experiment using a three-point calibration verifier (Digilab). Rabbits were kept in individual cages with free access to food and water. The compounds were administered as aqueous solutions (0.5–4% in dose-response studies and 4% in comparative studies) in phosphate buffer (pH 7.4) or normal saline into both eyes of a group of six rabbits. Before the IOP measurements, corneal anesthesia was provided by topical administration of 1 drop of 0.5% proparacaine (Allergan, Irvine, OH) to minimize discomfort from tonometry. Each measurement lasted between 5 and 10 s. An average value of three measurements was recorded at set time points (0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h) on a chart calibrated in millimeters of mercury. After allowing a washout period of at least 8 days, the same group of rabbits was administered 50 μL of saline or buffer without the drug into both eyes and the readings were served as control. The significance of the change in IOP was determined using Student's *t*-test.

In Vivo Disposition

A standard dose of 50 μL of 4% dipivalyl terbutalone in 0.9% saline was administered topically to each eye of the rabbits. After set time intervals (5, 30, 60, and 180 min), rabbits were sacrificed and their eyes were quickly enucleated. Five rabbits were used at each time point. From the enucleated eyes, aqueous humor was obtained by making a single puncture at the limbus using a 25-gauge × 5/8″ needle attached to a 1 mL syringe. After aqueous humor was removed, cornea and iris–ciliary body tissues were separated. The tissues were pooled and homogenized in ice-cold perchloric acid (0.05 *M*) containing 0.05% sodium metabisulphate, which served as an antioxidant. Homogenization was carried out by using a tissuernizer (Silverson Machines, Inc., East Longmeadow, MA). The resultant homogenates were suspended in methanol to obtain 10% homogenates. The homogenates were transferred to microcentrifuge tubes and subjected to centrifugation for 20 min at 10,000*g* to ensure protein precipitation. Aqueous humor was analyzed without additional dilution. Appropriate aliquots (ranging from 5 to 25 μL) were injected into HPLC and analyzed for terbutalone and terbutaline.

RESULTS AND DISCUSSION

The results of dose-response (IOP-reducing activity) relationships of dipivalyl terbutalone at four different concentrations (0.5, 1, 2, and 4% solutions in normal saline) are shown in Figure 2. The maximal IOP reduction was observed at all four dose levels 3 h after topical administration, and the IOP returned to normal between 7 and 8 h after administration. Increasing the dose level (0.5, 1, 2, and 4% solutions) exhibited a linear increase in maximal IOP reduction (−2.68, −3.61, −4.69, and −5.60 mm Hg, respectively) in the tested dose range at 3-h time point. The corresponding percent of reduction values from the base line are −11.17, −15.04, −19.54, and −23.33.

The comparative efficacy study was carried out for which IOP-lowering activity of dipivalyl terbutalone was compared with that of epinephrine in 4% concentration. In these investigations, change in IOP [(treated value − treated baseline) − (control value − control baseline)] in millimeters of mercury was plotted as a function of time (Fig. 3). Dipivalyl terbutalone was found to be the most

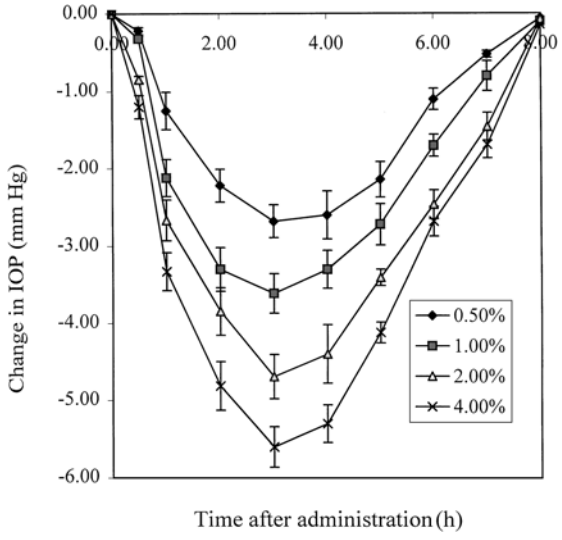

Figure 2. Dose-IOP lowering response of dipivalyl terbutalone in normotensive albino rabbits at 0.5, 1, 2, and 4% dose levels.

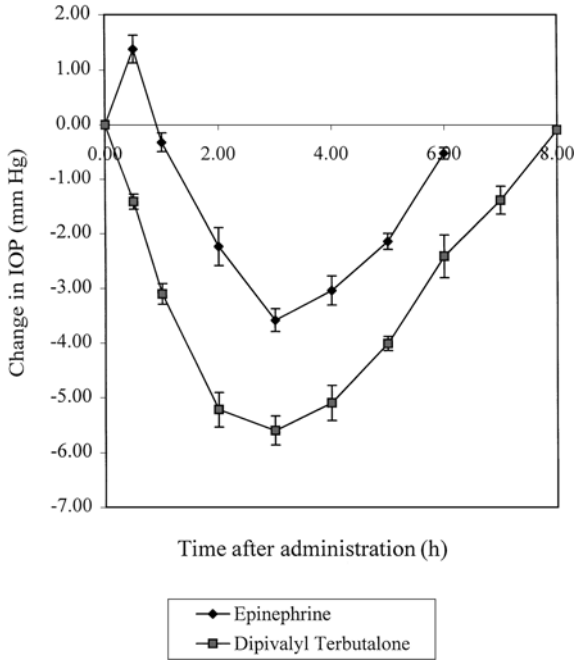

Figure 3. Comparative IOP lowering activity of dipivalyl terbutalone and epinephrine in normotensive albino rabbits at 4% dose level.

effective in decreasing IOP. At each time point, change in IOP was significantly ($p < 0.05$) lower than that with epinephrine. At 3-h time point, the percents of reduction in IOP from the baseline for epinephrine and dipivalyl terbutalone were 14.92 and 23.33%, respectively. The IOP-lowering data for epinephrine is consistent with the published findings. In comparison with epinephrine, dipivalyl terbutalone had faster onset of action. The time course of activity was found to be similar to epinephrine except that the duration of action was longer.

The in vivo disposition of dipivalyl terbutalone (**1**) in the anterior segment tissues and fluids was carried out in albino rabbits to assess its ability to reach the target site, iris–ciliary body. The two possible metabolites of dipivalyl terbutalone, terbutalone and terbutaline, were monitored in these experiments. The data from the investigations are summarized in Tables 1 and 2. The numbers reported in these tables represent the amounts of terbutaline and terbutalone per gram of tissue/fluid. From Table 1 it can be seen that the tissue concentrations of terbutalone increase and decrease quite rapidly. The highest levels of terbutalone were found in the cornea. This may be explained on the basis of the biphasic anatomical structure of the cornea, which makes it act as reservoir for the drugs entering across epithelial barrier. Relatively low levels of

Table 1.

Tissue Concentrations of Terbutalone in Albino Rabbits After Topical Administration of Dipivalyl Terbutalone[a]

	Concentration (Ng/g) of Terbutalone After Time			
	5 Min	30 Min	60 Min	180 Min
Cornea	384 ± 49	533 ± 55	475 ± 84	238 ± 51
Aqueous humor	25 ± 09	49 ± 14	31 ± 12	12 ± 05
Iris–ciliary body	39 ± 12	178 ± 44	67 ± 21	23 ± 10

Figures represent the mean ± SE of 5 rabbits.
[a]Standard dose of 50 μL of 4% dipivalyl terbutalone.

terbutalone were found in aqueous humor compared with that found in other tissues. This may be attributable to two reasons: 1) loss by continuous turnover of aqueous humor at the chamber angle and absorption by tissues of the uveal tract and the blood circulating through them; and 2) relatively lower enzyme levels in the aqueous humor compared with other tissues of the anterior chamber. The terbutalone levels were higher in the iris–ciliary body tissues than in aqueous humor. These findings are consistent with the reported data on adrenalone esters (13). In the ocular tissues, terbutalone is evidently formed in vivo by the hydrolytic cleavage of the diester by esterases. Terbutaline, the active species, was found exclusively in the iris–ciliary body tissues in vivo (Tab. 2), suggesting the site-specificity of dipivalyl terbutalone. The presence of terbutaline in the iris–ciliary body tissues is indicative of the bioactivation via reduction-hydrolysis processes as was observed by Bodor and Visor for adrenalone esters (14). The iris–ciliary body appears to be one of the major sites of drug metabolism in the eye (21,22).

Substitution of two hydroxyls on terbutalone (**2**) by ester groups apparently renders them more lipophilic,

Table 2.

Tissue Concentrations of Terbutaline in Albino Rabbits After Topical Administration of Dipivalyl Terbutalone[a]

	Concentration (Ng/g) of Terbutalone After Time			
	5 Min	30 Min	60 Min	180 Min
Cornea	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$
Aqueous humor	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$	UD$^{\blacktriangledown}$
Iris–ciliary body	24 ± 14	64 ± 18	148 ± 29	308 ± 96

Figures represent the mean ± SE of 5 rabbit.
[a]Standard dose of 50 μL of 4% dipivalyl terbutalone.
$^{\blacktriangledown}$indicates undetected.

thereby improving their penetration into cornea across epithelium. After penetrating corneal membrane barrier, the effectiveness and site-specificity of CDS depends on the ability of the target tissue to regenerate the parent compound (**3**) at an effective rate. Although the enzymatic processes frequently destroy pharmacological activity of drugs, the bioreduction followed by hydrolysis of dipivalyl terbutalone may actually enhance the biological response delivering terbutaline to the target (iris–ciliary body) tissues. With the proper selection of stearic bulk in the esterification, the duration of action of such site-specific chemical delivery system may be manipulated. The specificity of carbonyl reductases that were thought to be responsible for this bioreduction is still unknown. The results of our investigations suggest the potential use of dipivalyl terbutalone as topical ocular hypotensive agent.

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