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Ophthalmic Drug Design Based on the Metabolic Activity of the Eye: Soft Drugs and Chemical Delivery Systems

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ABSTRACT

Despite its apparent easy accessibility, the eye is, in fact, well protected against the absorption of foreign materials, including therapeutic agents, by the eyelids, by the tear-flow, and by the permeability barriers imposed by the cornea on one side and the blood-retinal barrier on the other. Most existing ophthalmic drugs were adapted from other therapeutic applications and were not specifically developed for the treatment of eye diseases; hence, they are not well suited to provide eye-specific effects without causing systemic side effects. A real breakthrough in the area of ophthalmic therapeutics can be achieved only by specifically designing new drugs for ophthalmic applications to incorporate the possibility of eye targeting into their chemical structure. Possibilities provided along these lines by designing chemical delivery systems (CDSs) and soft drugs within the framework of retrometabolic drug design are reviewed here. Both are general concept applicable in almost any therapeutic area. This review will concentrate on β-adrenergic agonists and anti-inflammatory corticosteroids, where clinical results obtained with new chemical entities, such as betaxoxime, adaprolol, loteprednol etabonate, and etiprednol dicloacetate, exist to support the advantages of such metabolism-focused, ophthalmic-specific drug design approaches.

KEYWORDS: beta-blockers, corticosteroids, eye-targeted delivery, glaucoma, intraocular pressure, oxime

OCULAR DRUG DESIGN AND DELIVERY: CHALLENGES

For the therapeutic treatment of most ocular problems, topical administration clearly seems the preferred route, because for systemically administered drugs, only a very small fraction of their total dose will reach the eye from the general circulatory system. Even for this fraction, distribution to the inside of the eye is further hindered by the blood-retinal barrier (BRB), which is almost as effective as the blood-brain barrier (BBB) in restricting the passage of xenobiotics from the blood stream.[1] At first sight, the eye seems an ideal, easily accessible target organ for topical treatment. However, the eye is, in fact, well protected against absorption of foreign materials, first by the eyelids and tear-flow and then by the cornea, which forms the physical-biological barrier. When any foreign material or medication is introduced on the surface of the eye, the tear-flow immediately increases and washes it away in a relatively short time. Under normal conditions, the eye can accommodate only a very small volume without overflowing. Commercial eyedrops have a volume of ~30 μL, which is about the volume of the conjunctival sac in humans; however, after a single blink, only an estimated 10 μL remains.[2] Consequently, there is a window of only ~5 to 7 minutes for any topically introduced drug to be absorbed, and in many cases, no more than 2% of the medication introduced to the eye will actually be absorbed.[2-4] The rest will be washed away and absorbed through the nasolacrimal duct and the mucosal membranes of the nasal, oropharyngeal, and gastrointestinal tract. For the remaining portion, the main biological barrier to penetration is represented by the cornea, which is very effective. The human cornea is composed of 5 tissue types with 3 of them, the epithelium, the endothelium, and the inner stroma, being the main barriers to absorption. The relatively lipophilic corneal epithelium, which has low porosity and high tortuosity due to tight annular junctions, is the main barrier for hydrophilic drugs, whereas the middle stromal layer, which consists mainly of water interspersed with collagen fibrils and accounts for most of the cornea's thickness, is the main barrier for lipophilic drugs.[2,5-7] This results not only in a low net eye drug delivery, but also in substantial systemic availability of ocular drugs after topical application,[8] which also results in systemic side effects. Furthermore, even a superficial analysis of existing ophthalmic drugs reveals that most of them were not developed for the treatment of eye diseases: drugs originally intended for other therapeutic areas were converted to ophthalmic applications following accidental observations or unrelated studies that indicated their potential usefulness. This further decreases

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the likelihood of achieving eye-specific delivery and reduced systemic side effects. Various approaches have been tried to circumvent these problems of low ocular delivery and potential for substantial systemic side effects.[9]

For example, prodrug approaches have achieved some limited successes. Prodrugs are pharmacologically inactive (or maybe weakly active) compounds that result from transient chemical modifications of biologically active species, so that following administration, they are metabolically transformed into the effective drugs.[10-13] Compared with the original structure, prodrug structures incorporate chemical modifications to improve some deficient physicochemical property, such as membrane permeability or water solubility, or to overcome some other problem, such as rapid elimination, bad taste, a formulation difficulty, or simply patentability/marketability. After administration, the prodrug, by virtue of its improved characteristics, is more systemically and/or locally available than the parent drug. However, before exerting its biological effect, the prodrug must undergo chemical or biochemical conversion to the active form. Marketed ophthalmic prodrugs include, for example, dipivefrine—the dipivalate ester prodrug of epinephrine; and latanoprost and travoprost—isopropyl ester prodrugs that are prostaglandin $F_2\alpha$ analogs for which the acid metabolites are active and are prostanoid selective prostaglandin F (FP) receptor agonists.

However, a real breakthrough in the area of ophthalmic therapeutics can be achieved only by specifically designing new drugs with their ophthalmic application in mind, so that the possibility of eye targeting with reduced systemic effects is already incorporated into their chemical structure. This possibility will be illustrated here along the lines of retrometabolic drug design[14-16] for 2 important ophthalmic drug classes, β-blockers and corticosteroids.

Eye-targeted Chemical Delivery Systems: Oxime and Methoxime Analogs of β-Blockers

Concept

Several oxime or methoxime analogs of known β-adrenergic blockers are of interest as potential antiglaucoma agents.[17-27] They represent an important class of potential drugs developed using general retrometabolic drug design principles and can be considered as site-specific enzyme-activated chemical delivery systems (CDSs).[14-16] In these compounds, a β-amino oxime or alkyloxime function replaces the corresponding β-amino alcohol pharmacophore part of the original molecules (Figure 1). These oxime or alkyloxime derivatives exist in alternative *Z* (syn) or *E* (anti) configuration. They are enzymatically hydrolyzed within the eye by enzymes located in the iris-ciliary body, and, subsequently, reductive enzymes also located in the iris-ciliary body produce only the active *S*-(–) stereoisomer of the corresponding β-blockers.[19] For aryl β-amino alcohol-type β-adrenergic agonists and antagonists, most of the activity is known to reside with the (–)-stereoisomer,[28-30] possibly because this isomer allows better interaction of all 3 important functionalities (aromatic, amino, and β-hydroxyl moieties) with the β-adrenoceptor.[31] These oxime and alkyloxime derivatives showed significant intraocular pressure (IOP) lowering activity, but even their intravenous administration did not produce the active β-blockers metabolically; therefore, they are void of any cardiovascular activity, a major drawback of classical antiglaucoma agents.

The oxime-type CDS approach proposed here provides site-specific or site-enhanced delivery through sequential, multistep enzymatic and/or chemical transformations (Figures 1 and 2). In the case of eye-targeting CDS, this is achieved through a targetor (T) moiety that is converted into a biologically active function by enzymatic reactions that take place primarily, exclusively, or at higher activity at the site of action (ie, Enz^2 in Figure 2) as a result of differential distribution of certain enzymes found at the site of action (here, the eye).

The basis of the successful site- and stereospecific delivery of IOP-reducing β-adrenergic blocking agents to the eye detailed here is a general metabolic process, which appears to apply to all lipophilic ketone precursors of β-amino alcohols. However, ketones of the phenol ether-type β-blockers are not good bioprecursors to produce the β-amino alcohols in these compounds because in aqueous solutions, they decompose to form the corresponding phenols. To stabilize them, they were converted to oximes, which need to undergo a facile enzymatic hydrolysis to the bioreducible ketone. The oximes are much more stable than the ketones, but for some of them, their aqueous stability still did not provide an acceptable shelf life (eg, for alprenoxime, even at pH 4.5, which can be considered the lowest acceptable pH-limit for ophthalmic vehicle solutions and where the oxime is more stable, $t_{90\%}$ of a 1% wt/vol solution was only 44 ± 5 days at room temperature).[25] For these compounds, significant additional stabilization could be obtained by using methoximes, methylethers of the corresponding oximes. For example, for the methoxime analog of alprenolol no significant decomposition was observed within 1 year at pH values around 6.5 during storage at room temperature.[25]

A variety of such oxime and methoxime analogs of alprenolol, betaxolol, carteolol, propranolol, timolol, etc, were synthesized and studied.[17-27] They were all shown to undergo the predicted specific activation within and only within the eye. Highest concentrations of the active β-antagonists were observed in the iris-ciliary body. When applied topically,

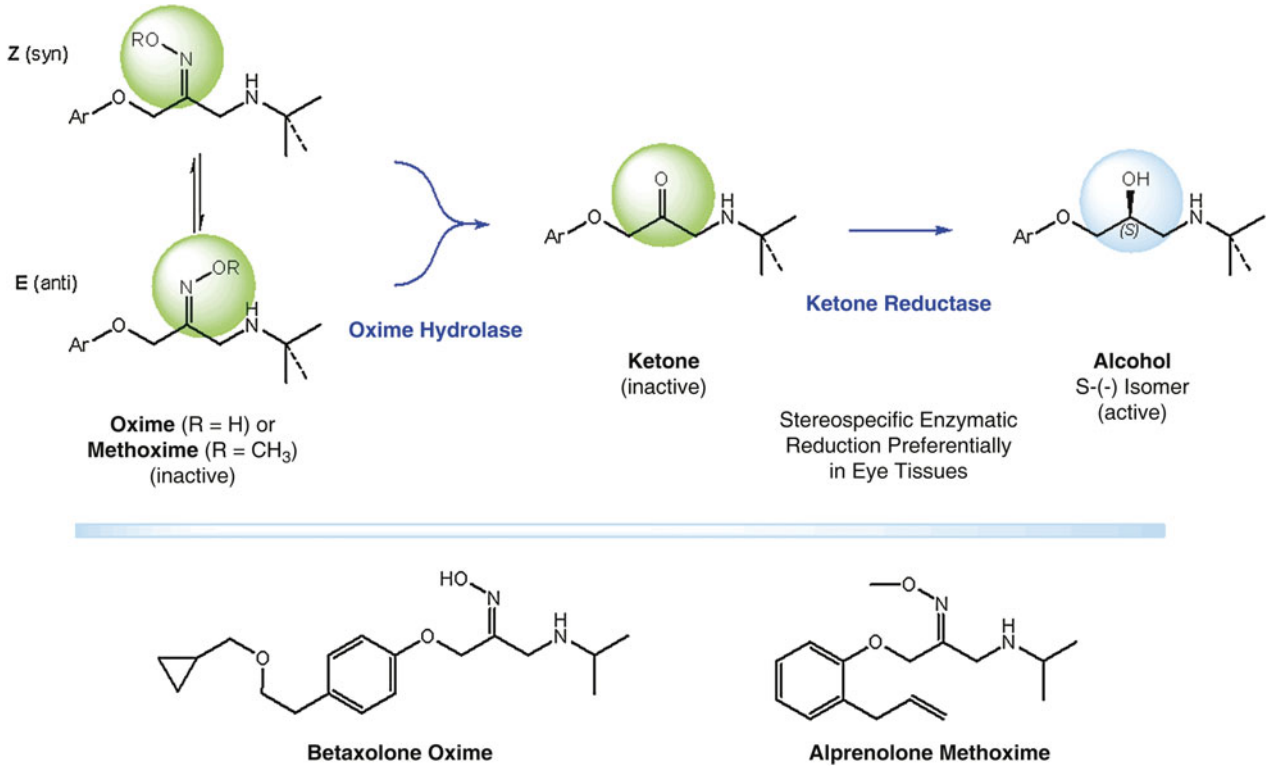


Figure 1. Sequential activation of oximes in the site- and stereospecific delivery of β-adrenergic antagonists to the eye. The original oximes or methoximes and the intermediate ketones are inactive; they are enzymatically converted into the active *S*-(–) β-adrenergic blocker alcohols in a site- and stereospecific manner.

both the oximes and the methoximes showed higher IOP-reducing activities in rabbits than the corresponding alcohols. On the other hand, topical or even intravenous (IV) administration did not produce any cardiovascular effect either in normal rabbits, dogs, and rats, or after inducing tachycardia. Following IV injection, the oxime disappeared rapidly from the blood, and at no time could the corresponding alcohol be detected. This result indicates that the required enzymatic hydrolysis-reduction activation sequence, which occurs in the eye, does not take place in the systemic circulation. Therefore, they are void of any cardiovascular activity, a major drawback of classical antiglaucoma agents.

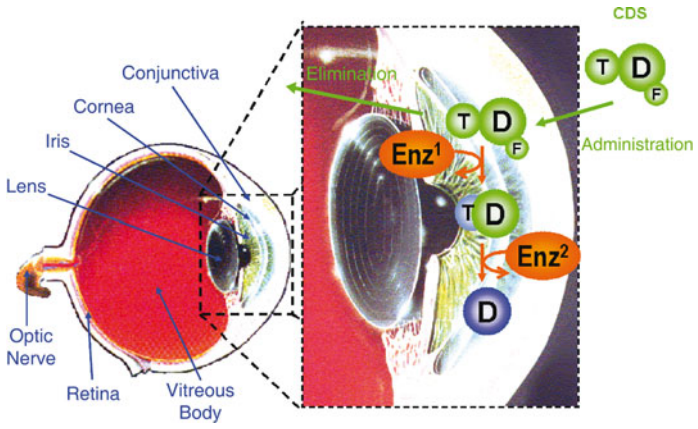


Figure 2. Schematic representation of the processes that provide eye-targeting for the oxime-type CDS approach used for eye-targeted delivery of active β-blocker drugs (D).

Potential Therapeutic Applications: Glaucoma

Glaucoma is a group of diseases of the eye characterized by progressive optic nerve cupping and visual field loss. The 2 glaucoma forms, open-angle and angle-closure glaucoma, are estimated to affect ~50 million people worldwide and to be responsible for bilateral blindness, defined by the World Health Organization (WHO) as visual acuity worse than 3/60, in more than 7 million people.[32] In the United States, glaucoma is responsible for visual impairment of 80 000 Americans, and at least 2 million people have the disease.[33] It is the leading cause of blindness in African-Americans and the third leading cause in whites. Persons most at risk for glaucoma are (1) persons with diabetes, (2) persons with recently controlled hypertension, (3) African-Americans (who have an incidence rate of glaucoma-related blindness that is 8 times that of non-African-Americans), (4) individuals with a family history of glaucoma, (5) persons with facial hemangioma, and (6) victims of eye injury. At present, the pathophysiological processes involved in the glaucomatus nerve damage and the relationship to aqueous humor dynamics are not fully understood.[34] Elevated IOP usually will lead to optic nerve damage; however, certain patients' optic nerves appear to tolerate elevated IOP in

the range of mid-to-high twenties (ocular hypertensives), while others have progressive nerve damage despite having IOP in the so-called normal range (normal or low tension glaucoma). Current medical therapies are targeted to decrease the production of aqueous humor at the ciliary body and to increase its outflow from the angle structures.

Since the introduction of timolol in the late 1970s,[35] β-adrenergic antagonists (β-blockers) have become the major class of drug used in the treatment of this disease.[36] Topical β-blockers were the first medications that had relatively few visual or ocular side effects, and today they still are widely used anti-glaucoma agents and are considered to be among the first choice of medications for initial therapy in open angle glaucoma.[37] They are effective in IOP-reduction but can produce systemic side effects through direct absorption in the tissues and via the nasolacrimal system. Just as any other drug, β-blockers administered topically to the eye are absorbed through the nasal, oropharyngeal, and gastrointestinal mucosa after passing through the nasolacrimal duct, and then only after absorption are they distributed to the systemic circulation. The systemic side effects of β-blockers are primarily related to their effects on the cardiovascular (β_1-receptor), respiratory (β_2-receptor), and central nervous systems. Inhibition of β_1-receptors in the cardiovascular system can lead to bradycardia, decreased myocardial contractility, and hypotension.[38] Among patients using topical β-blocker eyedrops twice daily, increased risk for nocturnal arterial hypotension has been reported, which represents a potential risk factor for the progression of glaucomatous optic neuropathy and anterior ischemic optic neuropathy in vulnerable individuals.[39] The blockade of β_2-receptors in the bronchi and bronchioles results in the contraction of bronchial smooth muscles; hence, β-blockers should not be used in patients with asthma or obstructive airway disease, heart failure, sinus bradycardia, and atrioventricular block greater than first degree.[38] Topically administered β-blockers might adversely affect plasma lipids, including decreased high-density lipoprotein cholesterol (HDL-C), an increased ratio of total cholesterol to HDL-C, and increased triglyceride, which may increase the risk of coronary artery disease.[37,38] From 1978 to 1985, 32 deaths were reported in association with topical timolol use, 85% of which were attributed to cardiovascular or respiratory problems.[40] In addition, central nervous system (CNS) side effects can include anxiety, confusion, depression, emotional lability, memory loss, sleep disturbance, and sexual dysfunction.[41,42] Site-directed β-blockers such as betaxoxime, specifically designed to be activated in the eye and only in the eye, should be particularly useful to avoid undesirable side effects.

Betaxolol is a relative β_1-receptor-selective adrenoceptor antagonist,[43] therefore it should reduce the risk of respiratory adverse effects by minimizing β_2-receptor inhibition. In several studies, betaxolol was found to be better tolerated than nonspecific β-blockers in patients with pulmonary disease[44,45]; nevertheless, it still can produce asthmatic attacks.[46] Betaxolol is the drug of choice in patients with compromised respiratory or cardiac functions if a β-blocker has to be used. However, betaxolol is more expensive than most ophthalmic β-blockers,[37] and it has been associated with more ocular burning and stinging. Also, IOP-control with betaxolol has not always been as effective as with timolol.[43] Betaxolol has been reported to block calcium channels and to protect neurons in vitro from glutamate toxicity. In humans, betaxolol, although not as effective in lowering IOP as nonselective β-blockers, has been reported to prevent visual field loss better than timolol. This could potentially be a reflection of its neuroprotective effect. A neuroprotective effect, combined with significant IOP reduction and safer systemic side effect profile, makes this β-blocker an excellent choice as first-line therapy for glaucoma.[37]

Oxime and Methoxime Analogs of β*-blockers*

The oxime or methoxime salts can be relatively simply prepared from the original β-blocker alcohols by oxidation of the secondary alcohol using activated dimethyl sulfoxide (DMSO) (Pfitzner-Moffat oxidation)[47] followed by coupling of the formed ketone with either hydroxylamine or methoxyamine in the same reaction medium. The oxime or methoxime derivatives exist in alternative *Z* (syn) or *E* (anti) configuration (Figure 1). Isomerization in buffer is usually relatively slow, being somewhat faster in alkaline than in acidic buffer. Equilibrium is pH-dependent (eg, for betaxoxime, equilibrium is reached within 2 weeks and the final ratios are *Z*/*E* 46:54 at pH 6.5 and 48:52 at pH 7.4, respectively).[27] Stability studies were performed for several analogs in buffer solutions at different temperatures. Methoximes are usually more stable than the corresponding oxime derivatives; at room temperature, they have t_{90}s (time within which 10% of the active drug is degraded) in the range of a few years. In biological fluids, the *Z*/*E* isomer equilibration is much faster and indicates involvement of enzymatic catalysis. For example, for betaxolone oxime and methoxime, isomerization is 300 to 500 times faster in biological fluids than in simple buffer.[27]

Following ocular administration of the oxime or methoxime analogs, the highest concentrations of the active β-antagonist alcohols formed by the predicted specific activation sequence were observed in the iris-ciliary body (Figure 3). This result seems to indicate that the iris-ciliary body is the primary site of the reduction of the intermediate ketone derivative. The oxime and methoxime analogs of alprenolol[20,25] and betaxolol (Figure 4)[27] were found to produce significant and long-lasting reduction in the IOP of rabbits

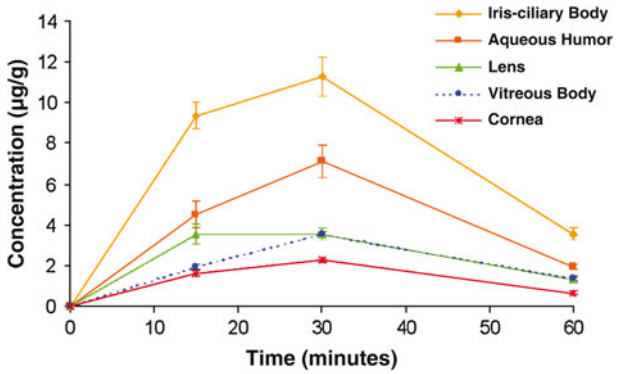


Figure 3. Concentration of betaxolol in eye tissues after topical administration of betaxoxime to rabbit eye. Data are average ± SD for 3 animals (topical administration of 50 µL doses of 2% wt/vol solution in saline to both eyes).[27]

following unilateral or bilateral administration. In most cases, the novel analogs produced more pronounced and longer-lasting effects than the parent compounds and were also less irritating.

In rats and rabbits, the IV bolus injection of alprenoxime led to insignificant transient bradycardia, while no activity was found after oral or topical administration.[20] Alprenolol in a similar dose produced sustained and significant bradycardia for more than 30 minutes. A study in dogs[24] confirmed that no significant cardiac electrophysiologic parameters are altered after systemic treatment with alprenoxime or its methyl ester analog, even at doses that far exceed that which is effective in reducing IOP. Drugs were administered as a loading 1 mg/kg IV bolus followed by a 150 µg/kg/min infusion for 20 minutes. Determined parameters included, among others, sinus cycle length (SCL), atrioventricular conduction time (AH), bundle of His conduction time (H), His-Purkinje conduction time (HV), sinus node recovery time (SNRT), sinoatrial conduction time (SACT), and various effective and functional refractory periods. None of them was affected more than 6% by the administration of the oxime or methoxime analog of alprenolol. In the mean-

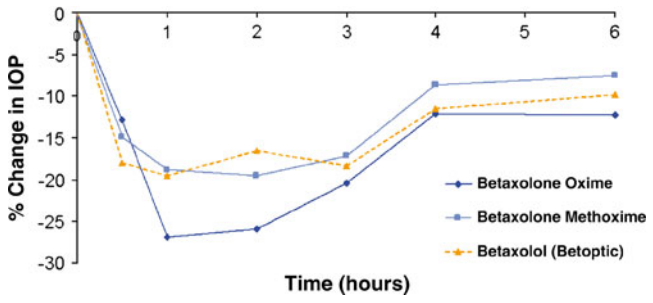


Figure 4. IOP-reducing activity of betaxolol (Betoptic) compared with those of its oxime and methoxime analogs in New Zealand albino rabbits. Doses of 100 µL solutions formulated in saline with 0.01% ethylenediaminetetraacetic acid (EDTA) and 0.01% benzalkonium chloride (pH 6.0) were administered in one eye (1.84-1.86 mg).[16]

time, administration of alprenolol itself exerted profound effects on cardiac function consistent with β-blocking activity and resulting in changes of 30% to 140%. Alprenoxime also did not alter isoproterenol-induced tachycardia in dogs.[24] Similarly, in contrast to betaxolol itself, its oxime or methoxime analogs had no effect on isoproterenol-induced tachycardia in Sprague-Dawley rats at doses up to 20 µmol/kg (administered as IV infusion of 40 µmol/mL solutions in 10% hydroxypropyl-β-cyclodextrin) (Figure 5).[27] Acute oral and IV toxicity studies have been completed in mice and rats for betaxoxime, the oxime derivative of betaxolol selected for proof of concept studies.

Clinical Investigations

For alprenoxime, an open single-dose escalating tolerance study in 14 male volunteers and a phase 1 study to evaluate safety and tolerance of multiple doses in 14 normal male volunteers have been completed. These studies found single-drop instillation of alprenoxime ophthalmic solution up to 1.0% administered up to 2 times daily for 14 days to be well tolerated in normal male individuals. No clinically significant medical events were observed. Alprenoxime had no apparent clinically significant effect on the subject's hematology, biochemistry, or urine-analysis values. The data obtained from systemic monitoring showed that neither the cardiovascular nor the respiratory systems were significantly affected by the administration of alprenoxime at the doses given in this study. There were no occurrences of symptoms of pain or foreign body sensation by subjects of any treatment group. No evidence of lowered IOP was observed in these groups of volunteers. In the multiple-dose study, the extent of systemic β-adrenoceptor blockade was evaluated by determining the effect of treatment on the dose of IV isoproterenol-induced tachycardia. No evidence of systemic β-blocking actions was evident in these studies.

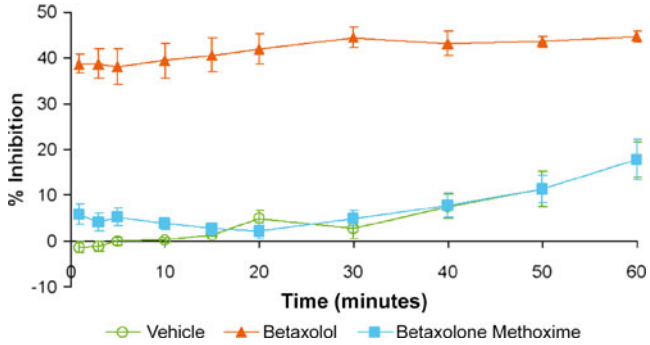


Figure 5. Effect on the isoproterenol-induced tachycardia in Sprague-Dawley rats, doses of 20 µmol/kg. Data represent mean ± SD for 4 to 6 animals per group.[27] Drugs were administered as IV infusion of 40 µmol/mL solutions in 10% hydroxypropyl-β-cyclodextrin 5 minutes after administration of isoproterenol (50 µg/kg, subcutaneously).

Further human development of alprenoxime was abandoned because it did not provide an acceptable shelf-life in aqueous solutions.

Betaxoxime, the oxime derivative of betaxolol, has been selected for proof of concept studies and has been synthesized and prepared as ophthalmic solution under good manufacturing practice (GMP) conditions. A phase-1 single-center, open, single-group study designed to evaluate the tolerability, safety, and efficacy of 2 concentrations (0.25% and 0.50%) of betaxoxime administered into one eye of ocular hypertensive subjects compared with placebo (vehicle) administered into the contralateral eye of the same subjects has been completed recently. Betaxoxime eyedrops were well tolerated at both concentrations applied; they did not irritate the eye and did not cause unwanted ocular or systemic (eg, cardiovascular) side effects. Statistically significant IOP reductions in the treated eye were found for both doses, whereas no consistent statistically significant reductions were found in the control eye (Figure 6). There was no significant change in the heart rate, and there were also no consistent, statistically significant changes in the systolic or diastolic blood pressures. Results indicate betaxoxime to be a safe and well-tolerated eyedrop with eye-targeted activity and promising IOP-lowering effects. Because of the advantages provided by its unique, eye-targeting profile, betaxoxime could replace the β-blockers currently used for ophthalmic applications.

Soft β-Blockers for Ophthalmic Use: Adaprolol

Soft drugs (SDs) represent a different, conceptually opposite targeting concept; whereas, eye-targeting CDSs, represented here by the above-described oxime analogs, are inactive compounds designed to achieve targeted effects via a multi-step activation process involving enzymes found primarily or found primarily at higher activity at their intended site of action. Soft drugs, represented here by the soft β-blockers and corticosteroids to be described below, are active compounds designed to achieve targeted effects via a single-step inactivation process involving enzymes found ubiquitously in the systemic circulation.

Eye-targeting Soft Drugs: Concept

SDs are new, active therapeutic agents, often isosteric-isoelectronic analogs of a lead compound, with a chemical structure specifically designed to allow predictable metabolism into inactive metabolites after exerting the desired therapeutic effect.[16,48,49] SDs are new therapeutic agents obtained by building into the molecule, in addition to the activity, an optimized deactivation and detoxification route. Therefore, in most cases, they produce pharmacological activity locally, but their distribution away from the site results in a prompt metabolic deactivation that prevents any kind of undesired pharmacological activity or toxicity. Because the desired activity is generally local, and the SD is applied or administered at or near the site of action, this approach is particularly well-suited for ophthalmic applications.[50,51]

With SDs, the goal is not to avoid metabolism, but rather to control and direct it. Inclusion of a metabolically sensitive moiety into the drug molecule makes possible the design and prediction of the major metabolic pathway and avoids the formation of undesired toxic, active, or high-energy intermediates. If possible, inactivation should take place as the result of a single, low-energy and high-capacity step that yields inactive species subject to rapid elimination. Most critical metabolic pathways are mediated by oxygenases, ultimately a result of the fact that "an organism's normal reaction to a

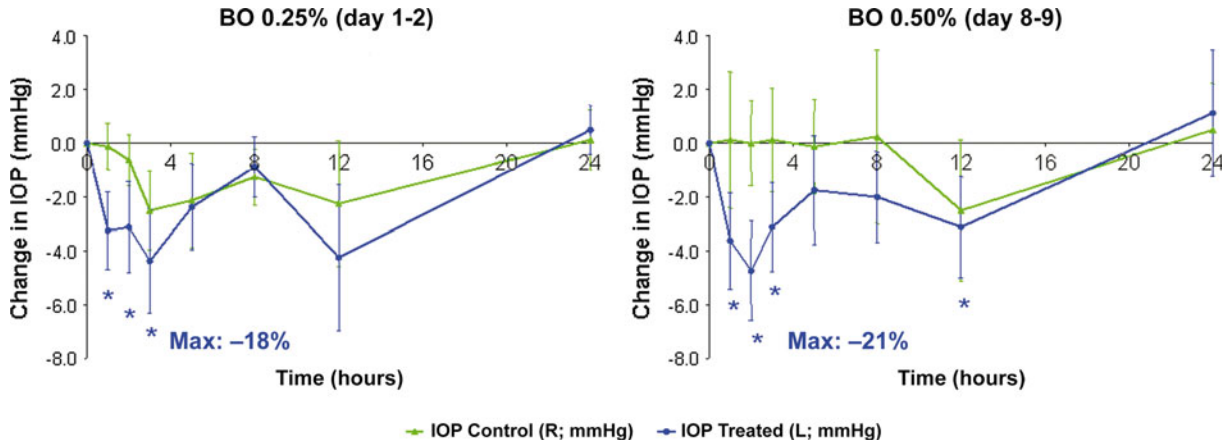


Figure 6. Effect of betaxoxime (BO) on the IOP of 4 ocular hypertensive patients in a phase 1/2 single-center, open-label, single group study shown as change in IOP versus baseline. Statistically significant reductions in the treated (L) eye ($P < .05$; paired t test versus predose at 0 hours; denoted with *) were observed at the following time points: BO 0.25%: 1, 2, and 3 hours; BO 0.50%: 1, 2, 3, 12 hours; no statistically significant reduction ($P < .03$) in the control (R) eye.

foreign substance is to burn it up as food."[52] Because oxygenases exhibit not only interspecies, but also interindividual variability and are subject to inhibition and induction,[53] and because the rates of hepatic mono-oxygenase reactions are at least 2 orders of magnitude lower than the slowest of the other enzymatic reactions,[54] it is usually desirable to avoid oxidative pathways as well as these slow, easily saturable oxidases. Therefore, the design of SDs should be based on moieties inactivated by hydrolytic enzymes. Rapid metabolism can be more reliably performed by these ubiquitously distributed esterases. Not relying exclusively on metabolism or clearance by organs such as liver or kidney is an additional advantage because blood flow and enzyme activity in these organs can be seriously impaired in critically ill patients.

Adaprolol

Because SD design is a general concept, topically applied SDs that show local activity but reduced systemic side effects could become useful therapeutics for almost any eye-related diseases. For example, in addition to the oxime or methoxime β-blocker analogs described here earlier, the development of soft β-blockers could represent another possible route toward improved, safer antiglaucoma agents. Because in this class, inactive metabolite-based SDs can be obtained by introducing the hydrolytically sensitive functionality at a flexible pharmacophore region, there is considerable freedom for structural modifications. Consequently, transport and metabolism properties are easier to control.

The metabolism of the well-known β-blocker metoprolol is compared with that of the soft β-blocker adaprolol that was designed starting from one of metoprolol's inactive acid metabolites in Figure 7. Metoprolol is extensively metabolized by the hepatic mono-oxygenase system both at the more restrictive β-amino alcohol pharmacophore region (pathway A) and at the more flexible pharmacophore region *para* to the phenol ring (pathways B and C).[55-57] Two of these metabolites, α-hydroxymetoprolol and *O*-demethylmetoprolol, have selective β_1-blocker activity but are 5 to 10 times less potent than metoprolol itself.[55] However, the main metabolites detected are the acids that are "inactive"; they are devoid of β-adrenoceptor activity or toxicity (median lethal dose LD_{50} > 500 mg/kg IV in mice).[55] Hence, the phenylacetic acid metabolite 2 shown in Figure 7 can serve as a good starting point for an inactive metabolite SD approach. Various soft β-blockers, with different receptor binding, transport, rate of cleavage, and metabolic properties, can be obtained by esterification and by the introduction of some additional flexibility in the design (eg, varying the separation between the ester moiety and the aromatic ring).[16,49]

From the various soft β-blockers developed along these lines in our laboratories, adaprolol, an adamantane ethyl ester (Figure 7), was selected as a potential candidate for a new topical antiglaucoma agent. Adaprolol was chosen based on the idea that if membrane transport (lipophilicity) and relative stability are important for pharmacological activity because they are needed to achieve good corneal permeability, then the ester group should be relatively

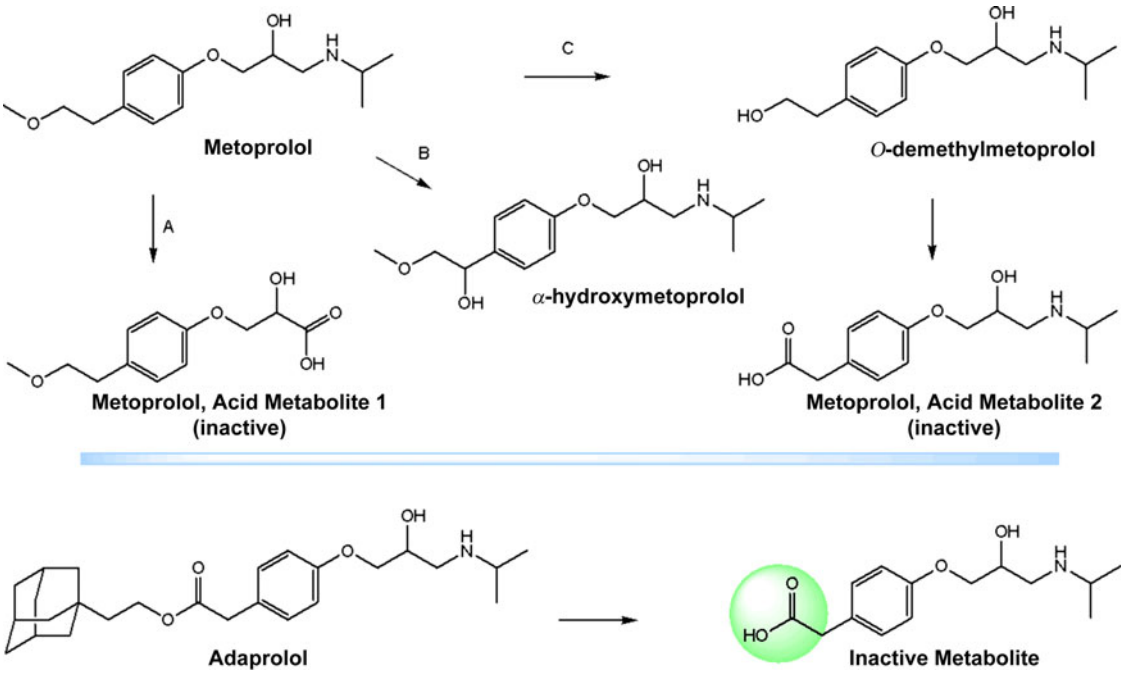


Figure 7. Structure and metabolism of the soft β-blocker adaprolol compared with that of the traditional β-blocker metoprolol. The known inactive acid metabolite of metoprolol served as the starting point of this inactive metabolite-based soft drug design.

lipophilic and impart ester stability.[58-62] Adaprolol did indeed produce prolonged and significant IOP-reduction, but hydrolyzed relatively rapidly.[59,60] Therefore, local activity could be separated from undesired systemic cardiovascular/pulmonary activity, a characteristic much sought after in the search for antiglaucoma therapy.[36] Following unilateral ocular treatment with adaprolol, no effects are produced in the contralateral eye because of systemic inactivation.

After performing the required toxicity studies, several clinical studies of adaprolol maleate have been completed already, and no severe or clinically significant medical events have been reported. A double-masked comparison of adaprolol and timolol performed on 67 ocular hypertensive patients with IOP > 21 mmHg demonstrated that intraocular pressure was significantly reduced throughout the study in all treatment groups. Adaprolol reduced IOP by ~20%, while timolol reduced IOP by 25% to 30%.[49] In patients over 70 years old, the IOP-reducing effects of 0.2% adaprolol and 0.5% timolol were statistically indistinguishable after 10 days of application (Figure 8).[49] On the other hand, timolol reduced the systolic blood pressure with statistical significance, whereas adaprolol did not (Figure 8). Timolol also showed a trend, though not statistically significant, to reduce the heart rate, while pulse was conserved in the adaprolol treatment groups. Therefore, adaprolol is effective in reducing IOP and has a safer cardiovascular profile than timolol.

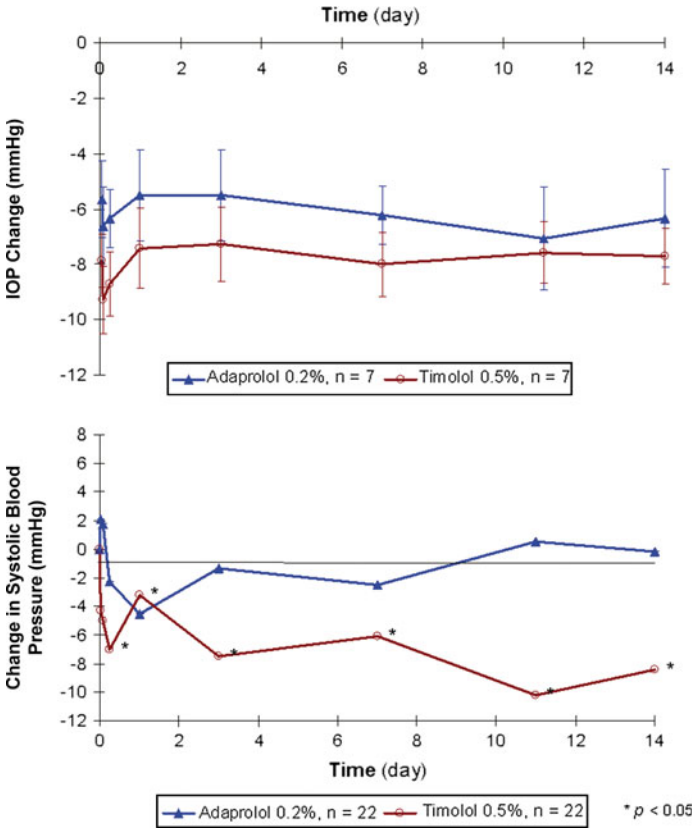


Figure 8. Comparison of the changes caused by administration of adaprolol (0.2%) and timolol (0.5%) in the IOP and systolic blood pressure of ocular hypertensive patients. IOP data shown are for patients over 70 years old.

Soft Corticosteroids: Ophthalmic Applications

Soft anti-inflammatory corticosteroids represent another, even more successful ophthalmic drug design area. Inflammation in the eye may result from surgery, injury, allergy, infection, conjunctivitis, or uveitis—conditions that can cause severe discomfort and lead to loss of vision. Topical corticosteroids represent an important class of drugs used to treat ocular inflammations and allergies as they are the most effective ocular anti-inflammatory compounds and offer the broadest range of treatment; however, several contraindications severely limit their usefulness.[63] In addition to the general systemic corticosteroid side effects,[64] they can also produce several ocular complications such as IOP-elevation and resultant steroid-induced glaucoma, induction of cataract formation, and secondary complications.[63] Ocular administration of corticosteroids usually produces increased IOP as a result of increased resistance to aqueous humor outflow, but the precise mechanism of decreased outflow is not known.[65] The mechanism of steroid-induced cataract is also not entirely clear,[66] but the most prominent hypothesis involves the formation of Schiff bases between the steroid C_{20} ketone group and nucleophilic groups such as ϵ-amino groups of lysine residues of proteins (Figure 9). Schiff base formation is potentially followed by a Heyns rearrangement[67] involving the adjacent C_{21} hydroxyl group and affording stable amine-linked adducts.[68-71] This covalent binding results in destabilization of the protein structure allowing further modifications (ie, oxidation) and leading to cataract.

Soft Corticosteroid

The design of soft anti-inflammatory corticosteroids has been one of the most active and most successful fields of SD drug design. A related, frequent misconception regarding soft steroids has to be clarified first. Often, the soft nature is associated with fast hydrolytic degradation, but this is not necessarily so. If hydrolysis is too rapid, then only weak activity may be obtained. The desired increase of the therapeutic index can be achieved only if the drug is sufficiently stable to reach the receptor sites at the target organ and to produce its desired effect, but the free, non-protein-bound drug undergoes facile hydrolysis to avoid unwanted, systemic side effects. In order to successfully separate the desired local activity from systemic toxicity, an adequate balance between intrinsic activity, solubility/lipophilicity, tissue distribution, protein binding, and rate of metabolic deactivation has to be achieved. In the case of slow, sustained

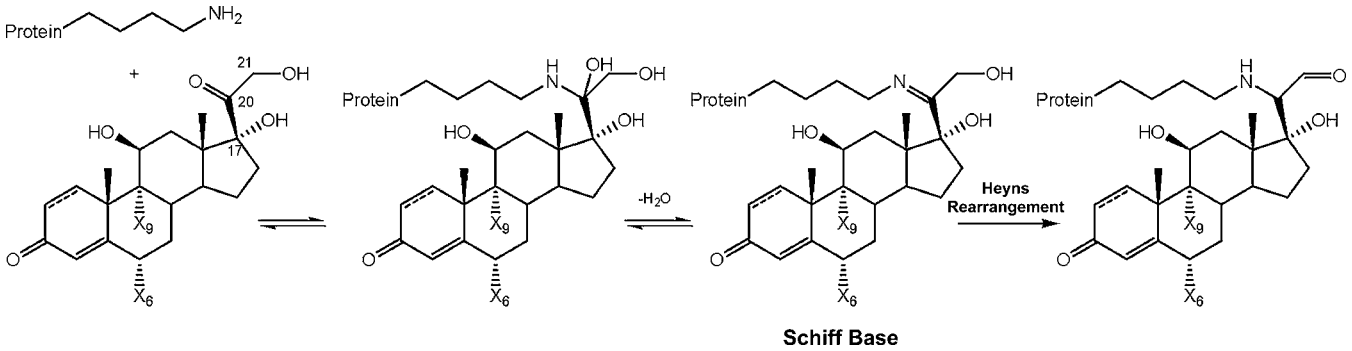


Figure 9. Mechanism of steroid-induced cataract according to the most prominent hypothesis. It involves first the formation of Schiff bases between the steroid C-20 ketone group and nucleophilic groups such as ϵ-amino groups of lysine residues of proteins and then a Heyns rearrangement involving the adjacent C-21 hydroxyl group that results in stable amine-linked adducts.

release to the general circulatory system from the delivery site, even a relatively slow hydrolysis could result in a very low, almost steady-state systemic concentration.

First-generation Cortienic Acid-based Soft Steroids: Loteprednol Etabonate and Analogs

Loteprednol etabonate (LE, Figure 10) is an active corticosteroid that lacks serious side effects and has been approved by the Food and Drug Administration (FDA) as the active ingredient of 3 ophthalmic preparations (Lotemax, Alrex, Zylet).[72-74] At present, it is the only corticosteroid approved by the FDA for use in all inflammatory and allergy-related ophthalmic disorders, including inflammation after cataract surgery, uveitis, allergic conjunctivitis, and giant papillary conjunctivitis (GPC). LE resulted from a classic inactive metabolite-based SD approach that used cortienic acid as starting point (Figure 10).[74-81] Hydrocortisone is known to undergo a variety of oxidative and reductive metabolic conversions.[82] Oxidation of its dihydroxyacetone side chain leads to formation of cortienic acid through a 21-aldehyde (21-dehydrocortisol) and a 21-acid (cortisolic acid). Cortienic acid is an ideal lead for the inactive metabolite approach because it lacks corticosteroid activity and is a

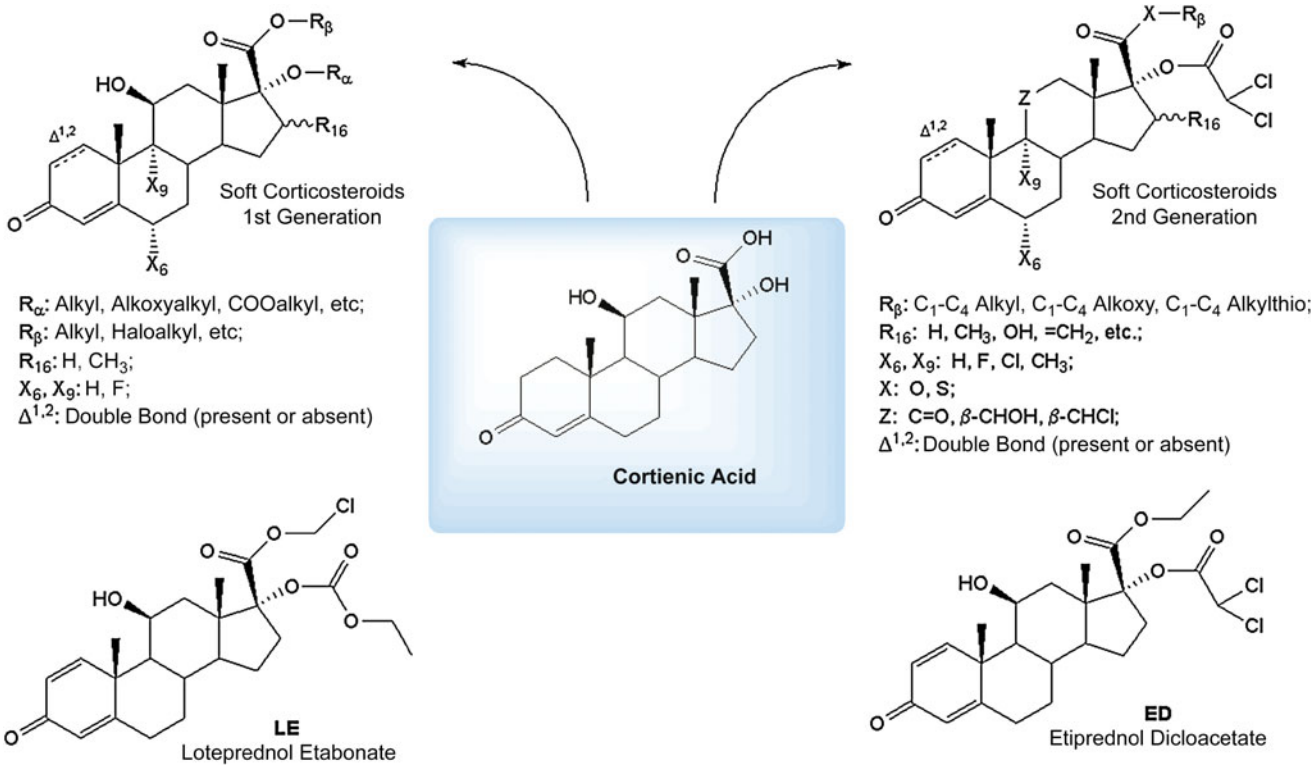
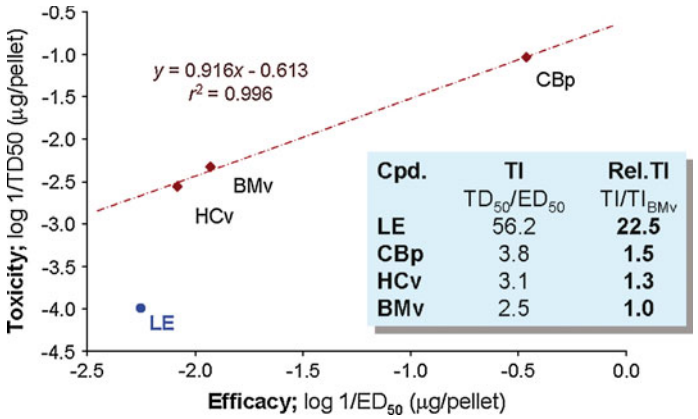


Figure 10. Design of first- and second-generation cortienic acid-based soft steroids, and their selected representative compounds LE and ED, respectively.

major metabolite excreted in human urine. To obtain new active soft compounds, the pharmacophore moieties of the 17α and β side chains have to be restored by suitable isosteric/isoelectronic substitution containing esters or other types of functions that restore the original corticosteroid activity and also incorporate hydrolytic features to ensure adequate metabolic properties. Modifications of the 17β ester function and the 17α hydroxy function, together with other changes (eg, introduction of $\Delta^{1,2}$, fluorination at 6α and/or 9α, methylation at 16α or 16β), led to a host of analogs representing the first generation of cortienic acid-based soft steroids (Figure 10). More than 120 of these soft steroids have been synthesized starting in the late 1970s[83] and during a systematic synthetic study performed in collaboration with Otsuka Pharmaceutical Co (Tokyo, Japan).[75,84,85] Critical functions for activity are a haloester in the 17β position and a novel carbonate[75,77] or ether[86] substitution in the 17α position.

Incorporation of 17α carbonates or ethers was preferred over 17α esters to enhance stability and to prevent formation of mixed anhydrides that might be produced by reaction of a 17α ester with a 17β acid functionality. Such mixed anhydrides were assumed toxic and probably cataractogenic. The carbonates were expected to be less reactive than the corresponding esters owing to the lower electrophilicity of the carbonyl carbon. LE, and some of the other soft steroids, provided a significant improvement of the therapeutic index determined as the ratio between the anti-inflammatory activity and the thymus involution activity.[50,74,87] Furthermore, binding studies using rat lung cytosolic corticosteroid receptors showed that some of the compounds approach and even exceed the receptor binding affinity of the most potent corticosteroids known.[88,89] LE was selected for development based on various considerations including the therapeutic index, availability, synthesis, and "softness" (the rate and easiness of metabolic deactivation). In traditional corticosteroids, efficacy and toxicity are closely correlated ($r^2 = 0.996$) as shown in Figure 11 using the relationship between the anti-inflammatory and thymus involution activities determined in the cotton pellet granuloma test; therefore, regardless of intrinsic activity, therapeutic indices, which represent the ratio between the median toxic dose (TD_{50}) and the median effective dose (ED_{50}), $TI = TD_{50}/ED_{50}$, are very similar. However, LE, the soft steroid, provides a significant improvement owing to its improved toxicity profile (Figure 11).

Early studies in rabbits[90,91] and rats[78] demonstrated that, consistent with its design, LE is indeed active and is metabolized into its predicted metabolites (Figure 12), and these metabolites are inactive.[77] The pharmacokinetic profile of LE indicates that, when absorbed systemically, it is rapidly transformed to the inactive 17β-carboxylic acid metabolite and eliminated from the body mainly through the bile and



Cpd.	TI TD_{50}/ED_{50}	Rel.TI TI/TI_{BMv}
LE	56.2	**22.5**
CBp	3.8	**1.5**
HCv	3.1	**1.3**
BMv	2.5	**1.0**

Figure 11. The relationship between the efficacy and toxicity of corticosteroids. Because of their close correlation in traditional steroids, the therapeutic index, which represents the ratio between efficacy and toxicity ($TI = TD_{50}/ED_{50}$), is very similar regardless of intrinsic activity (efficacy). LE indicates loteprednol etabonate (0.1%); HCb, hydrocortisone 17α-butyrate (0.1%); BMv, betamethasone 17α-valerate (0.12%); and CBp, clobetasone 17α-propionate (0.1%). ED_{50} indicates the median effective dose for the anti-inflammatory activity in the cotton pellet granuloma test (μg/pellet); TD_{50} indicates the median toxic dose determined from the thymolysis potency (μg/pellet). Relative TI computed with BMv as reference.

urine.[78,79,81] LE did not effect IOP in rabbits,[91] and later, various human studies (Figure 13)[92] also confirmed that it has no effect on IOP. Consistent with the soft nature of this steroid, systemic levels or effects cannot be detected even after chronic ocular administration.[93]

Clinical studies proved that it is a safe and effective treatment for contact lens-associated GPC, seasonal allergic conjunctivitis, postoperative inflammation (Figure 14), or uveitis.[72,73,94] A retrospective study found that even long-term (>1 year) use of LE causes no reported adverse effects.[95] Based on promising results from animal studies,[74,80,81,96,97] LE is also being developed for treatment of asthma, rhinitis, colitis, and dermatological problems.

Second-generation Cortienic Acid-based Soft Steroids: Etiprednol Dicloacetate and Analogs

More recently, a new class of soft steroids with 17α-dichloroester substituent has been identified (Figure 10).[98] This is a unique design; no known corticosteroid contains halogen substituents at the 17α position. Nevertheless, the pharmacophore portions of these second-generation cortienic acid-based soft steroids, including the halogen atoms at 17α, can be positioned so as to provide excellent overlap with those of the traditional corticosteroids (Figure 15).[88] Dichlorinated substituents seem required for activity and sufficiently soft nature, and 2 justifications seem likely. First, with dichlorinated substituents, one of the chlorine atoms will necessarily point in the direction needed for pharmacophore

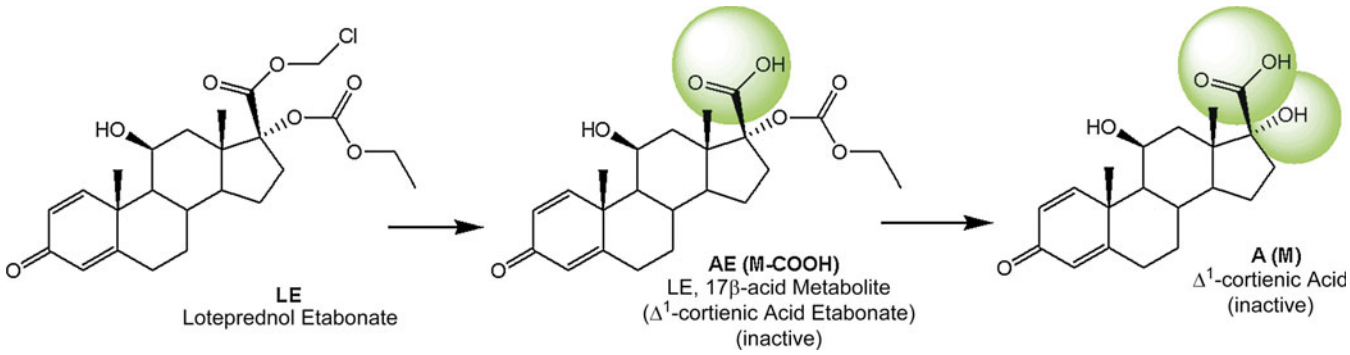


Figure 12. Metabolism of LE.

overlap, but with monochlorinated substituents, steric hindrance will force the lone chlorine atom to point away from this desired direction. Second, whereas compared with the unsubstituted ester, dichloro substituents cause an ~20-fold increase in the second-order rate constant k_{cat}/K_M of enzymatic hydrolysis in acetate esters, monochloro substituents do not cause any change.[99]

Contrary to the first generation of soft steroids, in this second generation, hydrolysis primarily cleaves not the 17β-, but the 17α-positioned ester. Nevertheless, the corresponding metabolites are also inactive. From this series, etiprednol dicloacetate (ED, Figure 10) was selected for development. ED has shown better receptor binding affinity (RBA) than LE and was proven as or even more effective than budesonide in various asthma models. In agreement with its soft nature, ED was found to have low toxicity in animal models and in human clinical trials.[98,100-102] The no observable adverse effect level (NOAEL) of ED after 28-day oral administration was found to be 2 mg/kg in rats and dogs, ~40 times higher than that of budesonide.[100]

The transrepressing and transactivating activity of ED and budesonide were also compared by measuring their inhibition in interleukin (IL)-1β production of a stimulated human monocyte cell line and by evaluating glucocorticoid-induced

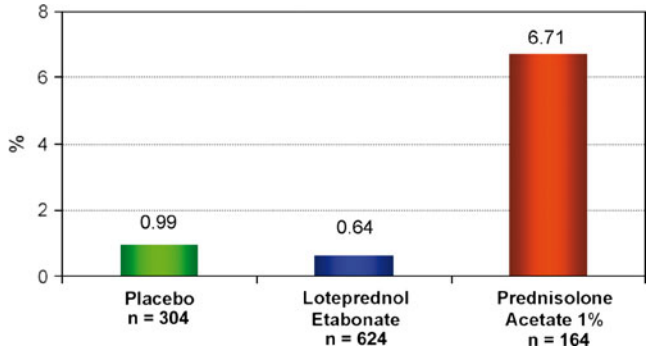


Figure 13. Pooled data showing the percentage of patients with IOP elevation greater than 10 mmHg among patients not wearing contact lenses and treated for more than 28 days with placebo, LE (concentration of 0.2% and 0.5%), and prednisolone acetate (concentration of 1%).[92]

increase in the activity of tyrosine-amino-transferase (TAT) of a rat hepatoma cell line, respectively.[101] Measured activities were expressed relative to dexamethasone. ED was found to be a dissociated glucocorticoid (ie, to possess reduced transactivating activity with a preserved transrepressing activity) (Figure 16). Glucocorticoids are signaling through a single receptor (GR), but the result of the signal can be either increase (activation) or inhibition (repression) of gene expression. Transactivation is mediated by binding of the hormone-activated receptor to a defined DNA sequence, called glucocorticoid response element (GRE). This process may account for some of the unwanted effects of glucocorticoids via the increase in expression of genes involved in gluconeogenesis and development of arterial or ocular tensions. Transrepression, which seems to be the main mechanism by which glucocorticoids suppress inflammation, may be the result of binding to negative GREs, but it occurs mainly by interaction with transcription factors (AP-1 and NF-κB), which control the genes of many inflammatory mediators from IL-1β to regulated upon activation, normal T-cell expressed and secreted (RANTES) chemokine. Hence, the dissociation of transactivating and transrepressing activity seen for ED is a likely advantage that may further help in separating the beneficial anti-inflammatory activity from the undesired side effects and is in line with the development of dissociated steroids, one of the novel mechanistic approaches pursued in development of new corticosteroids.[103,104]

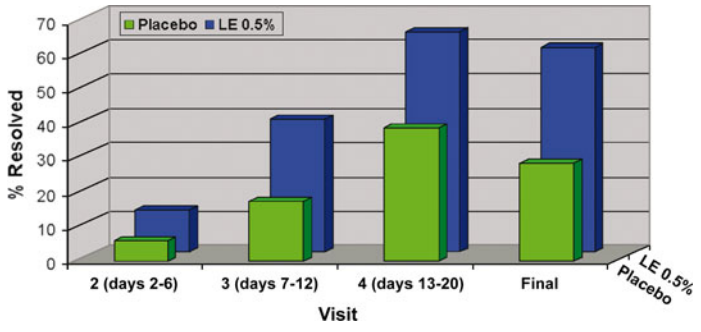


Figure 14. Resolution of anterior chamber inflammation (sum of cell and flare scores) at each visit during the treatment period in postcataract inflammation with intraocular lens implantation.[94]

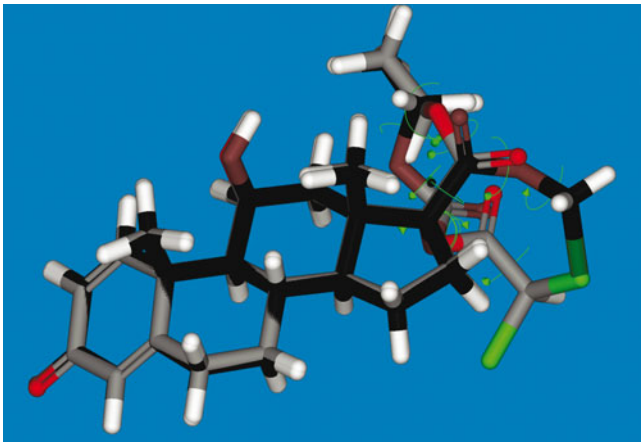


Figure 15. Overlap of LE (darker colors) and ED (lighter colors) generated by Discovery Studio's (Accelrys Inc, San Diego, CA) Molecular Overlay algorithm. To generate the overlap, individually AM1-optimized structures (CAChe 5.0, Fujitsu Ltd, Chiba, Japan) were used, and conformational change during a consensus overlap was allowed along the rotatable bonds shown. Atoms in the steroid ring structure, a pair of chlorine atoms, and the 2 pairs of carbon atoms in the alkyl side-chains were used as tethers. Note the good overlap even between oxygen atoms that were not required to overlap. The view is from the β side, from above the steroid ring system.

Conclusions

Successful eye-specific therapeutic agents can be obtained only by a drug-design process that thoroughly integrates the specific pharmacological, metabolic, and targeting requirements of ophthalmic drugs. CDS and SD approaches are both particularly well suited for this purpose, and they can provide flexible, generally applicable solutions. Their potential is well illustrated by the results obtained with several new chemical entities designed within this framework, such as betaxoxime, adaprolol, loteprednol etabonate, and etiprednol dicloacetate; all of which have already reached the clinical development phase in various ophthalmic areas, and one of them (LE) is already being marketed as well.

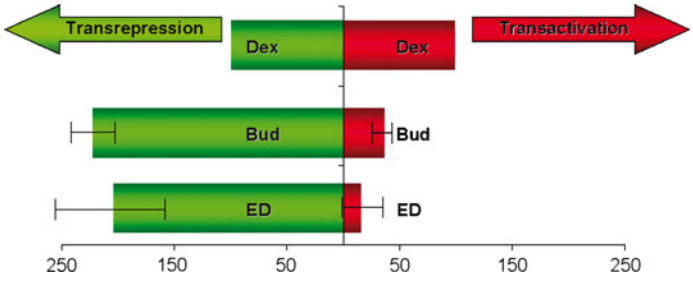


Figure 16. Comparison of transrepression (antiinflammatory effect) and transactivation (carbohydrate metabolism altering) effects of dexamethasone (Dex, used as 100% reference), budesonide (Bud), and ED. Averages of 2 experiments for concentrations of 10^{-7} are shown.[101]

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